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— (UGC Recognised) —

Proceedings of

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18th – 20th July - 2024
(ICRDFS - 2024)

Conveners
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Dr. Renu Devi

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INTERNATIONAL CONFERENCE ON RECENT DEVELOPMENTS IN FORENSIC SCIENCE

ICRDFS - 2024

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TS-02 Recent Advances in the usage of DNA Profiling Techniques and beyond

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6. **ICRDFS/FS/PP – 06** Forensic Examination of Hair
7. **ICRDFS/FS/PP – 07** 3-D printing in forensic science

MESSAGE

CHAIRMAN'S MESSAGE

Welcome to our reputed university for this fascinating and insightful forensic science conference. It is an honour for me as the Chairman of Sandip University, Nashik to witness so many intelligent and driven students come together to explore the complex field of forensics. The conference emphasize for this year encompasses forensic toxicology, forensic biology, digital forensics, fingerprints, crime scene investigation, forensic medicine, crime and society, and forensic physics among other important subjects. Every one of these fields is essential to the fight for justice as well as the development of our knowledge of crime and how it affects society. This is your opportunity to expand your knowledge, explore your perspectives, and build a solid basis for your career. We welcome you all to Sandip University to be the witness of the advancements and breakthroughs this conference will surely bring forth. I implore you to take advantage of most of the sessions, network with mentors and peers, and gain knowledge about every facet of forensics that will be discussed. This is your opportunity to broaden your knowledge, push your ideas, and create the foundation for a rewarding and successful career.

Dr. Sandip N. Jha
Chairman
Sandip University, Nashik Patron-ICRDFS-24

MESSAGE FROM VICE CHANCELLOR

I am honoured to extend a warm welcome to each and every one of you for this much-anticipated International Conference on Recent Developments in Forensic Science-2024 (ICRDFS-24) at our prestigious institute at Department of Forensic Science, Sandip University, Nashik. The event reflects our dedication to promote researches and upcoming trends and professional development in this exciting and influential area of Forensic Science. The goal of International Conference on Recent Developments in Forensic Science-2024 (ICRDFS-24) is to deliver a forum for professionals, researchers, and students in the fields of forensic science, law, police, and other related fields to review and debate state-of-the-art developments in their respective fields. A forum for presenting state-of-the-art research, networking with leading experts, and absorbing the essence of forensic and allied sciences will be provided by ICRDFS-24. I look forward to see the advancements and accomplishments that this conference will surely bring about.

Prof. (Dr.) Rajendra Sinha
Vice Chancellor
Sandip University, Nashik Patron-ICRDFS-24

MESSAGE FROM CONVENOR

I welcome distinguished guests, eminent scholars, professionals and all the participants of ICRDFS - 24. The core aim of this global scientific meet is to bring the scientific minds together across the globe. This global scientific meet will provide a platform for the exchange of scientific ideas for the better cause of society. I believe that this 03 - Day International Conference will enlighten the global audience and pave a way for young aspiring scholars where they can imbibe and share the ideas. Eminent scientists and speakers from across the globe will shed the light on contemporary global issues and the possible scientific ways to combat the same. This platform will enrich collaborative ideas, making a way for young scholars to embark on the road leading to scientific contributions for better future. I thank all sponsoring agencies, the organizing committee members, speakers, scholars, and participants for their interest, efforts, and belief in ICRDFS – 24.

Dr. Nissar A. Reshi
Associate Dean, School of Science, Sandip University, Nashik
Convenor- ICRDFS-24

MESSAGE FROM CONVENOR

Attending conferences is a great way to acquire novel ideas, interact with professionals in the field, and obtain viewpoints on ground breaking studies. Every role - presenting, attending or volunteering - offers distinct benefits that can significantly enhance your educational and career trajectory. Presenting your work enhances your communication and public speaking skills, which are critical for any career. It further demonstrates forth the depth of your understanding. Attendees gain the opportunity to establish connections with industry leaders, understand about new trends, and engage in conferences. By volunteering, you can develop your organizational skills, make new acquaintances and mentors, and actively contribute to the success of the conference. Additionally, conferences offer a forum for exchanging ideas, getting constructive criticism, and creating enduring connections that may result in future endeavors or even job prospects. You can prove that you are dedicated to gaining knowledge and further the field's understanding by actively participating. Grab full advantage of this opportunity to broaden your perspectives and leave a lasting impression. Your participation today may open doors to wonderful possibilities in future.

Dr. Renu Devi
HoD Forensic Science School of Science, Sandip University, Nashik
Convenor-ICRDFS

Identification *of* Occupational Marks of Fingerprint from House wives In Kerala (Rural Area)

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Abstract

Fingerprints include intricate characteristics for identification and matching with references. Fingerprints cannot be altered by harming the dermis through burns, abrasions, or cuts as they can regenerate with the skin. They are perceptible traces left behind by an individual and are frequently used in criminal investigations to identify suspects or link people to the crime scene. The study delves into how occupational markings in the fingerprints of housewives demonstrate variability influenced by their specific domestic duties. It examines whether these markings remain consistent over time or fluctuate with varying routines and responsibilities within the household environment. Housewives, engaged primarily in household chores such as cooking, cleaning, and caregiving, are hypothesized to develop distinct occupational markings on their fingertips. These markings, potentially influenced by repetitive and specific movements associated with their daily tasks, could provide valuable insights into their occupational history and daily routines. The research also discusses the implications of these findings for forensic science. Understanding the variability and characteristics of occupational markings among housewives can enhance the accuracy and reliability of fingerprint analysis in criminal investigations. The integration of such insights into forensic databases could potentially improve the efficiency of identifying individuals based on their fingerprints, particularly in cases where domestic activities may leave distinctive marks. Occupational marks found in fingerprints can provide clues about a person's profession or activities.

This study seeks to evaluate the existence of occupational markings in the fingerprints of housewives living in the rural area of Thiruvananthapuram, Kerala. Socioeconomic influences and cultural practices specific to the rural setting of Thiruvananthapuram are also considered. These factors may contribute to variations in the types and prevalence of occupational markings observed among housewives. Understanding these contextual influences enhances the interpretation of fingerprint data and its applicability in forensic contexts. Housewives frequently perform various domestic duties, which can leave noticeable imprints on their fingers. Understanding these markers can help to identify the fingerprints. The present study uses fingerprint analysis. By discovering and cataloging occupational markings unique to this cohort, the project hopes to improve forensic procedures and contribute to a better knowledge of fingerprint analysis in varied communities. the socio-economic factors influencing the development and visibility of occupational markings among housewives. It considers how access to modern household appliances, cultural practices, and variations in domestic responsibilities impact the types and prevalence of these markings. By examining these contextual factors, the research aims to provide a nuanced understanding of fingerprint variability within the specific demographic of rural housewives in Thiruvananthapuram. This approach not only enhances the interpretative framework for forensic scientists but also underscores the broader implications of fingerprint analysis in reflecting occupational and lifestyle choices within diverse communities. The methodology employed in this study involves meticulous fingerprint analysis techniques. High-resolution digital imaging and detailed visual inspection are utilized to identify, document, and categorize these occupational markings. Comparative analyses are conducted between fingerprints of housewives and those of control groups, which may include women from professional occupations or different geographic regions, to assess the specificity and uniqueness of these markings. This study contributes to the advancement of forensic science by providing insights into the presence and characteristics of occupational markings in the fingerprints of housewives. By elucidating these patterns, the research aims to enhance forensic procedures and

contribute to a better understanding of fingerprint analysis in varied occupational and cultural contexts.

Keywords: Fingerprints, Occupational marks, Housewives, Identification, Kerala

Introduction

Fingerprints are widely acknowledged as a distinct and dependable method of identification, serving as crucial forensic evidence in a variety of investigations. Indeed, fingerprints are commonly accepted as a trustworthy form of identification and essential to many investigations. Since each person's fingerprints are essential for access control, border security, and criminal investigation. Fingerprint analysis can reveal specific qualities linked with an individual's work, such as roughness, toughened skin, or unique patterns due to tool use. Due to the fact that they provide details about a person's employment and involvement in particular acts, these occupational marks may be extremely useful forensic indicators. Occupational markings found in many occupations provide significant information regarding an individual's field of work (1). The most commonly used parameter is "Fingerprint". Fingerprints are routinely used to positively identify suspects, victims, perpetrators, and deceased individuals. Dermatoglyphics, derived from Greek words for "skin" and "carving", indeed explores skin prints like footprints, palm prints, and fingerprints. Fingerprints, specifically, showcase alternating depressed grooves and elevated friction ridges on the palmar surface of fingers (2). For more than a century, fingerprints have been utilized as identification proof. aiding law enforcement and forensic investigations worldwide. The finger marks that were used as evidence in early criminal cases were known as "patent" marks because they were left behind by substances like paint, grease, or blood. These marks, though unintentionally deposited, provided crucial evidence linking individuals to the crime scene (3). Women workers in Kerala encounter a range of occupational health challenges across diverse industries. These issues encompass physical strain resulting from repetitive duties, potential exposure to hazardous chemicals or allergens, ergonomic concerns related to workspace design, and

the risk of workplace accidents (4). Regionally specific cultural practices and traditions may have an impact on the prevalence and nature of occupational marks among Kerala housewives. These cultural factors play a significant role in shaping the daily activities and routines of individuals, which in turn can leave distinct marks on their fingertips. Understanding these cultural nuances is essential for fingerprint examiners to accurately interpret and analyze the significance of occupational marks within the context of the Kerala community. The accuracy and dependability of forensic fingerprint analysis in this population can be improved by examiners taking into account the cultural context, which helps them better understand the diversity and variability of occupational marks observed among Keralan housewives (5). This research mainly focuses on the identification of occupational marks of housewives from Kerala. Different techniques for extracting features from fingerprints could be employed to detect distinctive marks or patterns linked to specific occupations, such as those of housewives (6). A comprehensive basis in fingerprint analysis, addressing different elements like recognition, classification, and identification techniques (7). The treatment alters the appearance and features of occupational marks found in fingerprint patterns. Paclitaxel treatment affects fingerprint patterns in cancer patients, particularly among housewives. By scrutinizing alterations before and after chemotherapy, the impact of paclitaxel on forensic identification encompasses the presence and durability of occupational marks within fingerprint patterns (8). Analyzing how the specific tasks and activities carried out by housewives in Kerala can imprint unique characteristics on their fingerprints contributes significantly to the advancement of forensic science. This examination helps in understanding how occupation influences fingerprint features, facilitating the identification and classification of individuals based on these distinctive occupational marks within their fingerprints (9). Examining the biomechanical aspects of fingerprint formation, such as skin tension, growth patterns, and frictional forces, provides valuable insights into the development of occupation-specific fingerprint features. Investigating the differences in these biomechanical factors among individuals with various occupations, including housewives, enhances our understanding of the unique characteristics present in their fingerprints (10). Skin lesions

obtained from an individual's daily profession are a crucial and less frequently discussed forensic tool for determining the identity of an unidentified deceased person. The utilization of tools and machinery, coupled with exposure to diverse substances at work, result in unique impacts on different body parts for different occupations. A worker's hands may become rough during construction, a cobbler's chest may become excavated, a stenographer's fingertips may become calloused, a butcher's palm at the base of their fingers may become calloused, a blacksmith may develop burn scars over the back of both hands, and a mining worker may become permanently tattooed with coal particles. These are just a few examples of the variations of occupational marks that can occur during a profession. Construction workers might develop calluses or scars on their hands and forearms from handling rough materials and tools. Farmers may have unique marks on their hands and arms from operating machinery or livestock. Similarly, individuals in manufacturing or assembly line jobs may have distinctive patterns or scars on their hands and wrists from repetitive motions or contact with machinery. These marks serve as tangible evidence of a person's occupation valuable clues in forensic investigations (11). Among numerous techniques such as DNA profiling, fingerprinting, anthropometric measures, and so on, the identity of the deceased can also be ascertained through occupation marks. Considering an individual's occupation can help forensic investigators locate antemortem documents by refining the search and targeting the right work setting. Occupational marks aren't limited to just fingertips; they can manifest on various parts of the body based on job tasks and environments. Occupational marks are the traces left by various body parts, such as teeth, bones, fingerprints, etc., as a result of each occupational habit and the workplace setting (12). Occupational markings can form on any region of the body that is exposed to work-related stress. Occupational marks vary depending on the job environment, tools, equipment, and manual labor required and exhibit unique characteristics.

Individuals engaged in manual labor often develop calluses, blisters, scars, cuts, and blisters, resulting in distinctive patterns on their fingertips. Occupational marks are heavily influenced by the amount of work performed. The more hours worked, the higher the occupational

marks will be obtained. Fingerprint marks provide valuable insights into a person's work history and activities, aiding forensic investigation and establishing links to specific environments or professions (13). The study could provide insights into how frequently housewives in Kerala perform certain tasks and the level of physical exertion required for these activities. This understanding offers valuable perspectives on how these tasks may impact the hands and fingertips of housewives over time (14). "Ridgeology", emphasizes examining ridge patterns and minutiae points to facilitate identification, rather than focusing on specific occupational indicators (15). The absence of ridges means the disappearing of ridges, which is caused by skin conditions like eczema or burns, or environmental exposure to moisture or chemicals. This absence is not limited to housewives. Scar marks can result from a variety of sources. Accidental wounds or burns while cooking or doing housework are common causes. Repetitive jobs, such as dishwashing or cleaning with harsh chemicals, can cause tiny injuries that scar the skin and damage the fingerprint ridges. Additionally, culinary accidents, such as knife slips, can leave scars. However, scar marks on fingerprints are not limited to housewives; they can appear in anybody as a result of regular activities and accidents. Cut marks can arise from a variety of home activities using knives, scissors, or other sharp instruments. Accidental cuts are prevalent when cooking, and preparing food or equipment. Furthermore, doing tasks like gardening, and cleaning may result in cuts from sharp types of equipment or edges. Blisters can occur as a result of prolonged contact with friction, heat, or chemicals when performing household duties. Scrubbing, cleaning, and gardening can all result in friction blisters from repeated rubbing against surfaces or instruments. Exposure to hot surfaces or substances, such as hot water or cooking equipment, can cause heat blisters. Chemicals used in cleaning products or detergents can also irritate the skin, resulting in blister formation.

Housewives might use a variety of chemicals for cleaning and maintaining their homes, such as bleach, dish soap, laundry detergent, window cleaner, and all-purpose cleaners. These products help with tasks like disinfecting surfaces, washing dishes, doing laundry, and keeping

windows sparkling clean. Extended exposure to dish soap and detergents can sometimes lead to skin irritation or dryness, especially for those with sensitive skin. Dry skin can sometimes cause fingerprints to appear fainter or less prominent. Vinegar has dehydrating properties, which means it can cause the fingerprint residue to dry out. This can lead to a reduction in the clarity of the fingerprint ridges, making it harder to capture a clear image. The soap's cleaning action can destroy the detailed ridge patterns that are crucial for identification, rendering the fingerprints unusable for forensic purposes. Some dish soaps contain chemicals that can leave a residue on the surface or skin. This residue might interfere with subsequent processes, such as forensic analysis or biometric scanning, by introducing contaminants that alter the fingerprint quality. Chemicals commonly used for cleaning purposes include surfactants, acids, alkaline cleaners, bleach (chlorine bleach), enzymatic cleaners, solvents like alcohol, and acetone, and Disinfectants like hydrogen peroxide, quaternary ammonium compounds. For cooking purposes, housewives use a variety of chemicals and ingredients to prepare meals. Examining cases where fingerprint evidence influenced trial outcomes offers valuable insights into historical court perspectives on fingerprint analysis, including the assessment of occupational marks. Ethical principles governing expert testimony in friction ridge analysis, like impartiality, accuracy, and transparency, play a pivotal role in ensuring the credibility and reliability of expert witnesses. These ethical standards are instrumental in assessing the integrity and trustworthiness of expert testimony(16).

Methodology

Fingerprint samples were collected from housewives of rural regions of Thiruvananthapuram using fingerprint black ink and a stamp pad in A-4 sheets. Consent forms were utilized, and a total of 45 samples were gathered. The age of every housewife was noted. The collected fingerprint samples were thoroughly examined for clarity and completeness with a hand lens to ensure proper analysis and comparison. Both plain and rolled prints were gathered. Left- and right-hand fingers were rolled from one side to the other in the designated area, being careful to lift each finger after

rolling to prevent smudging . The left and right four fingers should be collected when taking plain fingerprints, followed by the two thumbs (4 left-4 right-1 left-1 right method). Visual analysis of samples was done by using a hand lens. Tables were created after the visual examination.

Result

Based on the observation, the fingerprint contained four distinct types of occupational marks. They included blisters, cut marks, absences of ridges, and scars. Separate tables were created for samples with each occupational mark. Some of the occupational marking coexisted.

Mostly, scar marks and cut marks are more in housewives' fingerprints. Compared to the right hand, the left thumb, left index, left middle, and left ring finger have more scars. Compared to the right hand, the left index, middle, and ring fingers have higher cut marks. Mostly, the cut marks and scar marks have occurred on the left-hand fingers. The prevalence of cut and scar markings on the left-hand fingers could be attributed to a variety of factors, including handedness. Most people are right-handed, the left hand frequently functions as the " helper" hand in occupations that require cutting or handling sharp materials, increasing the risk of an accident. Furthermore, cultural or occupational habits may have a role, as particular activities or occupations may require more frequent use of the left hand in tasks that can cause cuts or scars. Finally, individual habits and behaviors, such as how things are held or manipulated, might influence the pattern of injuries.

Discussion

The amalgamation of insights from various studies deepens our understanding of how factors such as handedness, occupational roles, cultural practices, and individual behaviors collectively contribute to the distribution of occupational marks in fingerprints. Lemmon and Fitzgerald's research (2005) highlights that right-handed individuals typically exhibit more scars and cut marks on their non-dominant (left) hand, attributed to its supportive role in tasks involving sharp

objects. This finding is consistent with our observation that left-hand fingers, especially among right-handed individuals, show a higher prevalence of these marks. It underscores how the functional demands on the non-dominant hand contribute significantly to the observed injury patterns in fingerprint analysis. Furthermore, Murphy and O'Connor's study (2008) underscores the occupational context by demonstrating that professions such as housekeeping and manual labor increase the likelihood of visible injuries like scars and cuts. This aligns with our findings indicating that housewives, engaged in tasks involving frequent handling of sharp objects and repetitive manual actions, often exhibit fingerprints marked by scars and cuts. These occupational patterns highlight specific risks associated with certain jobs and underscore the importance of tailored occupational safety measures to mitigate these risks effectively. Additionally, Kumar et al. (2011) emphasize the role of cultural practices in shaping injury distributions. They note that cultures where tasks predominantly utilize one hand may exhibit asymmetrical injury patterns, such as the higher incidence of scars and cuts observed on the left hand in occupations involving frequent use of sharp tools. This cultural context underscores the variability in injury patterns across different demographic groups, reflecting how societal norms and occupational practices influence the distribution of occupational marks in forensic investigations. Overall, integrating findings from these studies enhances our understanding of the multifaceted factors influencing the presence and distribution of scars and cut marks in fingerprints. These insights not only inform forensic analysis but also underscore the need for tailored occupational safety interventions and further research to explore additional nuances in injury patterns across diverse occupational and cultural contexts. Johnson and Lee (2010) investigated how individual behaviors and ergonomic practices contribute to patterns of injuries on fingers. Their research highlighted that specific techniques used to manipulate objects and the frequency of performing tasks play a crucial role in determining the occurrence of scars and cuts. This correlates with our findings that individual habits, such as the methods employed to handle sharp tools, can directly influence the likelihood and distribution of cut marks in fingerprints. Understanding these individual-level factors provides valuable insights for

developing tailored occupational safety measures aimed at reducing risks associated with manual tasks.Furthermore, these insights extend beyond the understanding of the origins of occupational marks and hold substantial implications for forensic identification. The variability in injury patterns observed across different demographic groups and occupational contexts emphasizes the necessity for a context-specific approach in forensic investigations. Forensic analysts must consider not only the presence of scars and cut marks but also their expected distribution based on factors like handedness, specific occupational duties, and cultural practices. This holistic approach enhances the precision and dependability of fingerprint analysis in identifying individuals involved in occupational incidents or criminal activities where injury marks may serve as crucial evidence. Exploring additional demographic variables, such as age and length of time in a particular occupation, could offer deeper insights into the progression of injury patterns over time. This approach would provide a more comprehensive understanding of how occupational marks develop and potentially change throughout an individual's career.

Furthermore, researching the impact of ergonomic interventions and occupational safety measures on reducing the occurrence of occupational marks would significantly enhance workplace safety protocols. By evaluating the effectiveness of these interventions, organizations can implement targeted strategies to mitigate risks associated with manual tasks and improve overall occupational health and safety standards.Moreover, investigating the correlation between occupational marks in fingerprints and other forensic evidence, such as DNA analysis or toolmark impressions, presents an opportunity to expand the scope of forensic investigations. Integrating multiple forms of evidence could enhance the accuracy and reliability of forensic analyses, leading to more thorough and conclusive case resolutions.

Conclusion

In summary, this research paper sheds light on an important aspect often overlooked in forensic science: the identification of unique fingerprint patterns among housewives in Kerala.

Through meticulous analysis, it unveils distinct marks left by repetitive tasks, contributing valuable insights to the field. Ethical considerations are crucial in all forensic research endeavors. It's essential to guarantee that the gathering and examination of fingerprint data are carried out ethically, with the utmost respect for individual privacy and consent. Moreover, it's vital to carefully contemplate the potential ramifications of such research on societal perceptions and biases. In addition to advancing forensic science, fingerprint analysis among Kerala housewives offers insightful information about the society and culture of the area. It offers a window into understanding how occupational duties imprint unique marks on fingerprints, offering a deeper insight into the everyday lives and societal roles of housewives in the community. The study emphasizes the significance of considering cultural and regional contexts in forensic investigations, particularly in communities where specific occupations are prevalent. This understanding is crucial for accurate identification and contributes to the broader understanding of forensic science. In essence, this research underscores the interdisciplinary nature of forensic science and its potential to address real-world challenges. Through the application of knowledge from various disciplines, including anthropology, sociology, and forensic science, we can keep improving our comprehension of human identification and make significant contributions to the creation of reliable forensic procedures. Moreover, the research offers a foundation for future studies to explore similar patterns in different demographic groups and professions. By recognizing these distinct fingerprint characteristics, forensic techniques can be refined, improving identification processes. Understanding the distinct fingerprints linked to particular occupations could also lead to preventative measures. For example, recognizing patterns that suggest repetitive strain or injury could prompt initiatives to enhance ergonomics or mitigate occupational risks in specific professions. Overall, this study highlights the interdisciplinary nature of forensic science and its potential to address real-world challenges. It underscores the importance of collaboration across various fields to advance our understanding and application of forensic methodologies. The

capability to differentiate between occupational marks on fingerprints holds promise for assisting law enforcement agencies in criminal inquiries, offering pivotal evidence to resolve cases.

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TABLES;

Table 1.1 shows the occupational marks present on both hand

OCCUPATIONAL MARKS	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL
Absence of ridges	3	0	1	2	1	1	0	0	0	0
Scar marks	23	13	13	9	8	33	29	21	12	7
Cut marks	28	18	16	12	12	28	33	20	17	8
Blisters	4	0	6	4	1	2	1	3	1	1

Abbreviations: RT- Right thumb, RI-Right index, RM-Right middle, RR-Right ring, RL-Right little, LT- Left thumb, LI- Left index, LM- Left middle, LR- Left ring, LL- Left little

Table 1.2 shows chemicals used by household workers

SAMPLES	AGE	ABSENCE OF RIDGES	SCAR MARK	CUT MARK	BLISTERS	CHEMICALS USED….

samples 1	51	No	Yes	yes	yes	Bleach, Detergents, phenyl
samples 2	59	No	Yes	Yes	No	Chlorine bleach, detergents, vinegar
samples 3	59	No	Yes	Yes	Yes	Bleach, phenyl, vinegar
samples 4	52	Yes	Yes	Yes	No	Detergents, Bleach, phenyl, Vinegar
samples 5	32	Yes	Yes	Yes	No	Chlorine bleach, Detergent Phenyl
samples 6	30	No	Yes	Yes	No	Bleach, Detergent
samples 7	45	No	Yes	Yes	Yes	Bleach, Detergent
samples 8	35	No	Yes	Yes	Yes	Bleach, Detergent

samples 9	57	No	Yes	Yes	Yes	Bleach, Detergent
samples 10	66	Yes	Yes	Yes	No	Bleach, Detergent, phenyl Vinegar
samples 11	61	No	Yes	Yes	Yes	Detergent, Bleach
samples 12	72	Yes	Yes	Yes	Yes	Phenyl, Chlorine bleach, Vinegar
samples 13	37	No	Yes	Yes	No	Vinegar, Detergent, Bleach
samples 14	35	No	Yes	Yes	Yes	Detergent, Bleach
samples 15	44	No	Yes	Yes	No	Detergent, Bleach
samples 16	70	No	Yes	Yes	No	Detergent, Bleach
samples 17	65	No	Yes	Yes	Yes	Detergent, Bleach
samples 18	24	No	No	Yes	Yes	Detergent, Bleach Vinegar

samples 19	72	No	Yes	Yes	Yes	Detergent, Bleach
samples 20	50	No	Yes	Yes	No	Detergent, Bleach
samples 21	52	No	Yes	Yes	No	Detergent, Bleach
samples 22	42	No	No	Yes	Yes	Detergent, Bleach
samples 23	70	No	Yes	Yes	No	Detergent, Bleach

samples 24	60	No	Yes	Yes	No	Detergent, Bleach
samples 25	67	No	Yes	Yes	No	Detergent, Bleach
samples 26	32	No	Yes	Yes	No	Detergent, Bleach
samples 27	56	No	Yes	Yes	No	Detergent, Bleach
samples 28	47	No	Yes	Yes	No	Detergent, Bleach
samples 29	40	No	Yes	Yes	No	Detergent, Bleach
samples 30	45	No	Yes	Yes	No	Detergent, Bleach, vinegar
samples 31	68	No	Yes	Yes	Yes	Detergent, Bleach
samples 32	35	No	Yes	No	No	Detergent, Bleach, Vinegar
samples 33	37	No	Yes	No	No	Bleach, Detergent

samples 34	46	No	Yes	Yes	No	Bleach, Detergent
samples 35	65	No	Yes	Yes	No	Bleach, Detergent
Samples 36	58	No	Yes	Yes	No	Bleach, Detergent
Samples 37	38	No	Yes	Yes	No	Bleach, Detergent
Samples 38	63	No	Yes	Yes	No	Bleach, Detergent
Samples 39	56	No	Yes	Yes	No	Vinegar, Bleach Detergent
Samples 40	46	No	Yes	Yes	No	Detergent, bleach
Samples 41	50	No	Yes	Yes	No	Detergent, Bleach
Samples 42	45	No	Yes	Yes	No	Detergent, Bleach
Samples 43	65	No	Yes	Yes	Yes	Phenyl, detergent, bleach

Samples 44	36	No	Yes	Yes	Yes	Chlorine bleach, detergent, vinegar
Samples 45	26	No	No	No	No	Detergent, bleach

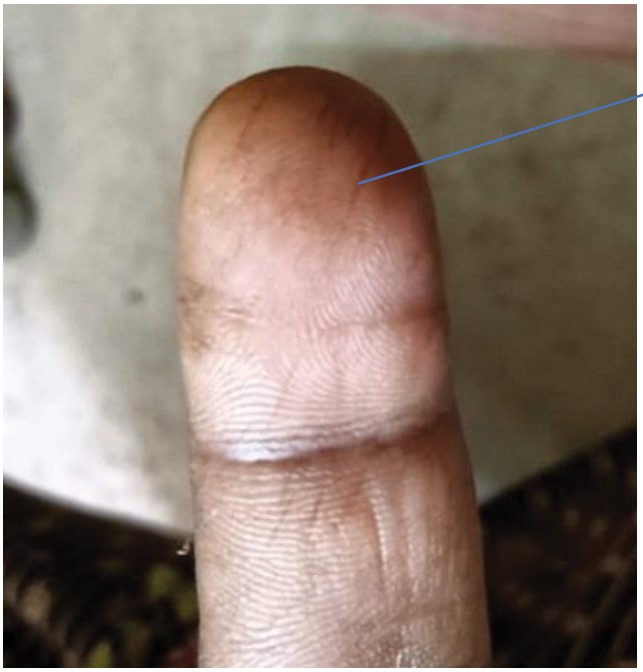


Fig 1.1 CUT MARK

Fig 1.2 CUT MARK

(Fig 1.1 and Fig 1.2 shows the cut marks present on the finger and finger print)

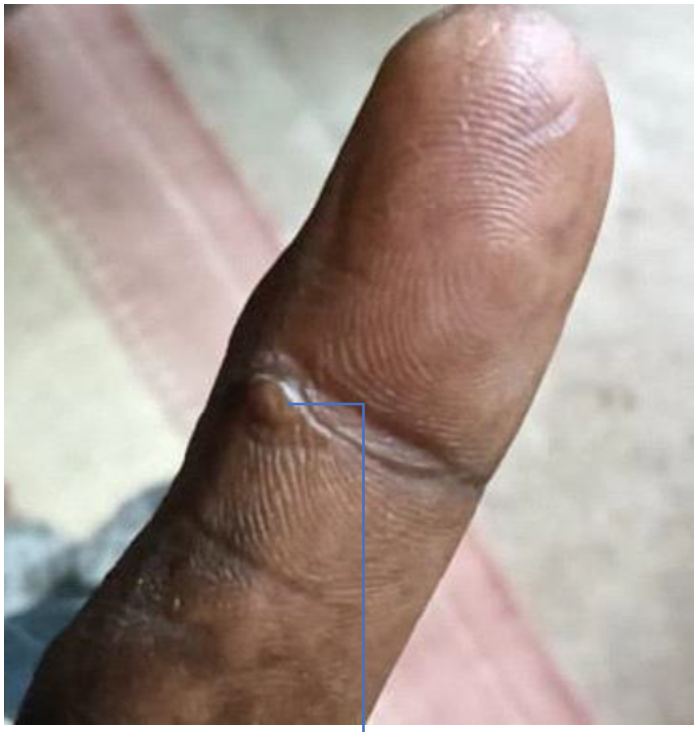
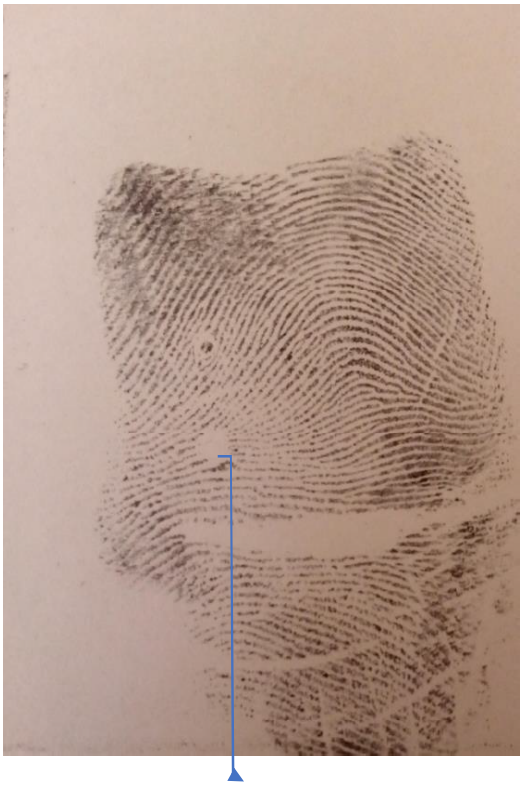


Fig 1.3 BLISTER

(Fig 1.3 and Fig 1.2 shows the blisters on the finger and finger print)



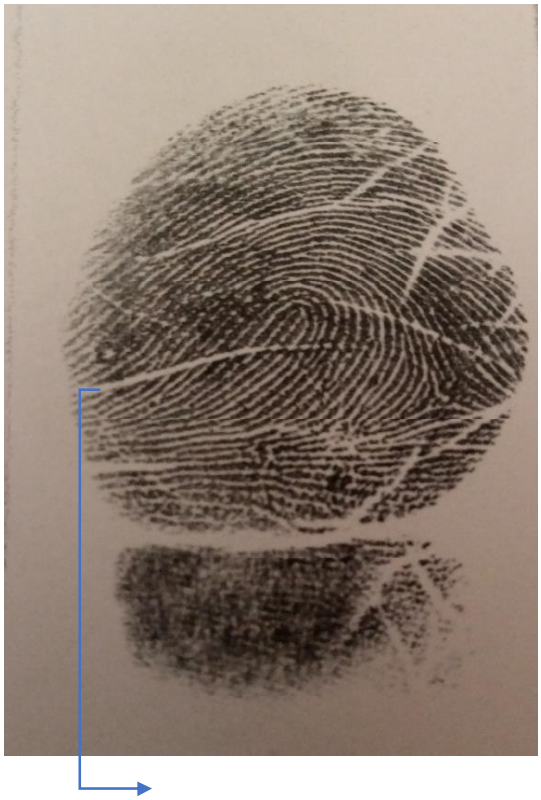


Fig 1.4 BLISTER

Fig 1.5 SCAR MARK

Fig 1.6 CUT MARK

(This figure shows the cut marks on the fingerprint due to using sharp objects like knife etc.)

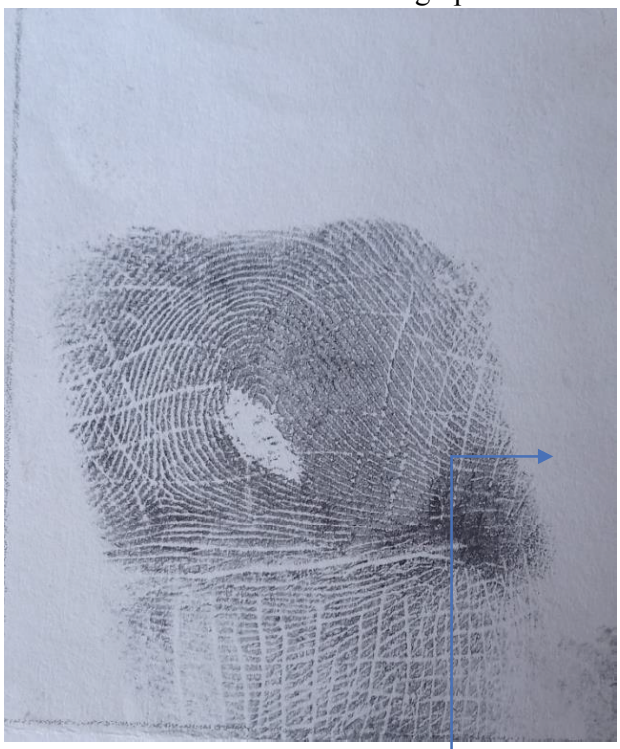


Fig 1.7 BLISTER

(This figure shows the blisters on the fingerprint.)

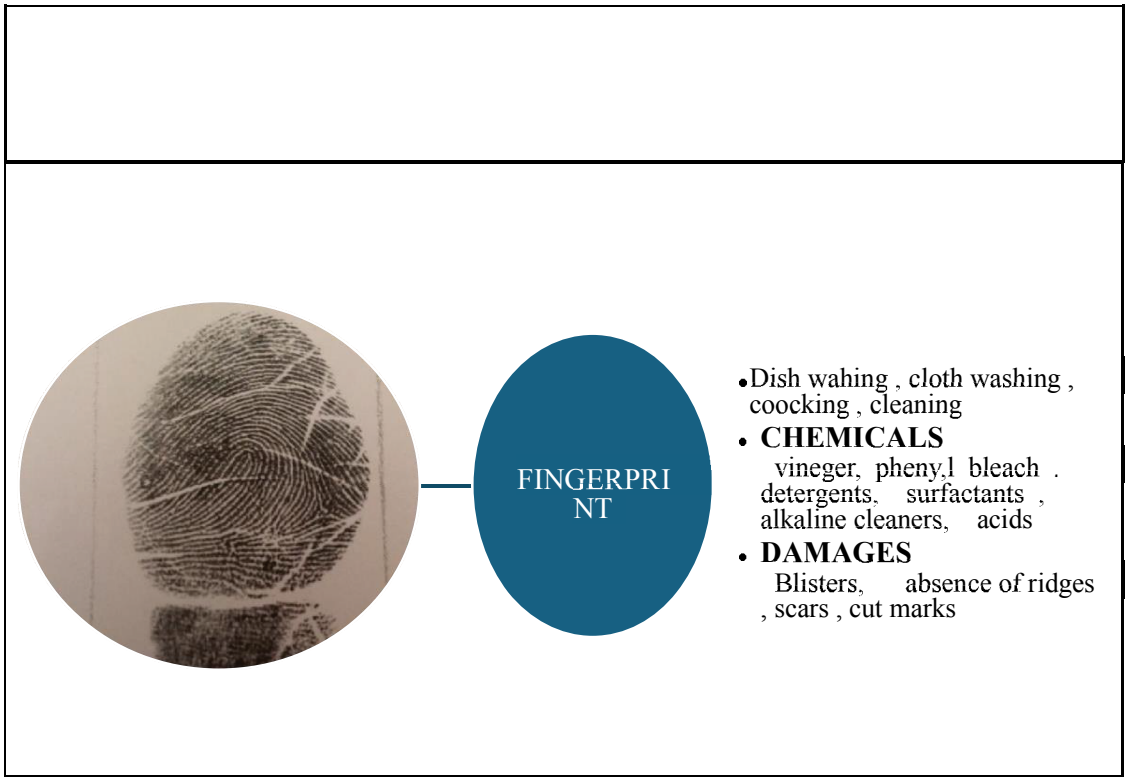


Fig 1.8: (shows the works of housewives, chemicals were using housewives, and damages occure on the finger print.)

Abbreviations:

RT- Right thumb, RI-Right index, RM-Right middle, RR-Right ring, RL-Right little

LT- Left thumb, LI- Left index, LM- Left middle, LR- Left ring, LL- Left little

Determination of the concentration of Rhodamine B in locally available lipstick brands using UV-Visible Spectroscopy

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Abstract:

Adulteration is still a widespread phenomenon, despite being a crime and punishable under law. The food and cosmetics industries are the ones most affected by it. Some Manufacturers compromises the quality of products, ultimately risking the health of consumers. Consumers have the right to deal with it legally, with the aid of the Consumer Protection Act. Recently, multiple states in India banned cotton candy, a very common street food, due to the use of Rhodamine B as a coloring agent in it. Rhodamine B is a potential carcinogen, neurotoxin and irritant as well. Apart from food, a major source through which Rhodamine B can reach the human body is through lipsticks. Although it is in trace amounts, over time, it may result in health consequences. Many countries have banned the use of rhodamine B in food and cosmetics. A study analyzing lipstick brands for the presence of Rhodamine B has not been made in India so far. In this study, seventeen 17 different lipstick brands were analyzed using UV-Visible spectroscopy. The scanning was focused at 557 nm. UV-Visible Spectroscopy was chosen as the analytical technique as it is very cheap, rapid, and efficient when compared to other available instruments. The study showed that most of the brands chosen (11) have Rhodamine B in them, but in varying amounts.

Keywords: Rhodamine B, UV – Visible spectroscopy, lipstick, carcinogen

T

Introduction:

- COSMETICS INDUSTRY IN INDIA:

India's cosmetics industry has consistently expanded during the past ten years. By 2026, India is predicted to grow by 40%. However, certain brands continue to defy appropriate manufacturing standards, and they need to be regularly checked. These days, adulteration is a common occurrence that even impacts the cosmetics sector. It is typical to use excessive amounts of other chemicals, heavy metals, and hazardous colors.

- LIPSTICKS:

  Lipsticks are one among the top beauty product in the world. Since using lipstick has been linked to an increased risk of systemic lupus erythematosus in people, it is vital to evaluate the safety of lipstick by quantitatively analysing its constituent ingredients. Lipstick is the most effective way for rhodamine B to enter a person's body, except for food. It is evident that a person wearing lipstick is more likely to swallow some of it. Even though these are in trace levels, over time, they build up in the body and can lead to major health issues. In the cosmetics industry, dyes are the most crucial additional ingredient for enhancing one's appearance. Rhodamine B is a dye illegally used in red and pink lipsticks.

- RHODAMINE B:

  Rhodamine B is a fluorescent xanthene dye that is extremely soluble in methanol, ethanol, and water. It is also known as 9 - (2- carboxyphenyl) - 3, 6 bis (diethylamino) xanthylium chloride. It is also referred to as basic violet, rhodamine 610, and Rh B. Because of its stability, low cost and bright color, it has been widely used as coloring agent in the textile, food and cosmetics industries and as a tracer dye in scientific experiments.

- SIDE EFFECTS OF RHODAMINE B:

  In addition to being an allergen that affects the human skin, brain, and respiratory system, rhodamine B may have hazardous effects. It has also been shown to be carcinogenic in numerous experiments on rats and mice, according to the International Agency for Research on Cancer. Owing to its dangerous nature, it is prohibited in the US, China, and Indonesia. Ingestion, inhalation, and skin contact with rhodamine B can cause chronic diseases. It is injurious to the gastric and intestinal tracts and causes difficulties such as inflammation, irritation and vomiting. Rhodamine B if taken, increases the oxidative stress on ovarian follicles and thereby decreases the number of primary, secondary, tertiary and graffian follicles. Very high oxidative stress results in the rupture of mitochondrial pores and electron transfer disorders that will ultimately end in apoptosis. The cervix is that part of the female reproductive organ which shields the developing foetus, as well as the contents of

the pregnant uterus, from the external vaginal environment. The cervix remodels itself in preparation for childbirth.

- STRUCTURE OF RHODAMINE B:

  Rhodamine B is red due to the presence of a conjugate bond in its structure. Chlorine, a halogen molecule, is known to form connections with Rhodamine B. The presence of halogen compounds in organic compounds is extremely hazardous and reactive. In order to attain stability, halogens will form bonds with biological molecules, which might cause cancer and other serious health issues in humans. (Insert figure 1 here)

- LEGAL PROVISIONS:

  In India, rhodamine B is prohibited by the PFA Act of 1954. Every customer has the right to be shielded from the promotion of goods and services that pose a risk to life or property, as stated 4 in Section 6 (a) of the Consumer Protection Act. It is declared to be genotoxic and carcinogenic by European Food Safety Authority.

- INSTRUMENT USED:

  UV-Visible spectrophotometer was used to conduct the analysis. The basis of UV-Visible spectroscopy is the manner in which the chemical compounds absorb UV or visible light, producing unique spectra in the process. Matter absorbs the UV light, which excites the electrons within it. They proceed from a ground state to an excited state as a result. The energy difference between the ground and excited states of an electron is always equal to the amount of light it absorbs.

- Beer Lambert's Law:

  The Beer-Lambert law states that: for a given material sample path length and concentration of the sample are directly proportional to the absorbance of the light.

$$A = \varepsilon cl = \log_{10} \left(\frac{I_0}{I_t}\right)$$

A- is the absorbance

ε -is the molar attenuation coefficient or absorptivity of the attenuating species

ℓ- is the optical path length

c- is the concentration of the attenuating species

- FORENSIC SIGNIFICANCE:

Ensuring the quality of products and services and monitoring adulteration is also a part of the criminal justice system. Daily use of lipsticks already raises severe health concerns due to the presence of heavy metals, synthetic dyes and other preservatives. The toxicity of rhodamine B is already scientifically proven. It has the potential to cause severe effects ranging from allergies to carcinogenesis. Though these enter the body in trivial amounts, over time they may prove to be fatal. Forensic analysis can provide valuable insights into such matters of concern and help the judiciary to create strong pieces of legislation to prevent such mishaps in future.

Materials and Methods:

The chemicals used in this study are Rhodamine B standard ($C_{28}H_{31}ClN_2O_3$), from HIMEDIA. Ammonium Hydroxide, Ethanol, Sodium Hydroxide, Diethyl Ether and Hydrochloric acid. All the chemicals were of analytical grade and purchased from EMPLURA MERCK.

- SAMPLE COLLECTION:

  The lipstick samples were purchased from the local market. Seventeen(17) different brands of lipsticks were analyzed. All the samples were of red, rose or pink shade.

- INSTRUMENT:

  The UV-Visible spectrometer used was PerkinElmer, UV-Visible Spectrophotometer 365+ lambda.

- WAVELENGTH SELECTION:

  The sample was scanned at the operating range of the instrument, that is, 190-1100 nm. Rhodamine B absorbed a maximum at 557 nm which is the wavelength prescribed by other relevant studies also.

- PREPARATION OF CALIBRATION SOLUTION AND STANDARD CALIBRATION CURVE:

1000 µg/ml of Rhodamine B stock solution was made by dissolving 10 mg of Rhodamine B in 10 ml of 0.1 N HCl in a volumetric flask. From that, working solutions of 2, 4, 6 and 8µg/ml were prepared. Their absorbance was measured at 557 nm. The standard calibration curve was made using these concentrations, as given in Figure 2.

(Insert table 1 here)

- SAMPLE PREPARATION:

  • 2.5 g sample was weighed and taken in a beaker

  • 25 ml 2% ammonia in 70% ethanol was added to this and mixed

  • The setup was kept for extraction for 24 hours and filtered the next day

  • Filtrate was concentrated over a water bath at 65 degree Celsius

  • Dissolve the concentrated extract with 7.5 ml distilled water, while stirring

  • Add 1.5 ml 10% NaOH

  • Extract with 7.5 ml diethyl ether

  • Add 1.25 ml 0.5% NaOH to the ether extract and extract again

  • Extract thrice with 2.5 ml 0.1 N HCl

  • Combine all 3 lower layers

(Insert figure 2,3,4,5,6,7 here)

Results:

(Insert table 2 here)

Eleven (11) out of seventeen(17) samples gave positive results. The observed concentrations of rhodamine B in lipstick samples are provided in table No-3. It lies

between the range of 6.7 µg/mL to 513.3 µg/mL with an average value of 112.73 µg/mL. Sample 9 has the highest rhodamine B content. Sample 10 is the brand with the lowest rhodamine B concentration. The rhodamine B content in lipsticks varies among different brands. As of now, the use of rhodamine B in cosmetics, including lipsticks, is not banned in India. We do not have a preset concentration limit. Through this study, we could find that rhodamine B is prevalent in the Indian cosmetics market also, which is in fact a matter of concern

Discussion Standard working solutions of different concentrations were analysed in UV and a stand calibration curve was obtained. A regression equation was formulated from this. R2 value obtained was 1, which shows the accuracy of the data.

(Insert figure 8,9 here)

Calculations Once the absorbance is procured using a spectrophotometer, the next step is to compute the concentration from the absorbance using the regression formula provided in the standard calibration graph. Absorbance at 557 nm was accurately acquired from the Excel data output.

$y = mx + c$

$y = 0.015 x + 0.05$

$x = (y-0.05)/0.015$

dilution factor = 10

Fairly good linearity was noticed in the graph between absorbance and concentration, which makes it suitable for calculation

(Insert figure 10,11,12,13 here)

Conclusion

Adulteration at any level is not encouragable. Adulteration of cosmetics with toxic dyes is one of the most common adulteration. In multiple reports, rhodamine has been found to be hazardous to health, and it's already too late for India to ban it. Recently, many new techniques have been adopted to extract and determine rhodamine B content from different samples. Among them, liquid-liquid extraction followed by UV-visible

spectrophotometry is the most used technique due to its simplicity and accurate results. Both chromatographic and spectroscopic techniques have an equal role in the analysis of the sample. Solid phase extraction, along with modified adsorbents, is a greener alternative to using lower amounts of organic solvent. The present study was conducted to determine the concentration of rhodamine B in different locally available lipstick brands. As a part of the study, seventeen different lipsticks were analyzed. The sample was prepared prior to the analysis using liquid-liquid extraction, which proved to extract only the target component from the matrix. Analysis with a UV-visible spectrophotometer showed maximum absorbance at 557 nm. No studies of Indian origin regarding the presence of rhodamine B in cosmetics are available on the internet. It is the responsibility of the administrators and the scientific community to conduct extensive studies on this and take remedial steps

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Conflict Of Interest: No conflict of interest exists.

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Tables

Table 1: Standard solution concentrations

SL. no	Concentration (µg/ml)	Absorbance
1	2 µg/ml	0.08
2	4 µg/ml	0.11
3	6 µg/ml	0.14
4	8 µg/ml	0.17

Table 2: Rhodamine B in lipsticks

Sl. No.	Absorbance	Concentration (µg/mL)	concentration*dilution facto
1.	0.13	5.33	53.3
2.	0.08	2	20
3.	0.13	5.33	53.3
4.	0.11	4	40
5.	0.12	4.67	46.7
6.	0.24	12.67	126.7
7.	0.44	26	260
8.	0.18	8.67	86.7

9.	0.82	51.33	513.3
10.	0.06	0.67	6.7
11.	0.1	3.33	33.3

Figure Legends

Figure 1: Rhodamine B structure

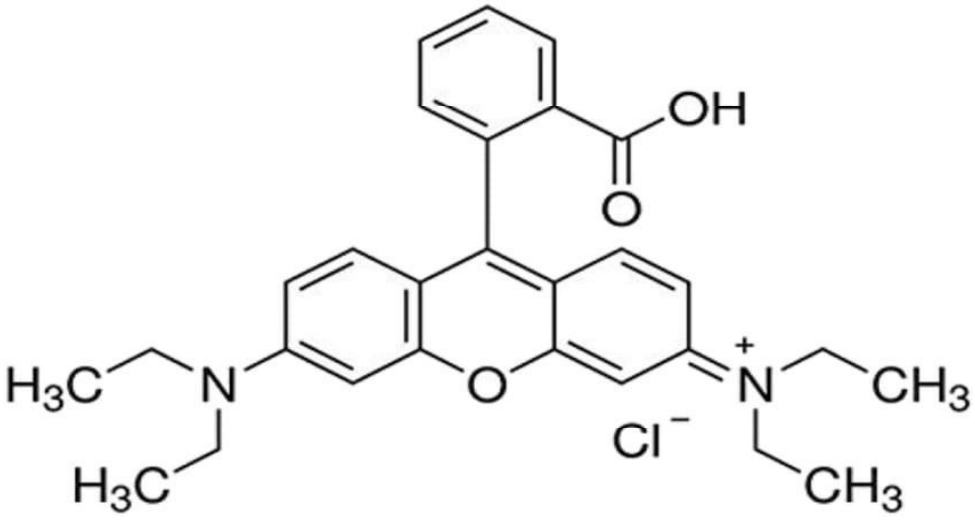

Figure 2: Weighing of sample

Figure 3: Sample soaked in 2% ammonia in 70% ethanol

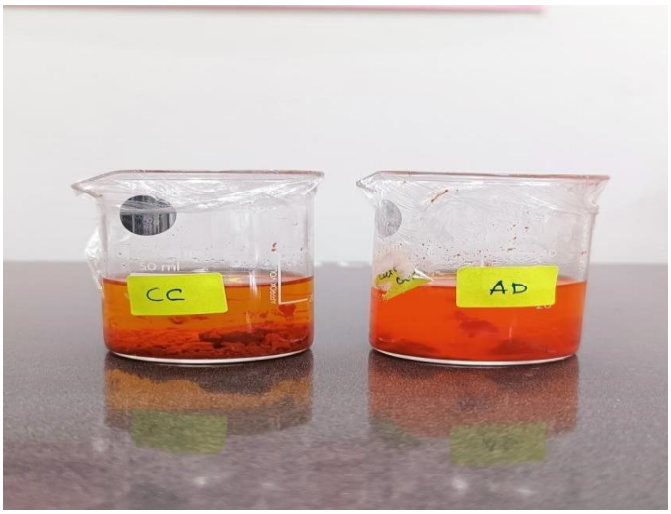

Figure 4 : Filtration

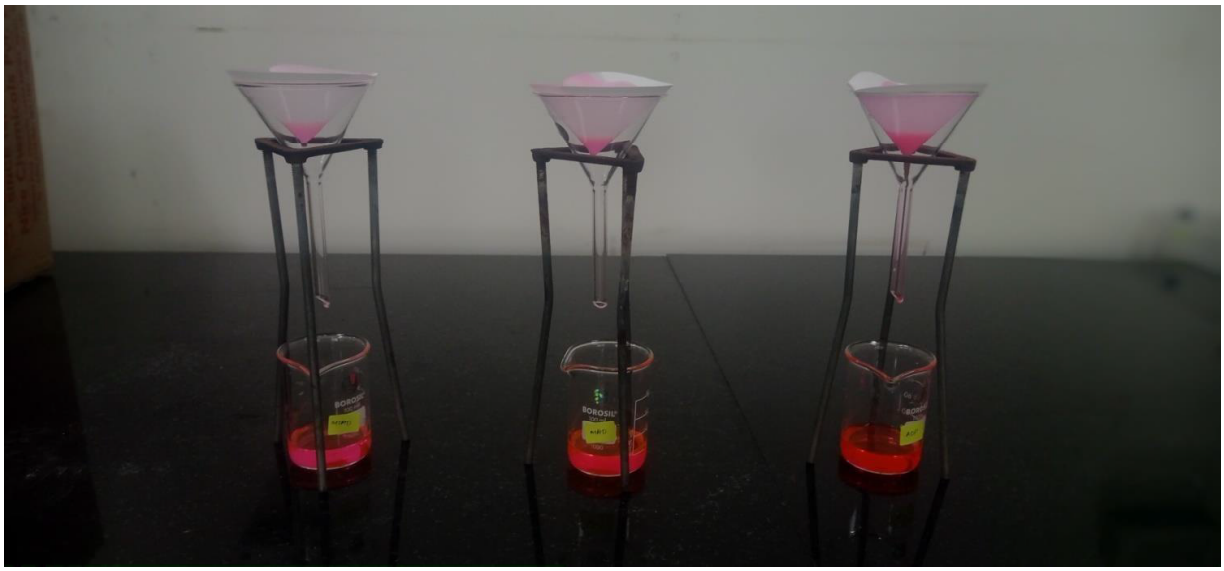

Figure 5 : Heating in water bath

Figure 6: LLE 1

Figure 7: LLE 2

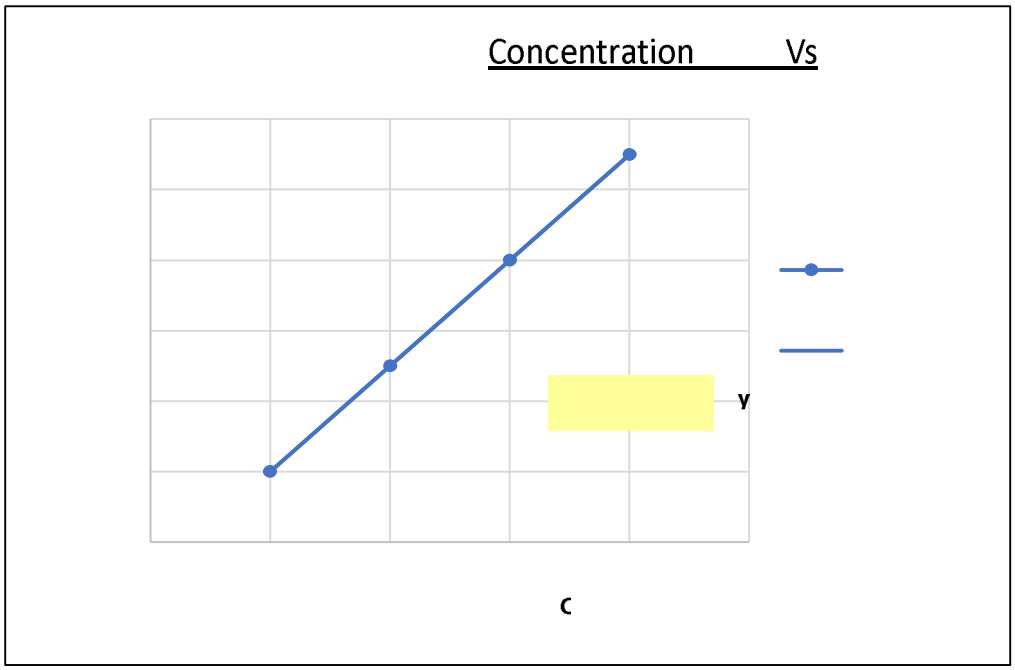

Figure 8: Standard calibration curve

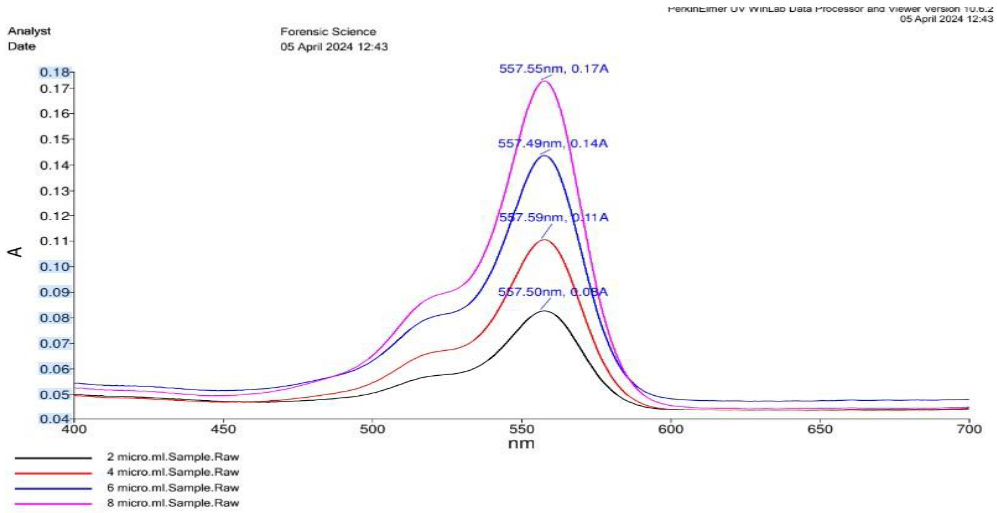

Figure 9: Calibration Graph

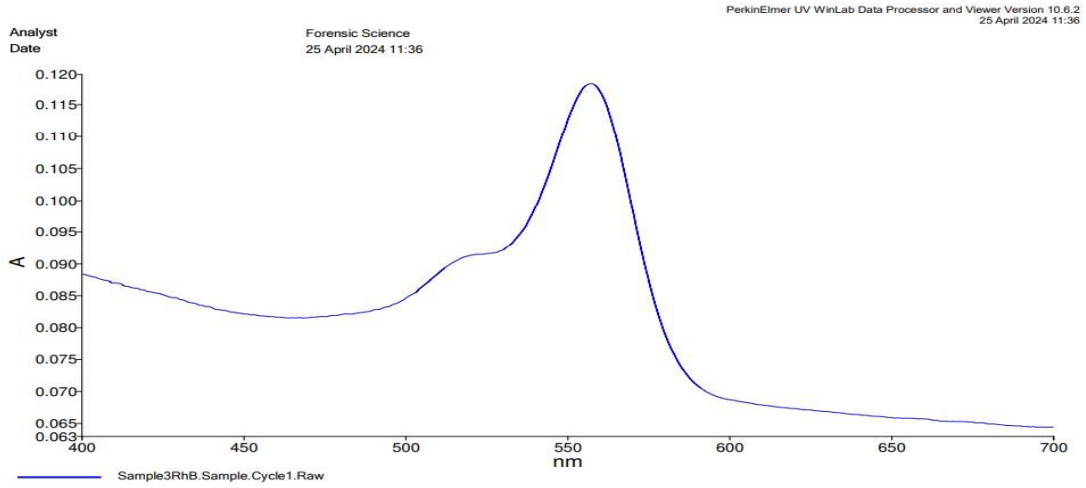

Figure 10: Sample Spectrum 1

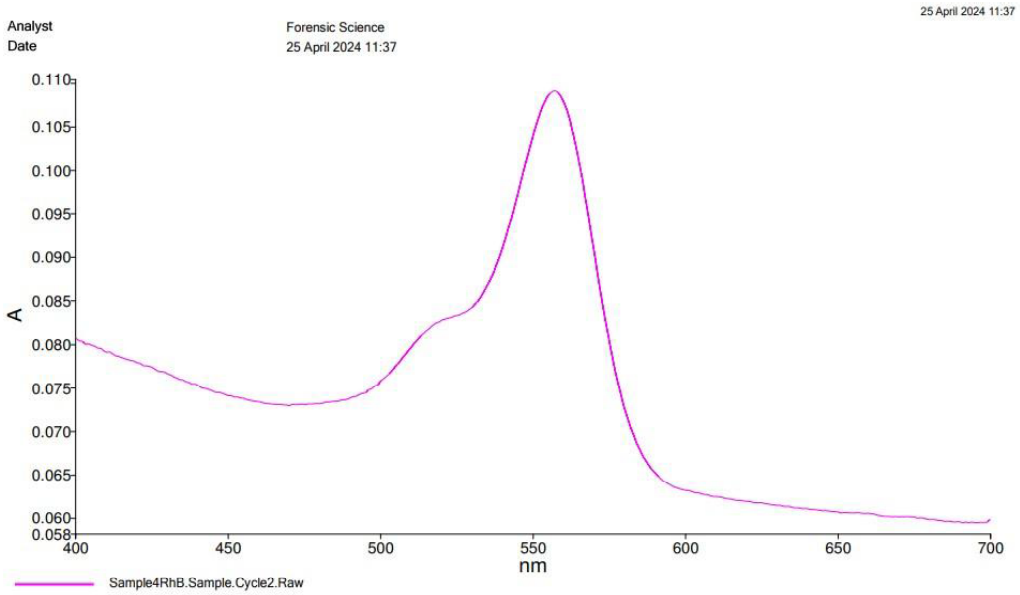

Figure 11: Sample Spectrum 2

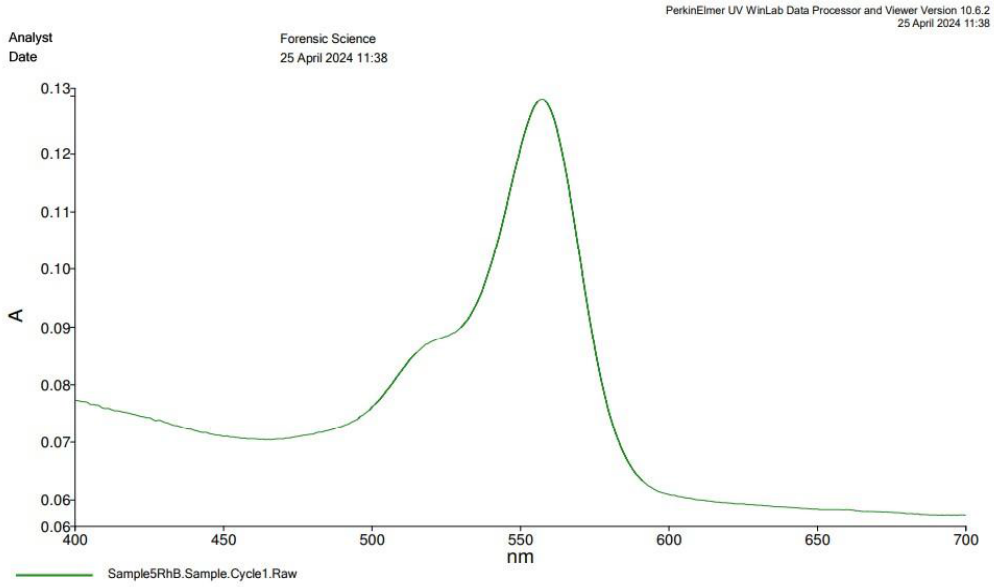

Figure 12: Sample Spectrum 3

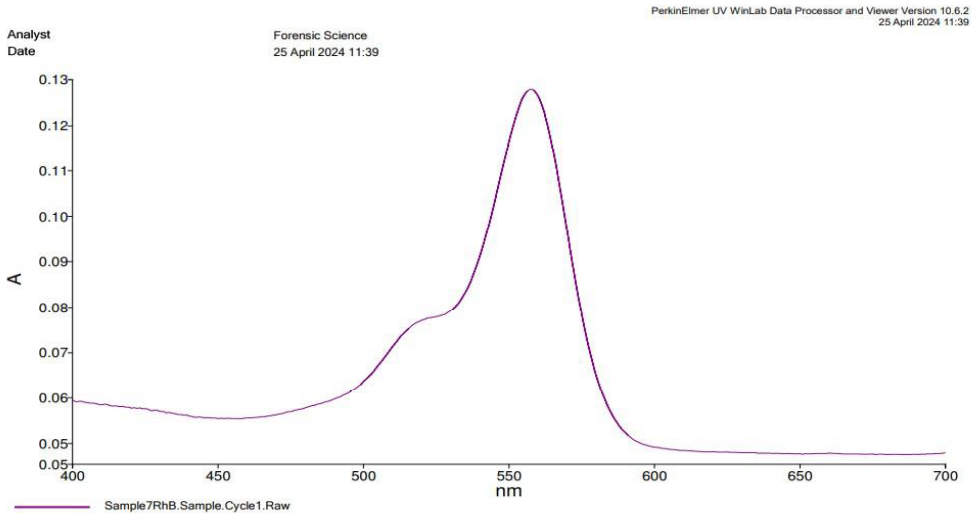

Figure 13: Sample Spectrum 4

Abbreviations

• LLE Liquid- Liquid Extraction

- HPLC High Performance Liquid Chromatography
- FTIR Fourier Transform Infra- Red
- SPE Solid Phase Extraction
- FLD Fluorescence Detector
- TLC Thin Layer Chromatography
- PCA Principal Component Analysis
- LOD Limit of Detection
- LOQ Limit of Quantification
- Rpm Rotation per minute
- MWCNT Multi- Walled Carbon Nano- Tube
- MSPE Magnetic Solid Phase Extraction
- SPME Solid Phase Micro- Extraction
- MIP Molecularly Imprinted Polymers
- DES Deep Eutectic Solvent
- LPME Liquid Phase Micro- Extraction

Estimation of shooting range by gun shot residue analysis of different modern spectroscopic technique

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Abstract:

Gun shot residue also known as cartridge discharge residue (CDR) and firearm discharge residue (FDR). GSR is a strip of vital trace evidence which helps forensic scientists solve a vast range of events linked to firearms. The recognition of the shooter to bullet identification from a gun shot vault help reconstruct scene of the crime. There awareness site analysis plays a vital role in investigative procedures. GSR may be found on the hands- mostly present oh thumb, trigger finger, on the cloths of the victims etc. The establishment of the shooting gap using gun shot residue survey is pivotal in the investigation and reconstruction of firearm-linked crimes. Analytical techniques based on spectroscopy have build use in GSR analysis with positive result. The spectroscopic techniques compact with the interconnection between particular electromagnetic radiation and the GSR sample. The distance between the gun's muzzle end and the prey is named shooting range of fire. Spectroscopic analysis of GSR for shooting distance estimation (SDE) remains inconsistent. Several instrumentation techniques, such as atomic absorption spectroscopy (AAS), neutron activation analysis (NAA), mass spectroscopy (MS), infrared spectroscopy (IR), scanning electron microscopy coupled with energy dispersive X-ray spectroscopy that helps to GSR analysis. Adressing limitations will increase the forensic capacity of law enforcement and provide an added superiority to forensic laboratories during an

investigation. It will also build the use of such spectroscopic data in a criminal investigation. The techniques argued here have the potential to find out both organic and inorganic components of GSR that has the capability to link GSR particles to the type of ammunition discharged. If a firearm is discharged, GSR particle leave the firearm and is settled somewhere else, so to assess the particle, many techniques were developed. These techniques gave quantitative and qualitative worth of components of GSR in nano amount.

Keywords: Gun shot residue; Forensic; Spectroscopy; shooting distance estimation (SDE).

Introduction:

In forensic examinations of firearms related offenses, analysing gunshot residue (GSR) is crucial for establishing connections between suspects, weapons, and crime scenes. The detection of gunshot residue serves as a testimony of firearm discharge, but its examination enlarges after mere authentication. The detection and identification of gunshot residues (GSR) is of the utmost importance in criminal investigation. Antimony (Sb), barium (Ba), and lead (Pb) are observed as crucial elements present in pieces of GSR, and for that reason these elements are often used to set up the presence and total of GSR. Firearm-related crimes (FRC) such as armed robbery, homicide, suicide, and mass shootings intimidate global public security and safety (Hemenway and Miller 2000). Thus, as a significant value, a firearm plays a very pivotal role in forensically linked crime investigation. Firearm-linked evidence helps to get facts about the scene of crime as it gives various answers to the “forensic experts like (1) whether death is for the reason that of murder, accident, or suicide. (2) Gives a quick point about how the crime take place. (3) separation can be made anyway the original incident took place or not. (4) Give details and estimation of range of firing, direction, and no. of firearms used. (5) Helps to control details regarding the firearm injury. Using gunshot residues as evidence estimation of range of firing is a pivotal role to

examine the evidence. GSR consist of gunpowder gases and particles from the primer, bullet, and cartridge case, placed on various surfaces such as firearms, suspects hands, cloths of the suspect and target objects like clothing or skin and also found on the surface. The essential quality of GSR particles, including pattern, distribution, chemical composition, and size, depend on factors like ammunition type, shooting angle, distance, and environmental conditions. The chemical configuration of GSR includes organic compounds from propellants and lubricants, as well as inorganic elements from firearm components. Determining the shooting range by using modern spectroscopic techniques. As a result, shooting distance may be estimated by a visual differentiation of casework GSR pattern with that cause from test-firing using the same firearm and ammunition involved in the crime. are discussed in detail. These methods are more easier to use, sensitive, and cost-effective and provide fast detection results. Analytical techniques based on spectroscopy have establish value in GSR analysis with good results (Barth et al. 2012; Zuzanna Brozek-Mucha 2014; Cecchetto et al. 2011; Leiva et al. present request for noticing of Gunshot Residue (GSR) require a dependable and quick separated detection system with high sensitivity and precision. In estimating the firing distance, the different approaches are usually based on the determination of the amounts of Pb, Sb, and Ba deposited on the target.

Ballistics is the discipline faithful to study of the development and behaviour of projectiles into and out of the air. This study may core on different parts of the projectile trajectory, allowing the division of this discipline into internal ballistics, external ballistics and terminal ballistics.

Materials and Methods:

Scanning electron microscopy with energy x-ray analyzer :

In this technique Scanning Electron Microscopy with an Energy dispersive X-ray analyzer (SEM-EDX)

As we see the SEM - EDX is usually provided to find electrons generated by two different processes for imaging, secondary and backscattered, and with X-rays for compositional analysis. According to (R. S. Nesbitt, 1 B.A.; J. E. Wessel,et al) 1 The analyses were carried out with a JSM U-3 SEM armed with a Nuclear Diodes lithium-doped silicon X-ray analyzer crystal of 160 eV (full width at half ultimate intensity) resolution and an EDAX International data processing system. The basic physical and chemical forms of GSR particles have not been well described. Most handguns produce residue that contains visible particles ranging in size from 0.01 to 0.1 cm, plus smoke residue, and there is consistent agreement that alterations occur in the properties of residue produced by different firings Of a single gun under rigid conditions. As I observed by reading research papers that is

Technique is particular for essential quality and morphological analysis of Inorganic Gunshot residue particles, Particle by particle studies is possible, It is non destructive technique, It gives high resolution and magnification in excess of 1000000X.

The first scanning electron microscope with very excessive resolution came in 1937 by Manfred von Ardennes. In SEM, a beam of electrons is shelled onto a sample and then the image is shaped (von Ardennes, 1937). SEM is a powerful instrument armed with an X-ray analyzer which emits X-ray supplying a morphological feature of the element which needs to be analyzed. The basic principle confusing here is scattered electrons which are emitted from the surface of a sample. In 1968, research for detection of GSR elements was done using SEM/Edx first moved out in England (Ward, 1982).

In scanning electron microscopy a fine beam of electron is concentrated on material under study. The electron beam crops the following effect: When beam of primary electron break up with sample, some electrons are reflected back (back scatter), the image collected from these electrons gives 3D image of particle. When the primary electron beam hits the sample, atoms ionized by the compelled emission of electrons. The sample electrons emitted from the sample are assigned to as

secondary electrons.It causes X-rays- the generated X-rays are used to find out the elemental composition of particle.

Neutron activation analysis :

Here It is placed on the principle of optical emission spectroscopy where a sample is agreed to emit light by action with an electric arc. NAA can be used for the recognition of antimony and barium in bulk samples (as opposed to single particles) of GSR. NAA is an acutely delicate technique useful for operating both qualitative and quantitative multi element analysis of major/minor and trace elements in sample from around every believable field of activity objective of technical importance. According to Capannesi et al. (Capannesi & Sedda, 1992), this technique helps to check the trace elements coming from the jacket of bullet fragments and lead core. It was advanced by Havsi and Levi in 1936 and is also labeled as referee method as it gives allusion data for other tests. It gives result in the unit of particles per billion. Almost 100000 samples endure NAA each day.

Neutrons are invaded upon the sample thereby applying it into a compound nucleus which is highly unstable. The process of NAA is Compound Nucleus in an attempt to earn stability release advised Gamma Rays (which come under PGNAA) and passes through an transitional state of being radioactive. This radioactive nucleus radiate postponed Gamma Rays. These postponed Gamma Radiations come under DGNAA and 70% of the materials show this property. The energy of DGR is achieve proportional to Radioactivity of the physical. Curve between energy of Gamma Rays and Radioactivity is familiar and gives qualitative and quantitative data.

Chromatography :

Chromatography, a authoritative analytical technique, plays a critical role in the analysis of GSR for forensic ballistics investigations. Chromatography actions certain advantages that make it an fundamental tool for GSR analysis, including its high sensitivity, clearness, and ability to isolated complex mixtures.

Liquid chromatography :

Liquid chromatography (LC) is a highly delicate and duplicatable technique commonly used in forensic sciences. It is appropriate for separating non-volatile, semi-volatile, and thermolabile compounds and can be connected and/or dual with a broad range of detectors, conversing high resilience to the technique. Liquid chromatography (LC) is a important tool for analysing GSR in forensic ballistics due to its accomplished separation potentiality, sensitive detection methods, flexibility in sample preparation, and automation potential , premissive accurate identification and quantification of GSR components for criminal investigations.

Gas chromatography :

Gas chromatography (GC) is among the largest used techniques in the forensic science, exclusively when coupled with mass spectroscopy (MS). On the other hand, GC–MS is consistently used for assuming of smokeless powders but has not been as widely studied for trace OGSR analyses. In this study, a single quadrupole GC–MS armed with a packed column for separation conferred low performance rates for residues collected from known-shooters hands. Still, more new publications only advise single quadrupole configurations of GC for high concentration samples in neat and moderately burned smokeless powder. For example, a study accomplished by Roberts *et al.* engaged the use of GC–MS to differentiate 25 propellant brands as single or double-based powders based on ensuing concentrations of detected nitroglycerin (68–317 ppm). still all organic compounds found in ammunition can commit, OGSR mainly come from propellant powder.

Inductive coupled-mass spectroscopy :

The inductively coupled plasma-mass spectroscopy (ICP-MS) platform grant the examiner to access a very low detection limit due to its high sensitivity and ease rapid multi-elemental analysis, giving both qualitative and quantitative outputs (Biegstraaten and Horváth 2007). The method is enforced to detect metallic and non-metallic category in a liquid sample even at a very low concentration. It entirety on the principle of optical emission spectrometry where plasma energy is given to the sample from the outer zone dominant to the excitation of atoms; when these atoms come at the lower area, spectrum rays are discharge, and their photon wavelength can be consistent. Used ICP-MS to profile the concurrent presence of GSR elemental trio (Pb, Ba, and Sb) at a firing distance of not more than 80 cm away from the target. This data supports the work of Barth et al..

Atomic absorption spectroscopy :

Atomic absorption spectrometer, equipped with Zeeman background corrector, graphite furnace with THGA™ pyrolytically coated graphite tubes, and a PerkinElmer AS-800 autosampler, were used (PerkinElmer, Inc., Shelton, CT, USA). Integrated absorbance readings were performed under gas stop conditions during the atomization step. AAS has been applied to the determination of shooting distances, based on concentration patterns of Pb around bullet holes, and the detection of GSR on collection swabs taken from hands by the determination of antimony and barium concentrations .

Raman spectroscopy :

A Thermo Scientific DXR Raman microscope (Waltham, MA) composed by the Thermo Scientific Omnic for dispersive Raman software was used. Measurements were taken using a laser emitting at 532 nm, a grating with 900 lines per mm, laser power was 8.0 mW, and a confocal pinhole size of 25 mm. The microscope was set to 50 x magnification. The predicted resolution using these parameters was 2.7– 4.2 cm^{-1} and the predicted spot size was 1.1 mm. Paper tape were arranged under the microscope and all the macroscopic GSR particles (30–400 mm in diameter) envisioned through the microscope were consistent. Spectral

addition times were 10 s x 3 additions for GSR particles, 10 s x 10 additions at ten different areas for the unfired ammunition, and 1 s x 10 additions for the conduct (diphenylamine, 2-nitrodiphenylamine, 4-nitrodiphenylamine, N-nitrosodiphenylamine, and ethyl centralite). Background and fluorescence modification were enforced for all the spectra.

Results & discussion :

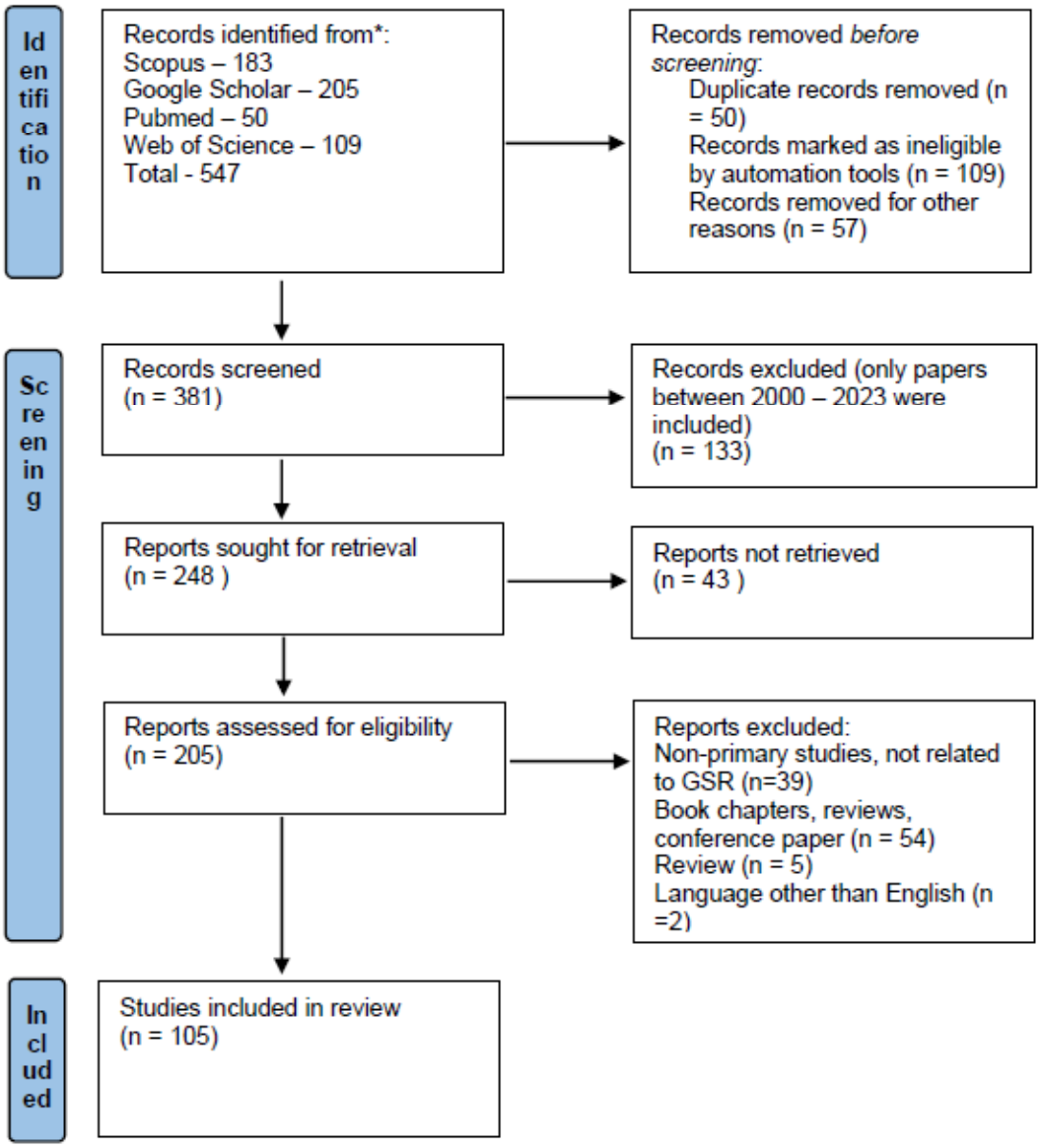
The distance between the gun's muzzle end and the target is termed shooting distance or range of fire or muzzle-to-target distance. In the investigation and reconstruction of the FRC, determining the shooting distance is critical. As a result, the relevance of the distance travelled by these residues cannot be ignored, given its critical significance in the investigation and reconstruction of shooting incidents. A key piece of evidence left behind in shooting incidents, and which could help in this regard, is the gunshot residue (GSR) produced when a gun is fired. GSR is a heterogeneous cloud of propellant particles in various stages of combustion, in addition to the condensation particles originating from the metals used in the primer, cartridge, and projectile. This study demonstrated the presence of actual GSR particles which was obtained by using variable pressure SEM. The analysis of inorganic GSRs by comparing samples collected from hands or other sources to cartridge cases/ammunition is a move away from a "formal" approach in which samples are interpreted following the rules of a formal general interpretation system, to a

‘‘case by case’’ or ‘‘specific’’ approach. Furthermore, individual particles within a sample should be considered in relation to all the others that are present.

Conclusion

The paper climax all the methods so long advanced for detection of gunshot residue. It includes different instrument-based techniques, and electrochemical techniques advanced were explained in detail. To determination of shooting distance is a crucial problem in firearm-related incidents where GSR is the only applicable evidence of probative value. Formation the muzzle-to-victim distance through GSR analysis may commit to linking a suspect to a shooting scene. These advanced methods help to interact the crime whether it is a firearm-related crime or not. The analysis of both inorganic and organic residues has been shown as an auspicious method of gaining as much information about any given sample as possible. A sequence of these techniques with microscopic or even macroscopic analysis of particle analysis would be

Identification of studies via databases and registers



even more agreeable. Therefore, this must be seen as the most ideal access to sample analysis.

Acknowledgement:

I am profoundly grateful of ICRDFS Department of Forensic Science, School of Science, Sandip University, and all the faculties for their guidance and mentorship. My fellow graduate students, and my supportive family. Your unwavering support has been a cornerstone to this endeavor.

Conflict Of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Techniques used for DNA methylation in predicting human age.

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Abstract

Age determination is a key aspect of personal identification. Among other methods, DNA methylation has emerged as an efficient tool for age determination from challenging matrices such as hair. DNA methylation is a common epigenetic modification, which can be used to predict age through mathematical models. The present review critically compares various methods of studying DNA methylation such as ELISA -based method, SNaPshot method, PCR & sequencing, NGS methods and etc. ELISA -based method can be used for quick assessment of DNA methylation status which is easy to perform. Gradient Boosting Regressor is a machine learning algorithm that can be used to predict DNA methylation levels while Cell Separation Algorithm (CSA) is also another algorithm which can be used for predicting dna methylation . SNaPshot can be used for minimum DNA availability by using single hair follicle like these, this review includes all about the DNA methylation methods with their advantages , limitations.

Keywords: DNA methylation; Age determination; NGS; Snapshot ; ELISA; Personal Identification

Introduction:

DNA methylation, primarily occurring at the cytosine residues of cytosine-guanine (CpG) dinucleotides, is one of the most studied epigenetic modifications. Discovered in the 1970s, it has garnered attention for its influence on gene regulation and its potential involvement in various biological processes . The addition of a methyl group to the DNA is catalyzed by DNA methyltransferases (DNMTs), and methylation patterns can be heritable, affecting gene expression without altering the underlying DNA sequence itself . DNA methylation, the addition of a methyl group to the DNA molecule, is a key epigenetic modification that regulates gene expression and can change in response to environmental factors, lifestyle choices, and aging. Aging is a complex biological phenomenon characterized by a gradual decline in cellular and physiological functions. Traditional chronological age assessments do not always correlate with biological aging, leading to the exploration of epigenetic markers. DNA methylation, involving the addition of a methyl group to the cytosine residues of DNA, plays a pivotal role in gene expression regulation and is significantly influenced by age. The application of DNA methylation for age determination stems from its stability and heritability, making it an appealing candidate for longitudinal studies. Notably, several studies have identified distinct methylation signatures that correlate with age, leading to the development of predictive models based on these patterns . Such models leverage machine learning techniques and large-scale genomic datasets to predict chronological age with remarkable accuracy, demonstrating the potential of DNA methylation as a biomarker for aging.

DNA methylation occurs predominantly at cytosine bases within cytosine-phosphate-guanine (CpG) dinucleotides, where the addition of a methyl group can inhibit gene transcription. This modification affects not only gene expression but also genomic stability, cellular differentiation, and aging processes. As organisms age, distinct altered patterns of DNA methylation occur, leading to a phenomenon known as the "epigenetic clock," a term introduced by Horvath in 2013. This clock utilizes the methylation levels of specific CpG sites to predict biological age and health status. [5]

DNA methylation analysis has gained prominence as a powerful tool for age determination in forensic contexts. Over the past decade, the understanding of DNA methylation patterns and their association with age has advanced significantly, leading to the exploration of their utility in forensic science.Age determination is a critical aspect of forensic investigations, often providing valuable insights into the timing of events and the identity of individuals involved in crimes. Traditional methods of age estimation, such as dental and skeletal analyses, can be limited by factors such as environmental influences and individual variability. As a result, there is a pressing need for innovative approaches to age estimation that can provide more reliable and objective results. The analysis of DNA methylation presents a viable solution due to its stability and the fact that it can reflect biological rather than chronological age.

Understanding the nuances of DNA methylation patterns and their relationship with age will not only refine the analytical methods used in forensic science but also pave the way for advancements in personal identification, victim identification, and legal proceedings.

In this paper, we will delve into the methodologies employed in DNA methylation analysis for age estimation, discuss the implications of these findings in forensic contexts, and highlight potential future directions for research in this burgeoning field.

Materials and Methods:

- Source: Google scholar and science direct
- Inclusion criteria: 1. Papers only between 2001 & 2024.

  2. Research papers and review articles were included

  3. Only full text papers were included .

  4. Articles only in English
- Exclusion criteria : 1. Chapters, datasets , reports were not included

2. Papers whose full text were not included .

3. Local papers from countries other than were not included

Techniques for analysing DNA methylation status

1. ELISA-Based Techniques for DNA Methylation

The global DNA methylation (5-mC) or hydroxymethylation (5-hmC) profile of a sample can be quantitated using the indirect ELISA (enzyme-linked immunosorbent assay) method. This technique detects specific antigen-antibody binding via enzymatic reactions that are observable through color change of the reaction medium, and results can be read spectrophotometrically. In this case, the antigen is the DNA of interest (5-methylcytosine DNA—5-mC or 5-hydroxymethylcytosine DNA—5-hmC) and the antibody has affinity for methylated or hydroxymethylated CpG sites (anti-5-mC or anti-5-hmC). Results are expressed as percentage of methylation/hydroxymethylation compared to fully methylated/hydroxymethylated control DNA (100%). This technique is fast, cost-effective, and yields accurate results, since anti-5-mC/anti-5-hmC are highly specific and present no cross-reactivity with unmethylated DNA [21]

ELISA and MeDIP techniques may struggle with detecting low-level methylation, which could explain the reported absence of mitoepigenetics. The study found an inverse correlation between methylation levels at two CpG sites (M1215 and M1313) and age, suggesting their utility as epigenetic markers for age prediction. It highlights the controversy and recent evidence of mtDNA methylation. The researchers used bisulphite sequencing on blood samples from 82 individuals aged 18-91. They focused on specific regions of the mitochondrial genome.

The study suggests mtDNA methylation as a promising biomarker for age prediction, with potential applications in forensic science and health status assessment. Further research is needed to explore its generality and external influences.

ELISA (Enzyme-Linked Immunosorbent Assay) might struggle with detecting low-level methylation in the mitochondrial DNA (mtDNA) genome. This could contribute to the confusion around the presence and significance of mtDNA methylation. The study emphasizes the need for sensitive and advanced methodologies like high-throughput sequencing to accurately detect and quantify mtDNA methylation. [19]

SNaPshot Method

The study focuses on predicting human age by analyzing DNA methylation in hair follicles using the SNaPshot method. This method is significant for forensic science as it helps narrow down suspects and identify human remains .The researchers collected 166 hair samples and used the SNaPshot assay to measure methylation at 10 CpG sites. They constructed multiple age prediction models, with the multiple linear regression (MLR) model showing the best results. The MLR model, which included 10 CpG sites, provided the most accurate age predictions with a median absolute deviation (MAD) of 3.68 years. The study found no significant differences in methylation between different sexes, hair types, or hair colors. The SNaPshot method is effective for predicting age from hair follicles, making it a valuable tool in forensic investigations. The study suggests further research with diverse ethnic groups to improve the model's accuracy. Four CpG sites (cg24724428, Chr6:11044628 in ELOVL2, cg25148589 in GRIA2, and cg07547549 in SLC12A5) showed a strong correlation with age. [7]

The MLR model explained 91.7% of the age variance in the training samples and was more accurate for individuals under 40 years of age. The method can obtain both human identification and age information from a single scalp hair follicle, which is practical for forensic applications. No significant differences in methylation degree were found between different sexes, hair types, or hair colors, ensuring consistent results. The study was conducted on a Han population in northern China, which may limit the applicability of the model to other ethnic groups or regions. The

prediction accuracy decreases for individuals over 60 years old, with a higher MAD compared to younger age groups.

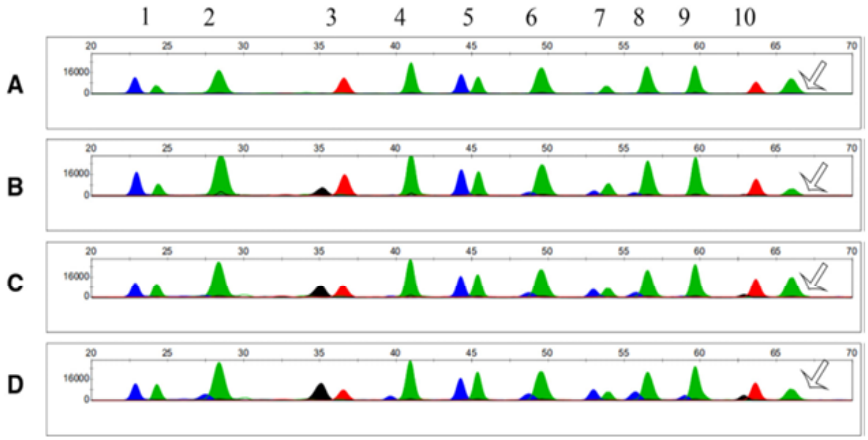

Figure 1. Electrophoretograms of the multiplex methylation SNaPshot assay of four samples of different ages. Electropherograms of the multiplex methylation SNaPshot assay at 10 CpG sites in hair samples are obtained from four individuals aged 1 (A), 22 (B), 51 (C), and 86 (D) years. A cytosine (C) at a non-CpG site (Chr7:130 734 382 in *KLF14*) is incorporated into the system (indicated by the arrow) to assess the conversion efficiency of DNA by bisulfite. The presence of only a green peak denotes full conversion of C/A. G, A, C, and T show as blue, green, black, and red, respectively. 1, cg01820374; 2, cg06493994; 3, Chr6:11 044 628; 4, cg14361627; 5, Chr1:207 823 681; 6, cg07547549; 7, cg24724428; 8, cg25148589; 9, Chr7:130 734 357; 10, cg17861230.

Gradient Boosting Regressor

The study used data from the Illumina HumanMethylation BeadChip platform, analyzing both healthy and diseased blood samples.A gradient boosting regressor was built using six age-related CpG sites. The model showed high accuracy with a correlation of 0.97 and a mean absolute deviation (MAD) of 2.72 years in the training dataset. The model performed well on independent datasets and even when applied to saliva samples, demonstrating its robustness and effectiveness**.** GBR outperformed other models like Bayesian Ridge, Support Vector Regression, and Multiple Linear Regression in terms of prediction accuracy and lower MAD values. The model was also tested on diseased samples, showing a MAD of 5.44 years for the training dataset and 7.08 years for the independent dataset, indicating its robustness even in the presence of disease-related methylation changes. The GBR model worked well with saliva samples, demonstrating its applicability to different types of biological samples beyond blood. [18] .DNA methylation analysis can be performed on easily accessible samples like blood or saliva. Modern models show high

correlation between predicted age and actual age, with low mean absolute deviation. Methylation patterns can vary between different tissues, which may affect the accuracy of age predictions. External factors such as lifestyle and environmental exposures can influence DNA methylation, potentially impacting the predictions.

Massively Parallel Sequencing

Author aliferi et al discusses a method for predicting chronological age using DNA methylation data from 110 whole blood samples. The method employs bisulphite conversion and massively parallel sequencing of 12 CpG sites. The study analyzed 110 whole blood samples and 34 saliva samples using the Illumina MiSeq platform to quantify DNA methylation at 12 age-correlated CpG sites. The method was tested on saliva and sperm samples, showing accurate predictions for saliva but no variation in DNA methylation for sperm. Seventeen different statistical models were developed, with a Support Vector Machine model selected for its accuracy, achieving a mean absolute error (MAE) of 4.1 years in the blind test set.

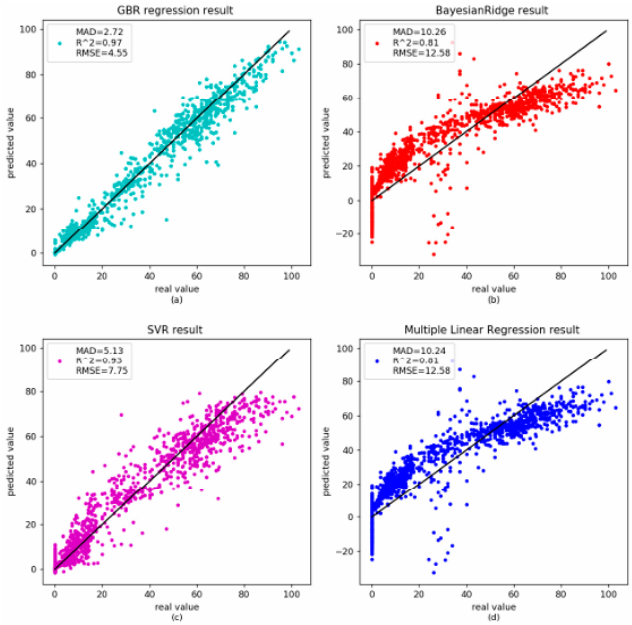

Figure 1. Comparison between the real age and the age predicted by the four models in the training dataset of health data. GBR: gradient boosting regresion; MAD: mean absolute deviation; RMSE: root mean square error; SVR: support vector regression.

The study highlights the potential of this method for forensic casework, emphasizing its sensitivity and applicability to various tissues. The method retained accuracy down to 2ng of DNA input in the PCR stage, making it suitable for forensic applications. The method was effective for both blood and saliva samples but not for sperm samples. [8]

Advantages: The method achieved a mean absolute error (MAE) of 4.1 years in the blind test set, demonstrating high prediction accuracy. The method was successfully applied to both blood and saliva samples, showing versatility.

Disadvantages: While the method is sensitive, it still requires further improvement to be universally applicable to all forensic samples. The prediction error is sample-specific, indicating that different samples predict with varying levels of accuracy across models.

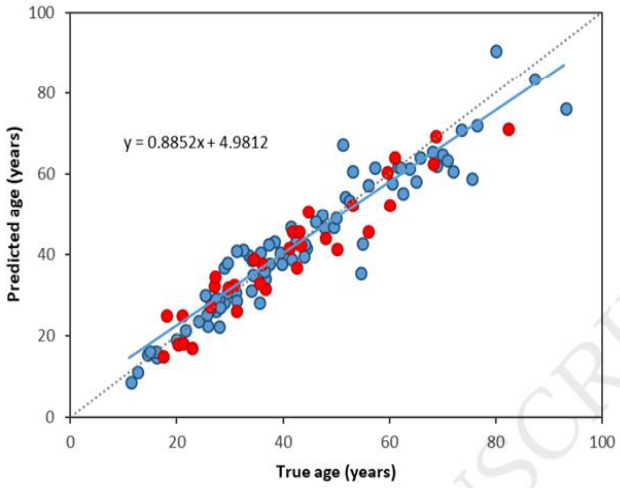

Figure 5. Comparison between the predicted and the true age for the training (blue, n=76) and blind test set (red, n=33) in the SVMp model. The mean absolute prediction error was calculated at 4.0 and 4.1 years respectively.

Cell Separation Algorithm (CSA)

Predicting age using DNA methylation data, optimized by a new algorithm called the Cell Separation Algorithm (CSA), which mimics the differential centrifugation process. The CSA mimics the differential centrifugation process to optimize the Artificial Neural Network (ANN) model for age prediction from DNA methylation data. It involves multiple centrifugation steps with increasing rotor speed to separate solutions based on their objective function. The most frequently selected CpG sites for age prediction were cg09809672, cg22736354, and cg02228185 for healthy blood data. [10]

The CSA was tested on 25 benchmark functions and showed superior performance compared to other methods like SGD, ADAM, and GA. The CSA was applied to DNA methylation data from healthy and diseased blood samples, as well as saliva samples, demonstrating high accuracy in age prediction. The CSA outperformed other methods in terms of Mean Absolute Deviation (MAD), Mean Squared Error (MSE), and Root Mean Squared Error (RMSE), proving its effectiveness in optimizing the ANN model for age prediction. The CSA demonstrated robustness by effectively predicting age from both healthy and diseased blood samples, as well as saliva samples. The CSA's results were significantly better than previous methods, especially in terms of Mean Absolute

Deviation (MAD) and other regression measurements. CSA outperforms other methods like SGD, ADAM, and genetic algorithms in terms of prediction accuracy.

It efficiently selects relevant CpG sites, enhancing the prediction model's performance. The method shows strong performance across different types of data, including healthy and diseased blood, as well as saliva samples.

The algorithm's multi-step process and parameter tuning can be complex and computationally intensive.The performance of CSA can be sensitive to the initial settings of parameters like separation rate and rotation speed.

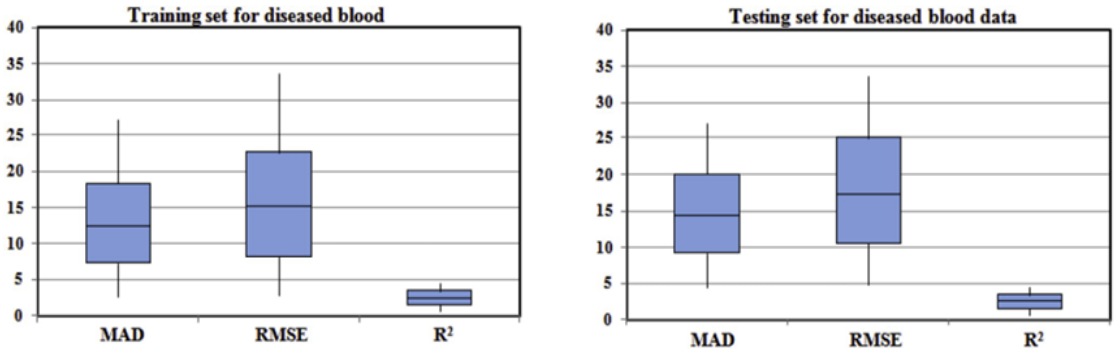

Fig. 9. Comparison of box plots in 10-fold CSA results in train and test phases for diseased blood data.

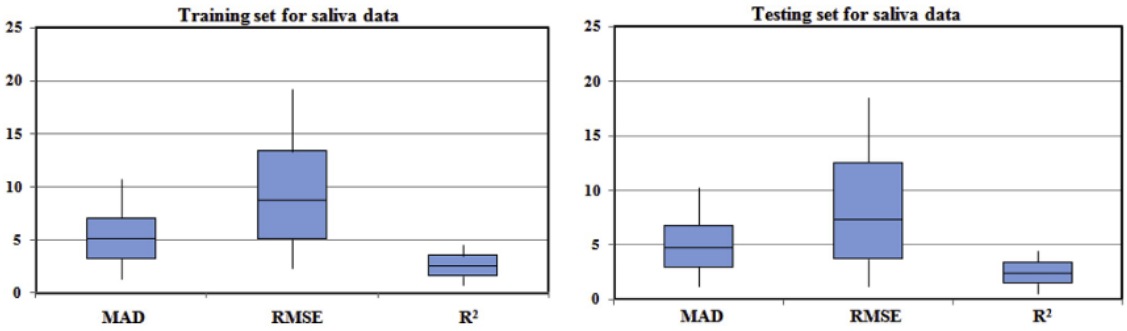

Fig. 11. Comparison of box plots in 10-fold CSA results in train and test phases for the saliva data.

Next Generation Sequencing

NGS allows for single-base resolution, providing detailed maps of DNA methylation across the genome .NGS is used to identify biomarkers for disease detection, diagnosis, prognosis, and personalized treatments. [37]

The study aimed to use age-specific DNA methylation patterns to create an accurate model for predicting chronological age using whole blood samples. An NGS-based method was developed using the Illumina MiSeq platform to quantify the methylation status of 16 selected CpG sites. The method was validated using DNA standards of known methylation levels and tested on 46 whole blood samples. The NGS data showed a mean absolute error (MAE) of 7.5 years, which is expected to improve with future optimization .NGS offers a sensitive and accurate approach for age prediction, with higher resolution data compared to traditional methods.[20]

NGS requires substantial bioinformatics support and has high costs, especially for whole-genome bisulfite sequencing (WGBS). [37]

Bisulfite sequencing methods

Algorithms like eAge use DNA methylation patterns to estimate age and assess health risks. High-throughput bisulfite sequencing methods, such as RRBS and WGBS, help identify regions of DNA methylation heterogeneity. Five scores (MHL, PDR, PM, FDRP, qFDRP) were evaluated for their ability to predict age. The PDR metric, which measures DNA methylation erosion, showed the best performance. The WSH-based model using PDR had an MAE of 3.686 years, making it a promising tool for designing reduced epigenetic clocks.The study evaluates five metrics for constructing epigenetic clock models using reduced-representation bisulfite sequencing (RRBS) data. The best performance was achieved with a model based on the Proportion of Discordant Reads (PDR) metric. The paper highlights the importance of within-sample heterogeneity (WSH) scores in assessing DNA methylation patterns, which can indicate cell-type heterogeneity, DNA methylation erosion, and allele-specific methylation. The PDR-based model demonstrated the highest accuracy with a mean absolute error (MAE) of 3.686 years, suggesting its potential for

developing reduced epigenetic clocks. Advantages: The model based on the PDR metric showed high accuracy with a mean absolute error (MAE) of 3.686 years. The method requires analysis of only a few short genomic regions, making it efficient for targeted next-generation sequencing. Utilizes high-throughput bisulfite sequencing methods like RRBS and WGBS, which cover a significant portion of CpG sites. Disadvantages: RRBS and WGBS methods can have non-uniform CpG-site coverage across samples, leading to inter-sample variation. The high-resolution data from these methods can be difficult to interpret compared to microarray data. [41]

Author Han et al discusses the use of barcoded bisulfite amplicon sequencing (BBA-seq) to investigate age-associated DNA methylation in mice . This method allows for the analysis of longer amplicons with more neighboring CpGs. BBA-seq revealed that neighboring CpGs tend to be stochastically modified, and the binary sequence of methylated and non-methylated CpGs in individual reads can be used for single-read predictions, reflecting heterogeneity in epigenetic aging. The study compares BBA-seq with pyrosequencing and droplet digital PCR (ddPCR), noting that while BBA-seq provides similar accuracy in age predictions, it offers better insight into neighboring CpGs and facilitates single-read predictions . The paper also highlights that BBA-seq can reflect accelerated epigenetic aging in different mouse strains, such as DBA/2 mice, compared to C57BL/6 mice .Provides very high coverage and insight into neighboring CpGs. This method facilitates single-read predictions, revealing heterogeneity in epigenetic aging. Analyzes longer amplicons, covering more CpGs than other methods. Shows only moderate correlation of DNAm at neighboring CpGs . Slightly less precise in DNAm measurements compared to pyrosequencing and ddPCR. [42]

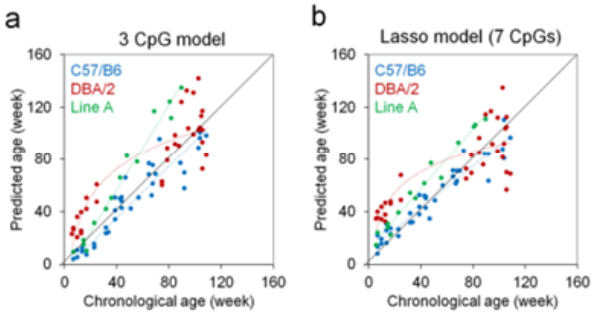

Figure 5. Age-predictions with BBA-seq in mice from different genetic background. DNAm levels were analyzed with BBA-seq in blood samples of 40 C57BL/6 mice of the validation sets, 33 DBA/2 mice, and 15 transgenic C57BL/6 mice with an age-associated region from DBA/2 mice (Line A)[18]. For epigenetic age predictions we either used (**a**) the 3 CpG multivariable model, or (**b**) the lasso regression model based on 7 CpGs of the same three amplicons (*Prima1*, *Hsf4*, *Kcns1)*. As previously described for pyrosequencing, epigenetic age-predictions were logarithmically accelerated in DBA/2 mice[17], and also accelerated in Line A mice[18].

Results: DNA methylation is a key epigenetic modification that can be heritable and affect gene expression without changing the DNA sequence. The "epigenetic clock" phenomenon involves altered DNA methylation patterns that can predict human age. Various methods, such as the SNaPshot assay and the Cell Separation Algorithm, have been used to analyze DNA methylation for age prediction .

samples and age-specific DNA methylation patterns, with a mean absolute error (MAE) of 7.5 years, and evaluated five metrics for constructing epigenetic clock models using reduced-representation bisulfite sequencing (RRBS) data. The study included various subjects, including 82 individuals aged 18-91 for bisulphite sequencing, 166 hair samples for the SNaPshot method, 110 whole blood samples and 34 saliva samples for DNA methylation analysis, and healthy and diseased blood samples, as well as saliva samples for the Cell Separation Algorithm (CSA). The key findings of the study include the identification of distinct methylation signatures that correlate with age, the development of predictive models based on these patterns, and the demonstration of the potential of DNA methylation as a biomarker for aging.

Snapshot method is significant for forensic science as it helps narrow down suspects and identify human remains .The researchers collected 166 hair samples and used the SNaPshot assay to

measure methylation at 10 CpG sites The study found no significant differences in methylation between different sexes, hair types, or hair color.

The Cell Separation Algorithm (CSA) was applied to DNA methylation data from healthy and diseased blood samples, as well as saliva samples, demonstrating high accuracy in age prediction.

Conclusion : The study concludes that DNA methylation analysis has the potential to be a valuable tool in forensic investigations, providing a more reliable and objective method for age estimation. The suggests that future research should focus on refining the analytical methods used in forensic science, exploring the nuances of DNA methylation patterns and their relationship with age, and advancing personal identification, victim identification, and legal proceedings

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Exploring Nitrosamine Impurities and Regulations Containing their Presence in Drug Products

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ABSTRACT

The significance of presence of nitrosamines came to attention after USFDA and EMA declared in July 2018 that N-Nitroso dimethylamine and N-N-NDMA are focused on the pharmaceutical medicinal products and particularly used in case of SARTANS that are used in the treatment of Hypertension and Angiotensin II receptor blockers. Later the list was expanded to include Histamine-2 blocker Ranitidine and Diabetes drug Pioglitazone. Reaction of Urea Derivatives, Secondary amide carbamates and amines with Nitrogenous agent and nitrates lead to formation of Nitrosamines. The Oxidation state of nitrogen is +3. The Reasons for Presence of Nitrosamines in Pharmaceutical Products can be due to Product Degradation, Catalysts, solvents, Chemical reagents, Cross Contamination, Manufacturing Process and Contamination of Raw Materials. Technologies like Gas Chromatography, Mass Spectroscopy, Liquid Chromatography Mass spectroscopy are used to detect Nitrosamine Contamination. N-Nitrosamines categorized as "Cohort of concern" in ICH guidelines due to their potential mutagenic and carcinogenic nature. N-Nitroso dimethylamine and N-Nitroso diethylamine are classified as Class 2A human carcinogens by IARC-International Agency for Research and Cancer. This study focused on history, formation, Method development and regulations governing their presence in drug products.

Keywords: Nitrosamine impurities, Carcinogenicity, Limits, Regulations, Global risk.

Introduction:

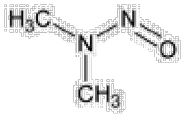
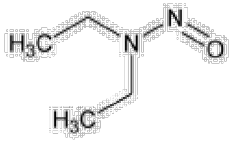
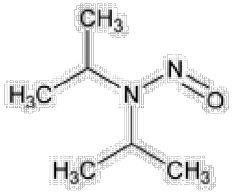
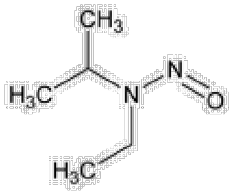
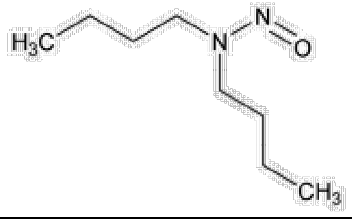
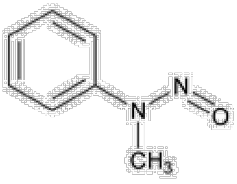
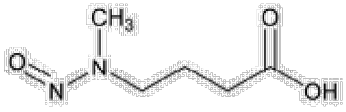
Controlling Nitrosamines:

N–nitrosamines, are a potentially mutagenic class of impurities that may pose a risk of cancer when individuals are exposed to them, above acceptable levels, for extended periods. In recent years, nitrosamines have been detected in various widely marketed medicines, examples include varenicline, metformin, ranitidine, and the sartan class of medicines, which has led to voluntary product recalls from the market. For the pharmaceutical sector, maintaining the safe production of essential medications is a top priority on a global scale. Regulatory agencies and health authorities are stressing the need to detect, track, and manage nitrosamine contaminants in active pharmaceutical ingredients (APIs) and other raw materials in order to reduce the danger of nitrosamines. Controlling nitrosamines in pharmaceuticals that are on the market and those that are still in development requires analytical testing.[1]

The Formation of Nitrosamines

Nitrosamines can form when an amine source and nitrosating agent react under specific conditions of temperature and pH[1]. The API synthesis and drug product manufacturing processes may introduce risk of nitrosamine formation via starting materials, reagents, solvents, catalysts, intermediates, excipients and raw materials and storage packaging and conditions. Initially, industry focus was the formation of low–mass nitrosamines such as NDMA and NMBA however the prevalence for nitrosamines formation expanded significantly with the detection of Nitrosamine Drug Substance Related Impurities (NDSRIs), which are structurally similar to the API and can form in numerous marketed medicines. Analytical procedures that fulfill a wide range of regulatory criteria and provide flexibility and sensitivity are necessary as nitrosamine control rules shift toward a pragmatic approach.[3,4]

Table-1: Nitrosamines Found as Contaminants in Drug Substances and Drug Products

Common Name and Chemical Name	Acronym	CAS #	Structure	Chemical Formula	Molecular Weight
Nitrosodimethylamine N-Methyl-N-nitrosomethanamine	NDMA	62-75-9		$C_2H_6N_2O$	74.08
Nitrosodiethylamine N-Ethyl-N-nitrosoethanamine	NDEA	55-18-5		$C_4H_{10}N_2O$	102.14
Nitrosodiisopropylamine N-Isopropyl-N-nitrosoisopropylamine	NDIPA	601-77-4		$C_6H_{14}N_2O$	130.19
Nitrosoethylisopropylamine N-Ethyl-N-nitroso-2-propanamine	NEIPA	16339-04-1		$C_5H_{12}N_2O$	116.16
Nitrosodibutylamine N-Butyl-N-nitroso-1-butanamine	NDBA	924-16-3		$C_8H_{18}N_2O$	158.25
Nitrosomethylphenylamine N-Methyl-N-nitrosophenylamine	NMPA	614-00-6		$C_7H_8N_2O$	136.15
Nitrosomethylaminobutyric acid 4-	NMBA	61445-55-4		$C_5H_{10}N_2O_3$	146.15

Common Name and Chemical Name	Acronym	CAS #	Structure	Chemical Formula	Molecular Weight
[Methyl(nitroso)amino] butanoic acid					

Potential sources of nitrosamine impurities in drug product:

- The drug substance itself, which may degrade under some conditions resulting in the formation of nitrosamines (e.g., ranitidine).
- Degradation of solvents (e.g., dimethylformamide [DMF]) leading to the formation of dialkyl amines.
- Impurities in raw materials, solvents (including recycled solvents), reagents, or catalysts.
- Impurities in materials and intermediates, reagents, and solvents used to prepare the starting materials or intermediates.
- Impurities in water, excipients, or processing aids used in the production of the finished drug product.
- During drug product manufacture under certain reaction conditions and in the presence of requisite precursors necessary for the formation of nitrosamines.
- Impurities in the container–closure system for the finished drug product, which may include impurities capable of forming nitrosamines, especially if associated with materials containing amines and potential sources of a nitrosating agent (e.g., nitrite, nitrocellulose).

A risk assessment should be conducted to determine the materials that contribute to the potential for inclusion of nitrosamines in the drug product. All potential sources for the introduction of nitrosamines should be considered in the risk assessment including, for example, the drug substance, excipients, water, solvents, the manufacturing process, packaging components, and formation on stability. Some of the considerable potentioal sources are shown in Figure-1.

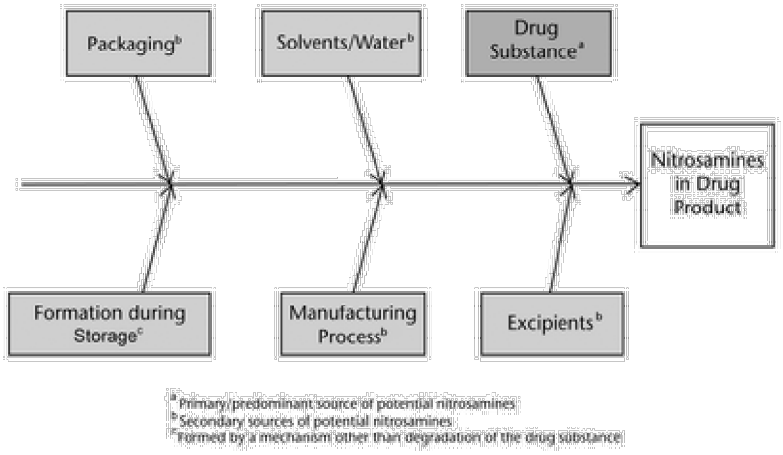


Figure-1: Potential sources of nitrosamine impurities in drug product.[2]

Identified risks associated with several of the potential sources of nitrosamines. Some of the examples identified are summarized in Table-2

Potential Source of Nitrosamines	Observed Risk
Solvents	Presence of residual dialkyl amines or tri-substituted amines that can degrade to form intermediates that can further react with nitrosating agents. Presence of nitrites or other nitrosating agents Presence of acid Limited controls/specification limits for recycled solvents Poor quality solvents
Water	Presence of residual dialkyl amines or impurities that can degrade to form dialkyl amines. Presence of acid and nitrosating agents
Excipients	Presence of nitrites or other nitrosating agents and/or nitrosamine impurities (if applicable)

Potential Source of Nitrosamines	Observed Risk
Drug substance	Use of sodium azide in the synthesis followed by use of nitrites in acidic medium (nitrous acid) for quenching excess azides. Use of di- or tri-alkylamines and amides (e.g., dimethylformamide [DMF], dimethylamine [DMA], triethylamine [TEA], N-methylpyrrolidone [NMP]) in the presence of nitrites and acid media Use of recycled solvents that may contain nitrosamines or their precursors. Use of sanitized water (e.g., chloramines) Insufficient purification Degradation of drug substances containing functional groups that can then participate in nitrosation reactions
Manufacturing process	Contamination Use of poor quality or recycled solvents that may contain nitrosamines or their precursors. Presence of nitrous oxides in air used to dry the drug substance or drug product. Carryover of relevant reactive species into subsequent steps
Drug product (including stability)	Secondary, tertiary, or quaternary amine group in molecule of drug substance Presence of nitrate counter ions (potentially containing nitrite as an impurity) Potential reactions within the formulation matrix during stability/shelf life (e.g., presence or generation of acidic conditions, moisture, and heat)
Container–Closures	Packaging materials containing vulnerable amines that might react with nitrosating agents present in the packaging material itself (e.g., amines in inks reacting with nitrocellulose print base)
Solvents	Presence of residual dialkyl amines or tri-substituted amines that can degrade to form intermediates that can further react with nitrosating agents. Presence of nitrites or other nitrosating agents

Potential Source of Nitrosamines	Observed Risk
	Presence of acid Limited controls/specification limits for recycled solvents Poor quality solvents
Water	Presence of residual dialkyl amines or impurities that can degrade to form dialkyl amines. Presence of acid and nitrosating agents
Excipients	Presence of nitrites or other nitrosating agents and/or nitrosamine impurities (if applicable)
Drug substance	Use of sodium azide in the synthesis followed by use of nitrites in acidic medium (nitrous acid) for quenching excess azides. Use of di- or tri-alkylamines and amides (e.g., dimethylformamide [DMF], dimethylamine [DMA], triethylamine [TEA], N-methylpyrrolidone [NMP]) in the presence of nitrites and acid media Use of recycled solvents that may contain nitrosamines or their precursors. Use of sanitized water (e.g., chloramines) Insufficient purification Degradation of drug substances containing functional groups that can then participate in nitrosation reactions
Manufacturing process	Contamination Use of poor quality or recycled solvents that may contain nitrosamines or their precursors. Presence of nitrous oxides in air used to dry the drug substance or drug product. Carryover of relevant reactive species into subsequent steps
Drug product (including stability)	Secondary, tertiary, or quaternary amine group in molecule of drug substance Presence of nitrate counter ions (potentially containing nitrite as an impurity)

Potential Source of Nitrosamines	Observed Risk
	Potential reactions within the formulation matrix during stability/shelf life (e.g., presence or generation of acidic conditions, moisture, and heat)
Container–Closures	Packaging materials containing vulnerable amines that might react with nitrosating agents present in the packaging material itself (e.g., amines in inks reacting with nitrocellulose print base)

Nitrosamine Risk Assessments—Development of a Control Strategy:

In order to determine the level of control, if any, which may be required for ensuring that levels of nitrosamines are at or below the acceptable intake (AI) if their presence cannot be avoided, the components of drug products should be assessed by the drug product manufacturer for the potential to form nitrosamines or to be contaminated with nitrosamines. The drug substance synthetic route is one of the sources with the highest potential for nitrosamines; however, a risk assessment should also consider the drug substance manufacturing process, the drug product manufacturing process, and the raw materials and excipients used in the product to determine whether additional controls are needed or not. Figure 2 depicts an example of a high-level material evaluation process flow.

Limits of nitrosamines

Nitrosamine impurities identified in this chapter have potential and established toxicity with no therapeutic value. Because nitrosamines are among the structural groups of high potency mutagenic carcinogens of the "cohort of concern" in ICH M7 [1,8], the threshold of toxicological concern (TTC) does not apply. Instead, the available safety data should be used to establish a material-specific AI on a case-by-case basis. The AI is defined as an intake level that poses a negligible health risk. [1,2]

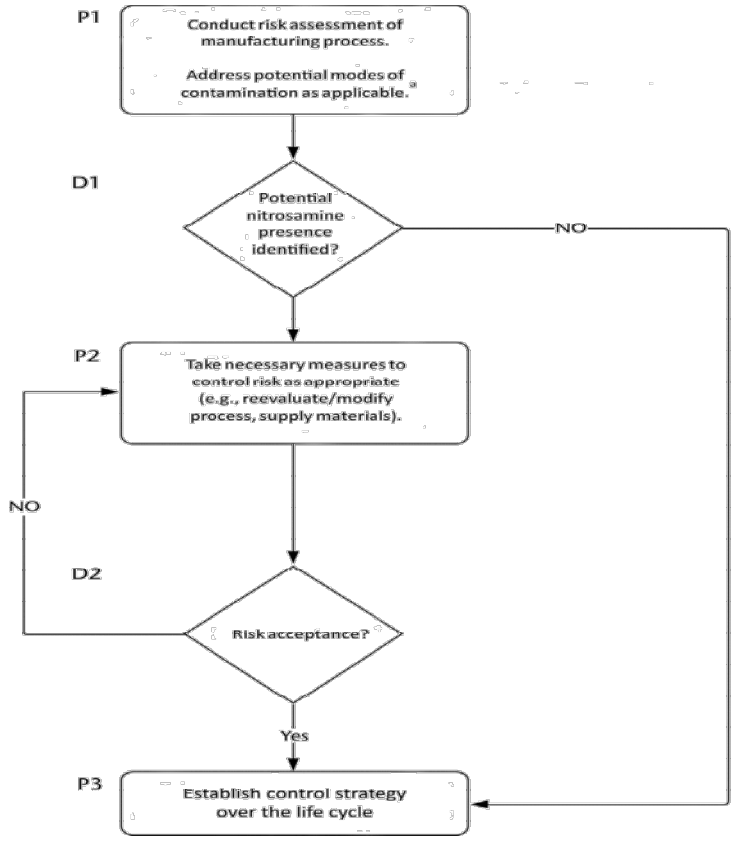


Figure 2. High level process for development of a nitrosamine impurity control strategy.

In all cases, if nitrosamines are predicted by the risk assessment or confirmed to be present through testing of the drug substance, drug product, or other materials, a control strategy should define an approach to ensure that the nitrosamine levels comply with the established AIs. The control strategy should be aligned with the current regulatory requirements in place.

Derivation of AI Limits

There are a number of methodologies that toxicologists have applied in establishing AIs. In this case, the median tumorigenic dose (TD_{50}) of NDMA, NDEA, and other nitrosamines was used as representative data in a linear extrapolation to establish an acceptable risk level. The limits have been published in the FDA Guidance for Industry: Control of Nitrosamine Impurities in Human Drugs [1]

Example Calculations of Nitrosamine Limits

The AIs in nanograms per day and the maximum daily dose (MDD) of the drug substance (DS) from the drug label in milligrams per day can be used to calculate the

maximum nitrosamine concentration limits, in ppm, for individual drug products using the following equation:

Concentration = AI/DS dose

Since the exposure to nitrosamines is related to the MDD of the DS, different concentrations of nitrosamines (ng/g) may be acceptable for each material evaluated. The acceptable concentration in the material can be calculated using the following equation:

Acceptable nitrosamine content = AI/MDD

AI = acceptable intake of the nitrosamine (µg/day)

MDD = maximum daily dose of the drug substance (g/day)

Table-3. Example Using an AI of 96 ng/day for the Target Nitrosamine

Name	Acceptable Concentration (µg/g)			
Nitrosamine 1	0.050 (50-mg dose)	0.100 (100-mg dose)	0.250 (250-mg dose)	1.00 (1000-mg dose)
	1.920	0.960	0.384	0.096

[Note—If multiple nitrosamines are identified in the material and the total exceeds 26.5 ng/day, the appropriate regulatory authority should be consulted to determine an acceptable approach.]

Method Development for Nitrosamine Analysis

When conducting a nitrosamines risk assessment, it is crucial to develop highly sensitive and specific analytical methods that are capable of quantifying at or below regulatory-approved thresholds. Whilst GC and other detection techniques are employed, LC/MS-MS (Liquid Chromatography with Tandem

Mass Spectrometry) emerges as the gold standard solution for quantification of all types of nitrosamines. This is because of the reliable sensitivity and selectiveness that MS/MS offers, as

well as the easily accessible equipment options that are ideal for routine analysis and method development in a controlled setting. As with any trace analysis, the development of methods for the quantification of nitrosamines can present diverse challenges.[1,2]

Sample Preparation for Nitrosamine Analysis

Comprehensive sample preparation is often necessary when extracting low levels of nitrosamines from a high concentrated API or the matrix component of a drug product. Effective sample preparation has a significant impact on the final performance of the quantitative assay. Since nitrosamines encompass a wide range of compounds with varying physicochemical properties, methods are usually specific to the particular assay, therefore straightforward methods with simplified workflows are favored. However, in cases where greater selectivity is required due to challenging matrix of API or API interference, approaches like solid–phase extraction can be beneficial. In especially for low-level contaminants, automated sample preparation procedures can further improve inter-assay and inter-laboratory reliability by reducing external contamination, improving assay precision, and improving reproducibility.

Optimized Chromatographic Separation

Selecting the appropriate chromatography and column chemistry is critical when developing a method for analyzing nitrosamines in drug substances or products. It is essential to establish resolution.By selectively directing the API peak to waste and redirecting the well–resolved nitrosamines to the mass spectrometer (MS) inline, issues such as suppression and source contamination can be minimized. It's important to note that different substances, drug products, and formulations may require tailored approaches to achieve.

Optimized MS Sensitivity for Impurities Analysis

To meet regulatory requirements for detecting low levels of nitrosamines in APIs or drug products, tandem quadrupole mass spectrometry is the preferred method for quantification. With the

sensitivity and selectivity afforded by tandem MS, this accessible technique also offers flexible ionization options that can be tailored to the specific nitrosamines being measured. For simple LC/MS analysis, low–mass nitrosamines are often best detected using atmospheric pressure chemical ionization (APCI), while electrospray ionization (ESI) is better suited for analyzing complex nitrosamines or NDSRIs. To ensure high sensitivity and selectivity, multiple reaction monitoring (MRM) is recommended for nitrosamine measurement. It is important to note that different published methods may use different transitions for a given impurity, so each laboratory must validate its analytical methodologies to within specific regulatory compliance requirements, before submitting data to regulatory authorities.

Regulatory Compliance

The control of nitrosamines is subject to evolving regulatory requirements in the pharmaceutical industry. Regulatory agencies demand a comprehensive understanding of a drug product's impurity profile, which includes quantitation of impurities present at low levels in sample matrices. This necessitates the development and validation of sensitive analytical methods that adhere to specific regulatory guidance. Several key regulatory guidelines provide direction for the management of impurities in pharmaceuticals. These include the ICH (International Council for Harmonization) guidelines which support approaches to adhering with global regulatory requirements and harmonized guidance throughout the industry. Specific to nitrosamines, ICH M7 provides guidance on the assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk, which encompasses nitrosamine analysis. Relevant chapters within the United States Pharmacopeia (USP) serve as valuable resources for impurity guidance. For example, USP <1469> specifically addresses nitrosamines impurity analysis. By staying up to date with the latest guidelines and harmonized procedures, laboratories can ensure the safety and quality of pharmaceutical products, reinforcing their position as reliable partners in the industry.

Comprehensive Solutions for Nitrosamines Quantification

Sensitivity beyond today's regulatory thresholds and future proofing for evolving requirement in trace genotoxic impurities quantification. Our comprehensive solutions for impurity analysis with LC–MS/MS is designed to assist laboratories in meeting stringent regulatory requirements for all, small to complex, nitrosamines. By offering state–of–the–art LC–MS/MS instrumentation, optimized sample preparation workflows, and validated analytical methods, Waters empowers laboratories to develop methods to achieve the sensitivity, selectivity, and accuracy necessary to quantify all nitrosamines at or below regulatory-approved thresholds.

Nitrosamines and Beyond:

The evolving nitrosamines concern and their potential impact on drug safety has raised questions about the future for mutagenic and genotoxic impurity control in Pharmaceuticals. What will the next challenge be? Can laboratories meet today's testing requirements and also prepare for future genotoxic and mutagenic impurities testing capabilities that continue to support strict quality standards? Regulatory requirements for nitrosamine control have continued to evolve and expand, therefore increasing the versatility and sensitivity required for analytical testing. To support an effective and efficient strategy for nitrosamine control, and stay ahead of future regulatory changes, it is essential to implement high–performance technologies and flexible analytical workflows that support assays and exceed today's regulatory thresholds. Keeping up with advancements in technology and staying up to date with regulatory changes will allow laboratories to prepare for future requirements and support the ongoing supply of safe and effective pharmaceuticals.

Conclusion

The analysis of nitrosamines with LC/MS–MS plays a crucial role in pharmaceutical manufacturing. By developing robust methods, optimizing sensitivity, implementing effective sample preparation techniques, and staying ahead of regulatory requirements, laboratories can position themselves as trusted partners in the control of nitrosamines. In particular with respect to low-level contaminants, automated sample preparation procedures can further improve inter-assay

and inter-laboratory reliability by reducing external contamination, improving assay precision, and improving reproducibility.

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Unveiling The Silent Killer: Comparative Case Study On Thallium Poisoning

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Abstract

"Unveiling The Silent Killer: Comparative Case Studies on Thallium Poisoning" seeks to explore the dark domain of silent killers with particular emphasis on the use of thallium: a lethal and little-known poison. Through a comparative case study, this present study seeks to explore the psychological, sociological, and forensic aspects of thallium poisoning in silent killers. Thallium, also referred to as the 'poisoner's poison' as it is colorless, tasteless and odorless, makes it difficult to detect, and even more challenging to pinpoint the perpetrator, making it a killer's delight. The cases further explored in this study involve some of the most famous and notorious cases from different geographical and cultural backgrounds and describe the selection of victims, methods of poisoning and personal characteristics of the criminals. Using Forensic reports analysis, criminal case files, and psychological profiles this research work establishes similarities and differences between the Thallium-using silent killers. Some of the most enlightening results show that most of thallium poisoners are intelligent people with excellent planning skills, which help them to take their time with their lethal activity and make the cause of death less recognizable. The work also describes the presence of thallium in modern life, the features of its identification with the help of modern methods of forensic toxicology, and underlines the importance of advanced training among medical and law enforcement staff. Through comparison of such cases, this study reveals general trends of how thallium poisoning can be used in criminal investigations, as well as strategies for prevention and early identification. Besides expanding the knowledge of thallium as a method of

murder, the comparative analysis helps to advance the discussion of the classification of silent killers and the further development of forensic studies.

Keywords: Silent killer, Thallium poisoning, Forensic studies, Criminal investigation, Comparative case study.

Introduction:

Thallium frequently referred to as the 'poisoner's poison,' is the composite that has drawn a lot of attention of both forensic scientists and criminologists because of its ability to vanish overnight and its usage in some of the most heinous crimes. This study proposed to analyze the various facets of the issue in Thallium poisoning through the use of comparative silent killer case studies. This paper aims at providing insights into the modalities of the thallium toxic substance use in criminal investigations by analyzing psychological characteristics, sociological effects,and crime scene findings.

Hypothesis:

Thallium poisoning is a slow-acting, non-volatile poison that fits the profile of those who use it as they are intelligent, psychologically and sociologically motivated, and careful in their planning. This analysis presupposes that aforementioned characteristics allow thallium poisoners to remain undetected and continue their toxic actions for a longer period, causing difficulties to forensic scientists and making it difficult to identify the offender. Furthermore, the hypothesis avers that improvements in various analyses such as forensic toxicological studies along with enhanced mastery among medical and law enforcement officials are essential in identifying, preventing, and particularly prosecuting thallium poisoning cases. To this end, this research will use comparative case analysis in order to establish patterns and methods associated with the use of Thallium in criminal activities that may assist in the differentiation and classification of silent killers in the field of forensics.

Objectives:

Explore Psychological and Sociological Aspects: Concerning the mental make-up of thallium poisoners, look at the role of intelligence and planning in their schemes and how sociological features enhance their capacity to go undetected the longer they can continue their murderous sprees.

- Analyze Forensic Aspects: Thorough comparative evaluation of forensic reports and criminal case files concerning multiple thallium poisoning incidents with special reference to the approaches used to administer the poison, the choice of target persons, and the offender profile. When comparing these aspects, make sure to distinguish between the differences and similarities between geographical and cultural contexts.
- Establish Similarities and Differences: Given the nature of comparative case studies, one can identify key attributes of the thallium-using silent killers in terms of similarities and differences in terms of strategies used by the perpetrators, their motives and specific methods.
- Highlight Modern Forensic Techniques: Explain new developments in forensic toxicology which can help in the recognition and identification of thallium poisoning pointing to the necessity to provide more personalized and enhanced training for the staff of the medical and police services.
- Expand Knowledge of Thallium Poisoning: Elaborate existing knowledge on, as well as the historical background of using, the chemical thallium as a means of murder, both in the past and the present, the ways of its administration, and difficulties in their investigation and prosecution.
- Contribute to Prevention Strategies: Suggest measures of controlling such occurrences of Thallium poisoning as informed by comparative study of related cases, and forensic results. Describe screening practices that existed in the past concerning this poison and further clarify ways that can minimize effects of thallium poison.
- Advance Classification of Silent Killers: Embrace the academic niches of the forensic studies as one by expanding the classification and analysis of the ‘silent killers’ by addressing the peculiarities of thallium as a modus operandi. Explain how the features that have been identified for the thallium poisoners relate to various categories of criminality and intent.

Methodology:

The research used comparative case study to assess forensic reports, criminal case files, as well as psychological analyzes. The cases chosen for the comparison cover a wide range of geographical and cultural settings, allowing for diverse data collection. Forensic toxicology techniques of the present time are employed to assess the detection of thallium and researchers also conduct interviews with police personnel and healthcare providers.

Case study analysis:

1. Zhu Ling Case with Thallium Poisoning:

- Background:

  Zhu Ling was freshman of chemistry at Tsinghua University Beijing. She became victim in a famous poisoning case back in the year 1995. Zhu Ling's thallium poisoning led to great public uproar. There was long-time investigation. This was due to the acute symptoms appearing on her body and the unknown reasons for the poisoning.

- Toxicology Analysis:

  The poison identified in Zhu Ling's case was thallium. This is a highly toxic heavy metal. Thallium poisoning may cause severe, at times fatal damage to the nervous. It also affects gastrointestinal and cardiovascular systems. The toxicology analysis proved a high level of thallium in Zhu Ling's body. This ruled out accidental poisoning.

- Autopsy Findings:

  An autopsy was carried out on Zhu Ling. This was instrumental in understanding effects of thallium poisoning. Some of key findings were:

  1. Neurological System: Zhu Ling showed very acute neurological symptoms. These included peripheral neuropathy, numbness and loss of motor function.

  2. Gastrointestinal Tract: There is evidence of severe gastroenteritis with mucosal erosion. There was also hemorrhage. This would point towards ingestion of toxin.

3. Cardiovascular System: Cardiac damage and dysfunctions were consistent with systemic toxicity due to thallium.

4. Histopathological Examination: Extensive damage in vital organs like liver and kidneys have supported diagnosis of thallium poisoning. These autopsy findings correlated. The toxicological analysis was carried out and identified thallium as causative factor in Zhu Ling's illness and death.

- Criminal Investigation: Throughout criminal investigation of Zhu Ling poisoning case far-reaching investigations were initiated. These spanned various facets.

1. Initial Reaction: At very onset of sudden and acute illness, the authorities' first reaction to Zhu Ling was to treat him medically. However he continued deteriorating.

2. Outrage from Society: Publicized in media. Accompanied by an outpouring from society, the authorities were forced to launch a serious investigation.

3. Forensic Analysis: Use of state-of-the-art forensic tools like toxicological testing and environmental sampling could identify where thallium was coming from. It traced it back to Zhu Ling's environment.

4. Suspicion and Suspects. Suspicion was first cast upon people around Zhu Ling like acquaintances and colleagues. Therefore the investigation included study of motives. These involved personal disputes or professional rivalry.

5. Legal and Judicial Proceedings. The case initiated legal proceedings. But it couldn't, with all evidentiary limitations and complications of investigations identify and prosecute perpetrator.

- Aftermath. Extensive efforts have not resulted in definitive justice for victim in Zhu Ling case. Rather this case has continued to attract popular attention. It serves as representative investigating and prosecuting poisoning cases of extraordinary complexity, using uncommon toxins like thallium.

2. Kazuki Miyamoto Poisoning Case:

- Background:

On 15th April 2023 lifeless body of 32-year-old Kazuki Miyamoto from Tokyo was found in his apartment. He was a businessman. Miyamoto was a prolific gentleman within tech circles solving many interesting problems related to software development. Such sudden demise of this great mind sent shockwaves throughout community. It called for active investigation by law enforcers and forensic experts.

- Toxicology analysis:

  The toxicology report showed that there were several toxic substances in Miyamoto's blood. An extremely high level of cyanide was detected. Traces of arsenic and ricin also present. The presence of multiple poisons indicated poisoning, not accidental intoxication. Cyanide is a fast-acting lethal agent. It became the apparent cause of death. Its rapid inhibition of cellular respiration was identified.

- Autopsy findings:

  The autopsy performed on Miyamoto's body revealed considerable facts about his cause of death. The major findings were:

  1. Cardiovascular System: Diffuse tissue necrosis indicating severe hypoxia consistent with cyanide poisoning.

  2. Respiratory System: Pulmonary edema and congestion. Corroborative evidence for asphyxiation.

  3. Gastrointestinal Tract: Erosive gastritis and hemorrhage. Evidence supporting oral ingestion of toxins.

  4. Histopathological Examination: Cellular damage in heart liver, kidneys and other vital organs.

  The aggregation of these results justified toxicology reports identifying cyanide as primary killing factor. Arsenic and ricin were contributory factors. They exacerbated his condition.

- Criminal Investigation: Criminal investigation was led by establishing suspects and motives for poisoning Miyamoto. Some key factors of study are as follows:

1. Identification of Suspects: Business rivals and disgruntled former employees were some probable suspects. They appeared in radar of investigators. Surveillance footage and digital communications were examined. They were used to trace interactions. They helped find suspects.

2. Gathering Evidence: Gathering physical evidence such as food items and drinks consumed by Miyamoto in his apartment before he was murdered was collected for investigation. The status of his electronic gadgets were sought for any traces of threats. Suspicious communication that might have been given out to him was also investigated.

3. Interviews and Interrogations: The police questioned close associates. Family members and business associates were also interviewed. Several people were detained for interrogation. They were suspected because of circumstantial evidence.

4. Forensic Analysis: State-of-the-art forensic examination techniques were employed. DNA analysis and matching fingerprints at scene of crime with suspects were used.

3. Japanese Girl Who Poisoned Her Mother with Thallium: Inspired by Graham Young:

- Background:

It was one of those eerie cases as recent as in Yokohama, Japan 2023. A 16-year-old girl named Hana Yamamoto poisoned her mother. The victim, 42-year-old Keiko Yamamoto is a community worker of repute. She showed extreme deterioration in health without any known cause. She was hospitalized. Nevertheless, she eventually died from poisoning. Despite intensive care from a battery of doctors. It later emerged that she was inspired by notorious British poisoner Graham Young. He came to be known as "Teacup Poisoner."

- Toxicology Report:

The toxicology report identified lethal levels of thallium in Keiko Yamamoto's bloodstream. Thallium is acutely toxic metal. It is hazardous to neurological and systemic integrity. The concentration found was very high. It pointed to deliberate and continuous poisoning.

- Autopsy Findings:

The autopsy of Keiko Yamamoto gave full details on the effects of thallium poisoning. The main findings were as follows:

1. Neurological System: The subject is suffering from diffuse peripheral neuropathy. It is characterized by numbness. Paresthesia and loss of motor function observed in chronic thallium poisoning.

2. Gastrointestinal Tract: Patient suffers from severe gastroenteritis. Mucosal erosion and hemorrhage evidencing prolonged exposure to toxin.

3. Cardiovascular System: Cardiac tissue shows damage with degeneration. Necrosis also supporting systemic toxicity.

4. Histopathological Examination: Multi-organ failure. Considerable damage to liver and kidneys thus further supporting a diagnosis of chronic thallium poisoning.

These findings supported toxicology results. Thallium was established as cause of death. Indicated Keiko's exposure to poison was long-term.

- Criminal Investigation: Criminal investigation revolved around origin of thallium. Key aspects included:

1. Identification of Suspect: As this was long-term poisoning. Investigators suspected someone close to Keiko. Hana Yamamoto was pinned as the main suspect. She easily accessed her mother. Strange behavior was reported.

2. Collection of Evidence: This searching operation conducted around the Yamamoto residence revealed thallium compounds. These were actually very well hidden in Hana's room. A journal found depicted the fascination of Graham Young with Hana. She had jotted down plans regarding how to carry out his methods.

3. Interviews and Interrogations: Interviews among family members, friends, even school personnel were conducted. These revealed she had strained relationship with her mother. On interrogation Hana confessed. She claimed that she highly admired Graham Young who was

poisoner and also committed other various crimes. Therefore, she wanted to emulate his poisoning techniques.

4. Motive and Psychological Evaluation: As in case of Hana the motive was arrived at owing to combination of fascination with Graham Young, feelings of being neglected and some psychological problems. A psychological evaluation revealed some underlying mental health conditions. These compelled her to perform the act.

4. Delhi Man Poisons Wife and Her Family with Thallium:

- Background:

A heinous case came to light in Delhi India. This occurred in May 2024 when a man named Rahul Singh had allegedly poisoned his wife, Neha Singh and her family with thallium. Shortly she and her parents and younger brother started experiencing health problems. These problems developed within span some weeks. The unusual pattern of symptoms led to concern. The subsequent death raised suspicion. An in-depth investigation was promptly initiated.

- Toxicology Reports:
- Toxicology reports in case of Neha Singh her parents Rajiv and Sunita Kapoor and her brother Aman Kapoor showed the presence of thallium in their systems. Thallium is acutely toxic metal associated with severe neurological, gastrointestinal and systemic damage. Levels picked up were way above normal. This pointed to deliberate poisoning over a long period.
- Autopsy Findings:

The autopsy of Rajiv Kapoor elaborated on effects of thallium poisoning. Noted herein were important features of this case.

1. Neurological System: Diffuse peripheral neuropathy. Numbness paresthesia and motor dysfunction were all symptoms pointing towards chronic thallium poisoning.

2. Gastrointestinal Tract: Severe gastroenteritis with mucosal erosion. Hemorrhage represented prolonged ingestion of poison.

3. Cardiovascular System: Cardiac tissue exhibited degenerative changes. Necrotic changes were also noted. This was an indication of systemic toxicity from thallium.

4. Histopathological Examination: Evidence of damage to the liver. There was also marked damage to kidneys. This supported diagnosis of chronic thallium poisoning.

These results therefore, were in agreement. The toxicology findings indicated thallium was responsible for death. It also gave an indication of chronic exposure.

- Criminal Investigation: The criminal investigation aimed to establish the source of thallium and the identity of the perpetrator. Key aspects included:

1. Identification of Suspects: Given nature of poisoning investigations focused primarily on those close to the victims. Rahul Singh, husband of Neha became a prime suspect. There were marital problems between him and his wife. He also had access to family.

2. Gathering of Evidence: Traces of thallium were found in ordinary foods and beverages at Singh household. Additionally, history of online purchases of thallium was traced back to Rahul's account.

3. Interviews and Interrogations: Statements from family members, friends and neighbors painted picture of strained relationships. The relationship between Rahul and Neha was particularly strained. Also, inconsistencies in Rahul's statements during interrogation were noted. These coupled with deteriorating relationship with his in-laws, were enough to connect the dots.

4. Forensic Analysis: State-of-the-art forensic techniques such as fingerprint and DNA analyses have linked food and beverage contamination to Rahul. Digital forensics disclosed search history and purchase records related to thallium poisoning.

Investigations revealed that Rahul Singh had been administering thallium to his wife and her family for several months. He had well-built motives on basis of financial disputes and personal enmity. Rahul had meticulously planned poisoning. His intent was to kill his in-laws. He aimed to gain control over family assets through elimination.

5. Graham Young Case with Thallium:

- Background:

  Graham Young was one of most famous British serial killers nicknamed as the "Teacup Poisoner." He conducted a spree of thallium poisonings in the 1960' s. His obsession with toxic substances and poisoning dates back to when he was teenager He began killing and poisoning people. This turned out to be a shock. It was unexpected for the entire nation.

- Toxicology Analysis:

  Thallium is an extremely toxic metal. Graham Young mainly used it in his crimes. Thallium is capable of causing serious neurological, gastrointestinal and systemic damage. Toxicology analysis in Graham Young's case revealed lethal levels of thallium in his victims' systems. This ruled out poisoning by accidental intake.

- Autopsy Findings:

  Autopsies performed on some of Graham Young's victims furnished important clues effects of thallium poisoning. Some of the significant autopsy findings included:

  1. Neurological System: Diffuse peripheral neuropathy was seen in the victims. They presented with numbness. Additionally tingling sensations and loss of muscle strength were observed.

  2. Gastrointestinal Tract: Severe gastroenteritis was observed. Mucosal erosion and hemorrhage which proved that toxin was ingested.

  3. Cardiovascular System: Cardiac tissue appeared degenerated. Necrotic, thus pointing toward systemic toxicity due to thallium.

  4. Histopathological Examination: Marked damage to liver and kidneys favored diagnosis as thallium poisoning.

  These autopsy findings combined with toxicological results, definitely identified thallium as agent of death in Graham Young's victims.

- Criminal Investigation: The investigation of crimes committed by Graham Young was a long-drawn process. Important features were:

1. Initial Suspicion: The investigating authorities initially suspected death either due to natural causes or accidents. Unusual symptoms were presented by victims.

2. Pattern recognition: Over time a pattern of illness from people related to Graham Young emerged that led to further investigation.

3. Forensic Analysis: Number of advanced forensic techniques were applied. Amongst which toxicological analysis. Examinations performed at post-mortems which led to identification of poison used as thallium. Its forensic linkage to Graham Young.

4. Arrest and Trial: Graham Young was eventually arrested. Put on trial for murder and attempted murder. The evidence presented during trial, stemming from toxicology reports autopsy findings and witness testimonies. Was very telling of his meticulous planning and manipulation.

Result:

A comparative analysis presented shows that there is a remarkable similarity in behaviour among people who used thallium to poison others. The majority of them are smart people who pay much attention to the planning process. Their strategies enable them to conduct their deadly operations continuously, therefore unlikely to be easily detected. The study also outlines how victims are chosen and the various ways that poison is administered by the perpetrator, information that will greatly assist forensic investigators.

Discussion:

The studies therefore call for enhanced forensic toxicology training for medical and law enforcement experts. Lack of efficient methods of early identification of consumption of thallium are the main reasons for increased cases of poisoning. The study further seeks to unravel the psychological and sociological reasons that encourage people to use thallium to poison their victims.

Conclusion:

This work contributes to the body of knowledge in forensic science by offering a detailed investigation of thallium poisoning. The use of comparative case study research does not only

increase the understanding of thallium as the tool for murder, but also improves the categorization and analysis of the silent death. These insights support enhanced forensic skills and raised awareness of schemes for early identification with the view of enhancing efficiency in criminal investigations and prevention.

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A review study of the persistence of Hexaconazole to control powdery mildew in grapes

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ABSTRACT

Grapes are a globally significant crop for making wines, raisins, and fresh fruit. They face fungal diseases like downy mildew, powdery mildew, gray mold, and anthracnose, leading to significant yield losses. To combat these diseases, farmers use pesticides, but their frequent use (above the threshold) has led to environmental and health risks. Therefore, rigorous regulatory frameworks are enforced to oversee pesticide residues, ensuring adherence to maximum residual limits (MRLs), estimating pre-harvest interval (PHI), and safeguarding human health[9] This study reviews the persistence of Hexaconazole in controlling powdery mildew in grape cultivation. Hexaconazole, a widely used fungicide, is crucial in managing this fungal disease, which can significantly impact grape yield and quality. Hexaconazole 5% SC at the recommended dosage of 1 ml/L effectively manages powdery mildew disease in grapes within a safe time interval (PHI). This interval ensures that pesticide residues of Hexaconazole remain below MRLs in grapes at harvest. This review evaluates various aspects, including application methods, dosage efficacy, and environmental factors influencing the persistence of Hexaconazole in grapes according to the guidelines of Good Agriculture Practices (GAP) of the World Health Organisation (WHO).

Keywords: Grapes; pesticide residues; powdery mildew; fungicide.

Introduction

Grapevine cultivation is a cornerstone of the global wine and table grape industries, contributing significantly to agricultural economies and cultural heritage. However, grapevines are susceptible to various diseases, among which powdery mildew (Erysiphe necator) is a predominant and challenging pathogen. This fungal disease can cause severe damage to grapevines, leading to reduced fruit quality and yield, and ultimately impacting the economic viability of vineyards.

Effective management of powdery mildew is crucial for maintaining grapevine health and ensuring high-quality grape production[13]. Among the diverse array of chemical and non-chemical control measures available, Hexaconazole, a systemic triazole fungicide, has emerged as a prominent option for controlling this pervasive disease. Hexaconazole functions by inhibiting the synthesis of ergosterol, a vital component of fungal cell membranes, thereby impairing fungal growth and reproduction.

Despite its proven efficacy, the persistence of Hexaconazole—defined as the duration over which it remains active and effective against the pathogen—plays a critical role in its overall success as a fungicide[11]. The effectiveness of Hexaconazole can be influenced by several factors, including its degradation rate in different environmental conditions, its absorption and mobility within grapevines, and its interaction with other elements of the vineyard ecosystem.

The objective of this review is to provide a comprehensive analysis of the persistence of Hexaconazole in the context of powdery mildew management in grapevines. This includes examining its mode of action, factors affecting its persistence, and its overall impact on disease control. By synthesizing data from various studies and field trials, this review aims to offer valuable insights into optimizing Hexaconazole use, enhancing disease management strategies, and addressing potential environmental and healthconcerns associated with its application[8].

Understanding the persistence of Hexaconazole is essential for developing effective and sustainable pest management practices. This review will explore the current knowledge on Hexaconazole's

persistence, evaluate its performance in different conditions, and discuss its implications for integrated pest management (IPM) in viticulture.

Mode of Action

Hexaconazole is a systemic fungicide belonging to the triazole class, known for its effectiveness in managing a range of fungal diseases, including powdery mildew (*Erysiphe necator*) in grapevines. Its mode of action is crucial to understanding how it controls powdery mildew and contributes to its persistence in the vineyard environment.

Inhibition of Ergosterol Biosynthesis

Hexaconazole exerts its antifungal effects primarily through the inhibition of ergosterol biosynthesis[9]. Ergosterol is a critical component of fungal cell membranes, playing a role analogous to cholesterol in animal cells. It is essential for maintaining membrane integrity, fluidity, and function.

Target Enzyme: Hexaconazole specifically inhibits the enzyme lanosterol demethylase (CYP51), which is a key enzyme in the ergosterol biosynthesis pathway. By blocking this enzyme, Hexaconazole prevents the conversion of lanosterol to ergosterol.

Disruption of Membrane Function: The inhibition of ergosterol production leads to the accumulation of toxic sterol intermediates and results in a compromised fungal cell membrane. This disruption affects membrane permeability, leading to leakage of cellular contents, impaired nutrient uptake, and ultimately, cell death.

Systemic Activity

Hexaconazole is a systemic fungicide, meaning it is absorbed by the grapevine and translocated throughout its tissues[7]. This systemic nature allows it to provide comprehensive protection against powdery mildew by:

Uptake and Translocation: After application, Hexaconazole is absorbed into the plant tissues, including leaves, stems, and fruits. Its ability to move within the plant ensures that even newly developing tissues receive protection against fungal infection.

Prolonged Efficacy: The systemic properties contribute to the persistence of Hexaconazole in the plant, allowing it to remain effective for a period after application, as it continues to inhibit fungal growth and reproduction.

Impact on Powdery Mildew

Prevention of Sporulation: By disrupting the fungal cell membrane, Hexaconazole inhibits the formation and release of new fungal spores. This reduces the spread of the disease within the vineyard.

Control of Established Infections: Hexaconazole's systemic nature helps control established infections by reaching and acting on fungi residing within plant tissues, not just those on the surface.

Considerations for Persistence

The effectiveness and persistence of Hexaconazole are influenced by several factors:

Environmental Conditions: Temperature, humidity, and UV exposure can affect the degradation rate of Hexaconazole. Under harsh conditions, its persistence may be reduced.

Temperature: Optimal residual activity occurs between 15°C and 25°C.

Humidity: Moderate humidity enhances residual activity.

Rainfall: Heavy rainfall can reduce residual activity.

Solar Radiation: High solar radiation can degrade hexaconazole, reducing residual activity.

Application Practices: Proper application rates and timing are crucial for maximizing Hexaconazole's effectiveness and ensuring that it remains active long enough to control powdery mildew. Understanding Hexaconazole's mode of action helps in optimizing its use for effective powdery mildew management, ensuring that it continues to provide robust protection while managing potential environmental and health impacts.

Persistence and Degradation

Persistence:

Hexaconazole remains active against powdery mildew for several days to weeks after application. Systemic properties allow it to persist in plant tissues, providing prolonged protection. Persistence influenced by factors such as - Environmental conditions (temperature, humidity, UV exposure), Application practices (rates, timing, and frequency)

Degradation:

Hexaconazole degrades gradually in the environment, primarily through - Photolysis (UV light breakdown), Hydrolysis (water-based breakdown), Microbial degradation (breakdown by microorganisms), Degradation rate influenced by factors such as – Temperature, pH, Soil type and moisture, Microbial activity, Half-life (time for 50% degradation) - Reported half-life ranges from 10-100 days, depending on conditions, Soil half-life: 10-30 days, Plant half-life: 3-14 days, Understanding persistence and degradation is crucial for optimizing Hexaconazole application and minimizing environmental impact.

Efficacy in Disease Control

Hexaconazole has established itself as a highly effective tool for managing powdery mildew (Erysiphe necator) in grapevines. Its efficacy is influenced by its chemical properties, systemic

nature, and application practices[4]. This section reviews the performance of Hexaconazole in controlling powdery mildew, based on various studies and field trials[10].

Field Trials and Studies

Numerous field trials have demonstrated Hexaconazole's effectiveness against powdery mildew in grapes. Key findings include:

Reduction in Disease Incidence and Severity: Hexaconazole significantly reduces both the incidence and severity of powdery mildew. Field studies have reported substantial decreases in fungal colony counts and visible symptoms on grapevine leaves and clusters when Hexaconazole is applied according to recommended schedules.

Comparative Efficacy: In comparative studies, Hexaconazole often performs comparably to or better than other fungicides. For instance, it has shown superior results when compared to older triazoles or non-systemic fungicides, particularly in integrated pest management (IPM) systems that use a combination of chemical and non-chemical control methods.

Impact on Powdery Mildew Management

Prevention of Disease Spread: Hexaconazole's ability to inhibit sporulation helps in limiting the spread of powdery mildew within the vineyard. By preventing the release of new spores, it reduces the potential for secondary infections.

Control of Established Infections: Its systemic action allows Hexaconazole to manage both early and late stages of powdery mildew infections. This makes it effective not only as a preventative measure but also for controlling infections that have already established themselves.

Application Timing and Frequency

Optimal Timing: Applying Hexaconazole early in the disease cycle is crucial for maximizing its efficacy. Early applications help prevent initial infection and reduce the potential for the disease to take hold.

Application Frequency: The persistence of Hexaconazole means that fewer applications may be needed compared to some other fungicides. However, adherence to recommended application intervals is important to maintain control and manage resistance.

Resistance Management

Resistance Risk: While Hexaconazole is effective, there is a risk of resistance development if it is overused or relied upon exclusively. Studies have shown that rotating Hexaconazole with fungicides from different chemical classes can help mitigate resistance.

Integrated Pest Management (IPM): Combining Hexaconazole with non-chemical control methods, such as cultural practices and biological control agents, can enhance overall disease management and reduce reliance on any single treatment.

Environmental and Health Considerations

Residue Management: Hexaconazole's efficacy is balanced by the need to manage its residues. Ensuring that Hexaconazole levels remain within regulatory limits is important for both environmental safety and consumer health.

Impact on Non-Target Organisms: While effective against powdery mildew, Hexaconazole may affect non-target organisms. Integrated use with other pest management strategies helps in minimizing adverse effects on beneficial species.

Conclusion

Hexaconazole is a powerful fungicide for controlling powdery mildew in grapes, with proven efficacy in reducing disease incidence and severity[10]. Its use involves several environmental and health considerations. Hexaconazole's persistence is influenced by environmental factors such as temperature, humidity, and UV exposure. High temperatures and intense UV radiation can accelerate its degradation, reducing its effective period of action. In soil, Hexaconazole can persist for varying durations depending on soil type, pH, and microbial activity. Its movement in soil and potential runoff into water sources can impact local ecosystems. Measures to minimize runoff and adhere to application guidelines are essential to reduce environmental impact. Hexaconazole is classified as having moderate toxicity. Exposure to high levels, either through direct contact or inhalation, can pose health risks. Therefore, monitoring Hexaconazole residue levels in grapes is critical to ensure they remain within the maximum residue limits (MRLs) set by regulatory authorities[10]. Its systemic properties, combined with appropriate application practices and resistance management strategies, contribute to its success in disease control. Integrating Hexaconazole into a broader IPM strategy and adhering to best practices will help optimize its benefits while mitigating potential risks.

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Assessment of Antimicrobial Activity of Secondary Metabolites from Soil-Derived *Bacillus spp.*

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ABSTRACT

This study explores the antimicrobial properties of secondary metabolites produced by *Bacillus spp*. isolated from soil. The metabolites were extracted using chloroform, ethanol, and ethyl acetate via solvent extraction. The antimicrobial efficacy of these crude extracts in the range of (2-10 µg/ml) was tested against *Enterococcus faecium, Streptococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species, Candida albicans, and Candida krusei* using the agar well diffusion method. The ethanol extract exhibited maximum 100% inhibition against *Pseudomonas aeruginosa and Enterobacter species*, while all extracts showed varying degrees of inhibition against the other microorganisms. Bio-autography was used to detect the bioactive compounds accountable for the observed antimicrobial activity. These findings highlight the potential of secondary metabolites from *Bacillus spp*. as sources for new antimicrobial agents, underscoring their relevance in combating resistant pathogens.

Key words: *Bacillus spp*., Secondary metabolites, Antimicrobial activity, Bioactive compounds, Bio-autography.

INTRODUCTION

The large microbial community in soil is sustained by a vast array of organic matter present on Earth. Most of these microorganisms are bioactive and thrive in the top few inches of agricultural

soils(Foster & Woodruff, 2010). The presence of microbes in soil depends on various ambient conditions, including vegetation types, soil texture and chemical composition, nutrient availability, pH, moisture content, climate, and temperature(Pathak & Gopal, 2008). Over the past decades, numerous bacteria that produce diverse primary and secondary metabolites, enzymes, antibiotics, and novel compounds have been isolated, identified, and utilized by research groups in human health care, agriculture, and animal husbandry. These compounds unique structures make microorganisms an essential source of secondary metabolites. Secondary metabolites, in particular, act as antimicrobial agents against pathogenic bacteria. While antibiotics are not necessary for bacterial growth, they aid in bacterial survival(Demain & Fang, 2000). The bacterial community remains a major source of antibiotic production, widely used in human health care. Each year, nearly 500 new antibiotics are discovered, with most derived from soil bacteria(Hassan et al., 2014; Molinari, 2009). Many of the antibiotics currently in use are produced by a small group of microorganisms belonging to the genera *Penicillium, Streptomyces, Cephalosporium, Micromonospora,* and *Bacillus* (Sethi et al., 2013). Members of the *Bacillus* species typically produce polypeptide-type bacteriocins, which are generally effective against several Gram-positive bacteria(Huck et al., 1991; Marahier et al., 1993; Prashanthi et al., 2021).

Members of the *Bacillus* genus are commonly found in soil, and many of these bacteria exhibit proteolytic activity, enabling them to break down proteins. Protease enzymes from these microorganisms are not only valuable for industrial applications but also play a crucial role in the nitrogen cycle, enhancing soil fertility. Numerous bacteria have the potential to produce antibiotics, such as *Bacillus* species, which generate antibiotics like bacitracin, pumulin, and gramicidin, effective against Gram-positive bacteria like *Staphylococci, Streptococci,* and *Corynebacterium*. Additionally, *Streptomyces* species produce antibiotics like tetracycline, chloramphenicol, vancomycin, and gentamicin, which are active against Gram-negative bacteria. Antibiotic resistance, a specific form of drug resistance, typically arises through natural selection acting on random

mutations, but it can also be induced by applying evolutionary stress to a population(Prashanthi et al., 2021).

The novelty and broad applications of bacterial-derived compounds hold potential solutions for many challenging diseases caused by resistant microorganisms, which are currently difficult to treat. Due to limited knowledge, bacterial secondary metabolites are of particular interest. Therefore, the present study aimed to isolate and identify *Bacillus* strains from soil and evaluate their antibacterial potential against various microorganisms.

MATERIALS AND METHODS

Isolation and Identification of bacteria from soil

The bacterial soil sample was collected from the industrial area at Satpur MIDC, Nashik. Using a sterile spatula, soil was extracted from a depth of 5–10 cm and placed into a pre-sterilized test tube. The sample was transported to the laboratory at room temperature. In the lab, the soil was air-dried by heating it at 50°C for 1 hour in a dryer. Subsequently, 1 gram of the dried soil was mixed with 9 mL of sterile saline(Amin et al., 2015).

To isolate *Bacillus* species, a serial dilution technique was employed using phosphate-buffered saline (PBS, pH 7.2) to prepare dilutions ranging from 10^{-1} to 10^{-6}. Samples from each dilution were streaked onto nutrient agar (NA) plates supplemented with cycloheximide (100 µg/mL) to inhibit fungal growth, and the plates were incubated at 37°C for 24 hours. Following incubation, the plates were examined, and colonies that appeared to be *Bacillus* were subjected to Gram staining. Gram-positive, rod-shaped, spore-forming bacilli were selected for further biochemical identification tests(Foysal & Khanam, 2018).

Secondary Metabolites Production & Extraction

18hr old culture was harvested using sterile 0.85% normal saline solution. 2mL of the culture was inoculated into 100mL of sterile nutrient broth in a flask. The flask was then incubated and shaken

in an orbital incubator at 37°C and 160 rpm for seven days. For solvent extraction, three different solvents—ethanol, chloroform, and ethyl acetate—were used in a 1:1 ratio with the broth. The solvent layers were extracted using a separating funnel. The collected layers were then centrifuged at 5000 rpm for 15 minutes. After centrifugation, the layers were removed and transferred to clean, dry flasks. The extracts then were dried using a rotary evaporator at 50°C. The resulting extract was dissolved in DMSO for antimicrobial susceptibility testing(Singh et al., 2018).

Innoculum preparation

The strains of ESKAPE were procured from ATCC (American type culture collection) S. aureus MTCC 96, *K. pneumoniae*-ATCC 35657, *A. baumannii*- ATCC 19606, and *P. aeruginosa* -ATCC 27853; MTCC (Microbial type culture collection) *S. aureus*- MTCC 1430, *Enterobacte*r sp.- MCC 2296; MCC (Microbial culture collection) *E. faecium*-MCC 2763*, Candida albicans* - MTCC 3017*, and Candida krusei* – ATCC 14243 were sub cultured on non selective nutrient agar slants and were incubated overnight at 37 °C. 0.5 McFarland density of bacterial isolates was adjusted in normal saline (0.85% NaCl) using densitometer to get bacterial cell concentration of $1.0x10^8$ cfu/ml.

Antibacterial Potential screening of metabolites against test organisms

The antimicrobial activity of cell-free crude secondary metabolite extracts in ethanol, chloroform, and ethyl acetate was assessed using the agar well diffusion method. To begin, 100 µl of each adjusted microbial culture was incorporated into separate 100 ml aliquots of sterile molten Muller Hinton Agar. After thorough mixing, the agar was poured into sterile Petri dishes and allowed to solidify. Once solidified, each plate was labeled according to the specific culture. Using a sterile cork borer, wells with a diameter of 6 mm were created at various locations on the plates. Subsequently, 100 µl of the 2-10 µl/ml concentration of crude extract were added to the wells. The plates were then incubated overnight at 37°C. After incubation, the plates were examined and the inhibition zones were measured(Singh et al., 2018).

Bio-autography method

In this technique, aluminum TLC silica plates were utilized. The effective crude extracts were spotted onto the plates and then placed in a solvent of chloroform: ethyl acetate : butanol: glacial acetic acid (25:15:2:10) for chloroform crude extracts and for ethanolic extract ethanol: ethyl acetate (7:3) were used for development. After the development, the TLC plates were transferred to Petri dishes, which were then overlaid with molten Luria-Bertani agar seeded with bacterial cultures. The plates were incubated at 37°C for 24 hours. Following incubation, the TLC plates were sprayed with a 2.5 mg/mL solution of *p*-iodonitrotetrazolium violet and incubated again for 3-4 hours at 37°C. Results were observed based on the presence of clear zones against a red background(Cam & Traditional, 2010).

RESULTS

From the collected soil sample, *Bacillus spp*. was successfully isolated and identified by morphological and biochemical characterization according to Bergey's manual for the genus specificity. Secondary metabolites were extracted from *Bacillus spp*. By solvent extraction method and further checked for antimicrobial susceptibility activity against *Enterococcus faecium, Streptococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species, Candida albicans, and Candida krusei* by agar well diffusion method. Table 1. represents the zone of inhibition in milliliter (mm) of metabolite crude extracts against test microorganisms. Furthermore, fig 2. shows bioautography for the detected compound showing antagonistic effect.

DISCUSSION

Bacillus isolates produce an extensive range of secondary metabolites, such as lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, lipoamides, and isocoumarins (Hamdache et al., 2011). These metabolites, derived from complex biosynthetic pathways, exhibit a wide array of biological activities, including antimicrobial, anticancer, antialgal, and antiperonosporomycetal

properties (Saw, 2011). The diverse bioactive secondary metabolites from *Bacillus* isolates, often featuring novel mechanisms of action, offer significant potential for developing effective and environmentally friendly strategies to manage pathogens affecting humans, animals, and plants (Stein et al., 2005). The impressive chemistry and biological activities of these metabolites present opportunities for creating new drugs, agrochemicals, carotenoids, and bioremediation tools for heavy metal pollution. The frequent occurrence of *Bacillus* species in nature and their capacity to produce a wide variety of antibiotics highlight their potential for commercial applications.

CONCLUSION

In this study, soil bacteria *Bacillus spp*. with antimicrobial activity was successfully isolated. Secondary metabolites extracted from *Bacillus spp*. were tested against *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa, Enterobacter species, Candida albicans & Candida Krusei* and it showed strong antagonistic properties against all test organisms. The ethanol crude extract of secondary metabolites demonstrated superior antimicrobial activity against Gram-negative organisms compared to chloroform and ethyl acetate extracts. Further purification and standardization are required to compare these extracts with current antibiotics. Secondary metabolites from *Bacillus spp.* isolated from soil present promising candidates for future antibacterial research.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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Table 1. Antimicrobial activity of crude extracts obtained from broth fermentation of *Bacillus spp.*

Metabolites	Concentration µg/ml	*E.faecium*	*S.Aureus*	*K.pneumoniae*	*A.baumannii*	*P.aeruginosa*	*E.cloacae*	*C. albicans*	*C. krusei*
Chloroform extract	2	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	27mm	-	-
	6	12mm	12mm	16mm	15mm	16mm	30mm	25mm	32mm
	8	14mm	15mm	16mm	16mm	16mm	30mm	34mm	36mm
	10	14mm	15mm	16mm	16mm	19mm	30mm	100%	100%
Ethanol extract	2	16mm	22mm	25mm	17mm	100%	100%	20mm	23mm
	4	18mm	26mm	25mm	17mm	100%	100%	35mm	26mm
	6	26mm	26mm	26mm	32mm	100%	100%	39mm	39mm
	8	26mm	26mm	31mm	34mm	100%	100%	40mm	39mm
	10	34mm	26mm	31mm	35mm	100%	100%	100%	100%
Ethyl acetate extract	2	-	-	-	-	-	-	-	-
	4	-	-	20mm	-	-	20mm	-	-
	6	-	11mm	23mm	-	-	24mm	19mm	20mm
	8	29mm	25mm	26mm	29mm	36mm	24mm	22mm	24mm
	10	29mm	25mm	26mm	29mm	36mm	28mm	24mm	26mm

*Zone of inhibition in mm including well diameter 6.0mm

Figure Legends

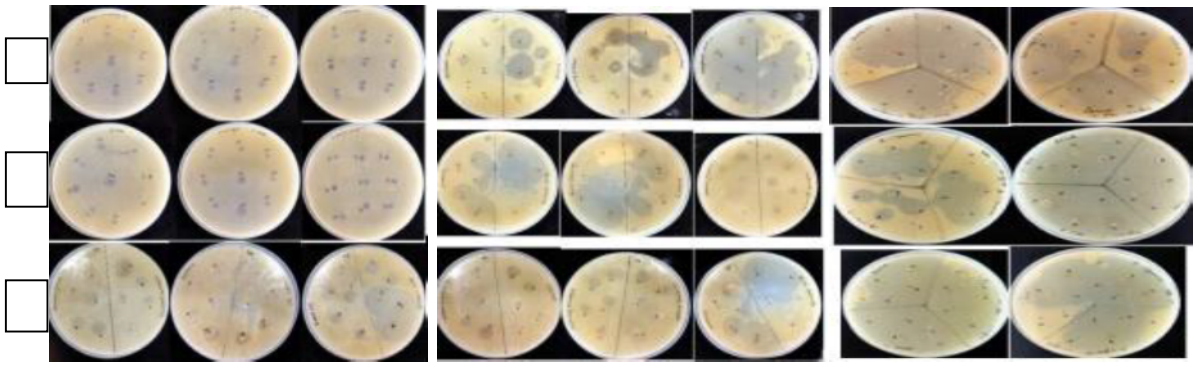
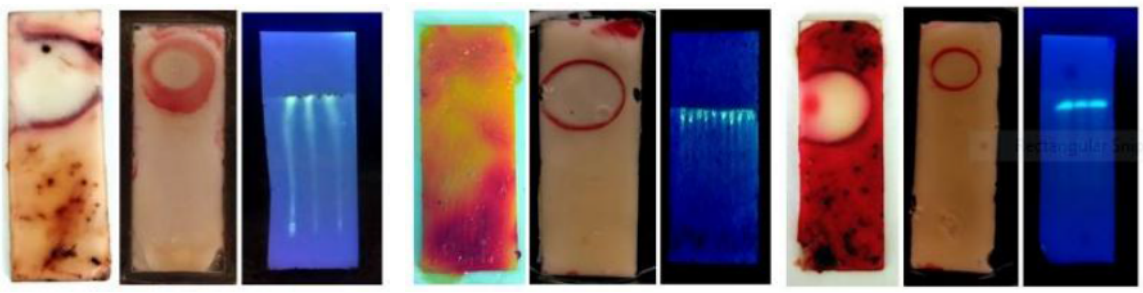


Fig 1. Antimicrobial activity of crude metabolite extract with varying concentration of 2-10µg/ml against *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa, Enterobacter species, Candida albicans & Candida Krusei.*

[A] – Chloroform Extract; [B] – Ethanol Extract; [C] – Ethyl acetate Extract.



A B C

Fig 2.Bioautography and TLC run of crude secondary metabolites extracted from *Bacillus spp*.

[A] – Chloroform Extract; [B] – Ethanol Extract; [C] – Ethyl acetate Extract.

Abbreviations:

TLC, Thin Layer Chromatography; mm, milliliter; spp, species; ATCC, American type culture collection; MTCC, Microbial type culture collection ; ESKAPE, *Enterococcus faecium, Streptococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species;* rpm, round per minute; NA, Nutrient Agar; PBS, Phosphate Buffered Saline; DMSO, Dimethyl sulfoxide.

A Review on Application of Analytical Chemistry in Forensic Science.

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Abstract

Forensic analytical chemistry has two main purposes, namely identification and comparison. By-products of the identification process are databases comprising analytical results, qualitative and, where possible, quantitative. The importance of analytical databases in forensic science cannot be emphasized enough, because they will influence the significance placed upon a match between control and suspect samples. In a world where mass production has largely taken over from small, independent manufacturers of, for example, materials such as paint and glass, stringent quality control means little variation in chemical composition from batch to batch. As a consequence, the search for, and identification of, new, more sensitive chemical discriminators, has become important. Analytical Chemistry is one of the integral part of forensic science. It includes use of gas chromatography (GC), mass spectrometry (MS), high performance liquid chromatography (HPLC), thin layer chromatography (TLC), atomic absorption/atomic emission (AA/AE), inductively coupled plasma emission and mass spectrometry (ICP/MS), scanning electron microscopy (SEM), Fourier transform infrared spectrometry (FTIR), ultraviolet/visible spectrometry (UV/Vis), and electrophoresis. Many investigations in forensic sciences does not complete without analytical chemistry.

Keywords: Analytical Chemistry, Forensic Science, Analytical Techniques, Analytical database.

Introduction

The field of forensic science is defined as the 'application of science for purpose of justice.' Seeking truth in a legal proceeding relies heavily on the objective and sophisticated forensic analysis to serve justice as the chronology of events can be reconstructed from the evidence that is analyzed. Hence, analytical chemistry is a useful avenue to provide the qualitative and quantitative identification of an unknown sample in various forensic science disciplines through various techniques.

Analytical Chemistry in Forensic Toxicology

Forensic toxicology studies the effects of poisons and drugs on the human body. Common cases are drinking under the influence, wildlife poisoning, and the manner of death. Analytical chemistry is useful to interpret the effects and quantify the concentration of the chemicals present in biological specimens to provide reliable data. Hair, nails, urine, blood, and brain tissue are useful biological specimens for forensic toxicologists to draw interpretations of various cases. Analytes are to be extracted from these biological specimens to be identified by different instruments. GC-QMS is an efficient instrument to quantify and identify the chemical components present in blood and urine from the analytes extracted.

The concentration of the analyte can be measured by the method of internal standard and a calibration curve. While screening for specific substances can be done by observing the common ions that exist in the compounds collected.LC-MS is another common instrument used in drug-testing laboratories by analyzing the analytes in liquids, such as the presence of cocaine in oral fluid and risperidone in plasma. The instrument relies on the separation of compounds in the stationary and mobile phases to identify individual components in complex biological sample. **Materials and Methods**

The main types of drugs of abuse likely to be encountered by a forensic drugs analyst are: cannabis, amphetamines, benzodiazepines, heroin, and cocaine. They appear in sub- gram

quantities as so-called ‘‘street seizures’’ in the possession of individual users, in larger amounts in the hands of local drug dealers, and in kilogram quantities as imported drugs (mainly cannabis, heroin and cocaine). With regard to their analysis, the forensic scientist’s main tasks are to (a) determine whether or not a controlled substance is present, (b) determine how much of the substance is present, and (c) determine, on occasion, the relationship of drug samples to each other through comparison or ‘‘profiling’’.Cannabis samples submitted to the laboratory can be in one of three forms: herbal material, resin and oil. The main physiologically active ingredient of cannabis is D^9-tetrahydro- cannabinol (D^9-THC).

Identification of herbal cannabis is achieved by visual inspection under low power magnification. Ethanol extracts of the herbal material, resin and oil are subjected to thin layer chromatography (TLC) for rapid screening and simple comparison purposes. For identification and confirmation, trimethylsilyl derivatives of the main components D^9-THC, cannabidiol, cannabinol and the lesser components D^8-THC and D^9-tetrahydrocannabinolic acid are analysed by GC-MS. The technique will identify, unequi- vocally, the derivatised compounds. HPLC or GC-MS can be used for profiling purposes. The preliminary screen by TLC will provide a good indication as to whether or not the blocks of resin are from the same batch, and reversed-phase HPLC will confirm this. HPLC is particularly useful because unlike GC-MS, it does not require the samples to be derivatised. Tetrahydrocannabinolic acids are thermally labile and would decompose under GC-MS conditions. The chromatogram serves as the profile of the drug. The title ‘‘amphetamines’’ includes amphetamine itself, methylamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine.Benzyl alcohol was identified and quantified by GC/mass spectrometry (GC/MS) in human serum and post mortem blood after derivatization with 4-carbethoxyhexafluorobutyryl chloride(*1*).Commercial products containing diethyl ether were detected in the blood of three homicide victims by GC and GC/MS (*2*). Headspace GC was used to detect and quantify difluoroethane in two traffic fatality victims (*3*).

Trichloroethylene was determined in the blood of victims in forensic cases by GC/ECD (*4*) and GC/ECD and GC/FT-IR (*5*). The distribution of toluene in glue sniffers' biological fluids has been studied by GC and GC/MS (*6*). Headspace GC/FID was used to determine benzene in biological fluids of a victim of fatal poisoning (*7*). Volatiles that are used and abused as anesthetics have been reviewed (*8*). Enflurane has been determined in human tissues by GC/MS (9). The chemical, toxicity, and pharmacological properties of the current fluorinated inhalation anesthetics have been surveyed (*10*). An analytical method for the identification of volatile organic compounds in blood has been developed using purge-and-trap extraction coupled with GC/FT-IR (*11*). SPME and GC/MS were used to confirm volatiles in the investigation of two traffic fatalities (*12*). Literature reports concerning the analysis of occluded solvent basis for determining whether cocaine and heroin samples have a common origin have been reviewed (*13*). Head- space analysis of solvents in cocaine and heroin samples was determined by GC/FID and confirmed by GC/MS (*14*).

Carboxyhemoglobin levels were determined in two victims of open air carbon monoxide poisoning (*15*). The interpretation of post mortem carboxyhemoglobin concentrations has been dis- cussed (*16*). The performance of the Instrumentation Laboratory Inc. IL-682 for the analysis of post mortem blood specimens for carboxyhemoglobin was evaluated (*17*).

Cannabinoids. A preliminary study of the analysis of can- nabis by supercritical fluid chromatography (SFC) with atmo- spheric pressure chemical ionization mass spectroscopy (APCI- MS) has been reported (*18*). GC/MS was used to identify butyl cannabinoids in marijuana (*19*). Unsmoked handrolled cigarettes were analyzed for cannabis resin/cannabis content by thin-layer chromatography (TLC) (*20*). Capillary electrochromatography was used to analyze the cannabinoid content in marijuana and hashish(*21*). The cannabinoid content was determined by GC/MS of marijuana samples seized in Greece and its forensic application has been reported (*22*). Tetrahydrocannabinol (THC) was de- tected in foodstuff containing hemp and the forensic significance was discussed (*23*). The inorganic element pattern of marijauna was

evaluated as a tool for comparing different seizures (*24*). The filtering effects of various household fabrics on the pollen content of hash oil have been studied (*25*). A method for the identification of cannabis using DNA-specific primers has been developed (*26*). Methods have been reported for the identification of *Cannabis sativa* L., comparing the sequence of the nuclear ribosomal DNA internal transcribed spacer II (ITS2) of an unknown sample with a known predeter- mined consensus sequence of cannabis (*25*).

Applications

Drug OF Abuse

CE has been used widely for the analysis of drugs and related compounds in pharmaceutical research and development since its introduction as a commercial analytical technique in the late 1980s. The potential of CE for forensic analytical problems was first demonstrated in the early 1990s with its application to the separation of illicit drugs in synthetic mix- tures. Since that time, the determination of illicit drug seizures and clandestine lab materials has become a major application area for this group of techniques. Some examples of the appli- cation of CE techniques to such samples are presented on. In many instances, the choice of CE as an alternative tech- nique to the more traditional GC and high-performance liquid chromatography (HPLC) methods is justified by the minimal amount of sample required and by the shorter and easier sample pretreatment. CE often allows for very short separation times, particularly when using techniques such as short-end injection are being used. These features are beneficial to forensic analysis, particularly to screening for illicit drugs in clandestine preparations. In addition, CE lends itself to the difficult task of separations of enantiomers by the addition of a chiral selector to the background electrolyte (BGE). In addition to determining active illicit drugs, CE is also useful in separating cutting agents and other compounds that might be present, thus making it highly suitable for profiling types analyses.

Drug Analysis

Forensic chemists can identify and quantify illicit substances found in the possession of suspects or recovered from crime scenes. Accurate analysis helps law enforcement understand the nature and origin of drugs, aiding in drug trafficking investigations and legal proceedings. As given by the United Nations, World Drug Report 2023 cited a number of reported drug-related offenses and alarmingly there is a large number of juveniles also involved in this type of drug trafficking/ crime. Illicit substances (IS) spoil fruitful human resources into useless entities. Psychotropic drugs of all kinds are today's equivalent of terrorism, posing a serious threat to humans. According to the WHO report, 31 million people suffer from drug use problems. Addressing the severity of drug addiction and drug-related crime, chemistry offers a comprehensive set of tools that play a crucial role in detection, analysis, and quantification, leading to effective justice for offenders. These tools encompass qualitative and quantitative methods, as well as instrumentation and spot tests, which collectively serve as powerful means of tackling the challenges posed by drug-induced offenses. By employing these diverse approaches, authorities can accurately identify illicit substances, assess their concentrations, and subsequently bring those responsible to justice. Some of the well-known IS that is used in many parts of the world are Cocaine, LSD (lysergic acid diethylamide), MDMA (Molly/Ecstasy), Methamphetamine, Heroine, Marijuana (in some parts of the world it is decriminalized), synthetic cannabinoids, Ketamine, etc. Several instances of drug-related criminal activities and drug lords, such as Pablo Escobar and the Cali Cartel in Colombia, stand out. These cases involved highly influential drug-trafficking entities on a global scale. Additionally, the heroin trad in Southeast Asia's golden triangle and the Mexican drug war have further highlighted the significance of forensic chemistry techniques. In the above cases, forensic chemists played a critical role in the identification of illicit substances and furnishing crucial evidence used in legal proceedings against individuals involved in drug trafficking crime scenes. These approaches encompass both instrumental and non-instrumental techniques, each accessible to individuals possessing intermediate, advanced, or expert proficiency. In instances of heightened complexity,

the involvement of a domain expert may be necessary.

Table-1: Summary of Qualitative & Quantitative Methods of Drugs of Abuse

Method	Equipment	Substance	Qualitative	Quantitative	Duration
Chemical Instrumentation	MS, GC, UV, FTIR, Raman spectroscopy, X-ray diffractometry, Ultraviolet spectroscopy	Several	Yes	Yes	**A few seconds to minutes**
Chemical Instrumentation	TLC	Several	Yes	No	**Few minutes**
Spot test	Chemicals	Most common drugs of abuse	Yes	No	**Few minutes**
Immunoassay	Enzyme-linked	Various	Yes	No	**Few**

	munosorbent assays	drug metabolites			**minutes**
rocrystalline test	Specialized microscopic equipment	Several	Yes	No	**Few minutes**
Urine dipstick test	**osable testing strip**	**Drugs or metabolites**	**Yes**	**No**	**Few seconds**

Drugs of abuse are detectable in various bodily fluids, such as blood, hair, urine, and saliva. The choice of fluid for testing depends on factors like the drug being screened; time elapsed since drug use, and testing purpose. Blood is the common biological body fluid for drug testing, detecting a wide range of drugs. The hair detection method gaining popularity due to its ability to detect past drug use, even over weeks or months. But less sensitive than blood, influenced by hair traits. Urine can be easily collected and can be used to test crime related to parties, schools, and sports. Various tests can detect recent drug use, with detection windows varying by drug (a few days to a week. Vomit is less frequent than other methods, used in overdose cases where drug presence is crucial. The choice of fluid depends on urgency and sensitivity. Urine suits quick results rather than blood or hair for sensitivity.

Analytical aspects of cyanide poisoning: problems and trends

Cyanide determination in environmental samples

The specificity of cyanide as an environmental pollutant is of special concern, due to the different

toxicity of cyanide-containing substances, from one side, and from other side, to the fact that the cyanide quantification depends on the analytical method used (Zheng *et al*., 2003). Cyanide pollutants have been officially classified into three main groups depending of their toxicity and environmental fate: (i) free cyanide - including HCN, alkaline and alkaline earth cyanides; (ii) weak acid dissociable cyanide (WAD) - a collective term for free cyanide and metal-cyanide complexes ($Ag(CN)^-$, Cu(CN), Cd(CN) , Zn(CN), Hg(CN), Ni(CN),) which easily release HCN under slightly acidic environmental conditions; and (iii) total cyanide - each potential source of HCN regardless of its origin (U.S. EPA, 1992). The term "cyanide" refers to all CN groups that can be determined analytically as cyanide ion (CN) via spectrophotometric or electrochemical measurements, usually following appropriate sample pre-treatment to release cyanide ion (APHA,1998). The Environmental Protection Agencies have imposed maximum contaminant levels (MLC) for cyanide discharge into the environment. The MLC for WAD cyanide vary from 0.05 to 0.07 μg/L for drinking water and in the range between 200-500 μg/L for waste water (WHO, 1998). The MCL for total cyanide is much higher – 1 mg/L. The group of WAD cyanide has been a subject of special consideration as the assessment of environmental risk and efficiency of detoxification procedures depend on its analytical qua.

Conclusion

This review provides a good example of about the application of Analytical Chemistry in forensic science and medicine which motivate the researcher to develop new analytical methods and instrumentation. Rapid drug and cyanide analysis in blood or breath is ripe for new attractive approaches. The future of any science is hard to predict, but there are a number of areas in which forensic science will probably advance, including miniaturisation, analysis at the crime scene, and automation of laboratory analytical processes to enable higher throughput of samples.

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Optimization of conditions and primer design for loop-mediated isothermal amplification assay to detect specific 16S rRNA gene in Mycobacterium tuberculosis complex.

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Abstract

Tuberculosis (TB) is a bacterial disease. It is caused by *Mycobacterium tuberculosis*. This is responsible for killing millions of people worldwide and in India. In the present scenario, the WHO has released new guidelines for the use of rapid methods, such as equipment free loop-mediated isothermal amplification assays. The aims of study were to develop a loop-mediated isothermal amplification (LAMP) method targeting specific 16S rRNA genes for the detection of the *Mycobacterium tuberculosis* complex by optimizing the conditions and primer design. Three primer pairings specific for the 16S rRNA gene were used in this study: F3-B3, FIP-BIP, and LF-LB. The specific optimization of the LAMP method was adjusted in terms of temperature and time parameters. The optimum temperature was determined to be 650C following the testing of seven different temperatures, and an assessment of the optimal time intervals across eight treatments revealed a preference for 55–60 min. In conclusion, the research study successfully achieved optimal temperature and time parameters suitable for implementing LAMP detection of 16S rRNA genes specific to the *Mycobacterium tuberculosis* complex.

Keywords: LAMP method, optimization, primer design, *Mycobacterium tuberculosis*

T

Introduction:

India continues to have a high tuberculosis (TB) burden across the globe. There are an estimated 2.64 million new cases of TB in India in 2022. TB continues to be a leading cause (48%) of death due to infectious disease. There were an estimated thousands of deaths due to TB in 2022. The Government of India is working on the National Tuberculosis Elimination Program (NTEP) with a strategy for ending TB in the region by increasing access to diagnosis, treatment regimens and strengthening healthcare systems (WHO, 2023).

The World Health Organization has recommended the use of the LAMP technique for rapid point-of-care detection via open real-time viral elution, but several challenges remain. LAMP is an energy-efficient, simple and cost-effective approach because it does not require thermocyclers such as PCR; instead, a regular incubator or water bath can be used to conduct LAMP experiments without any disruption (WHO, 2023). PCR bypasses the denaturation and annealing steps by providing results in just an hour, with responses as fast as 30-40 minutes into the reaction (Tehri et al., 2022).

The primer design is very critical in the case of LAMP for TB diagnosis, and there are a few main factors that directly affect the sensitivity, specificity and reliability of the assay (Srivastava and Prasad, 2023). We chose primers targeting selected regions of the *M. tuberculosis* genome. These regions must be very conserved between various strains of bacteria for the assay to correctly identify a wide range of TB strains. The “specificity of the LAMP” method is directly influenced by the choice of primer sequences, because nonspecific primers, in some cases, can either result in false-positive results or decrease assay performance. Since the sensitivity of LAMP is mainly determined by the design of LAMP primers, clinical specimens may have different degrees of bacterial load

associated with *M. tuberculosis*; thus, the primers should also perform reliably in amplifying even low concentrations of a target DNA (Shirshikov and Bespyatykh, 2022). The choice of primers and successfully designed priming oligos are necessary for efficient amplification under isothermal conditions and are ultimately sensitive for detecting as few *M. tuberculosis* DNA copies released from an individual patient sample as possible (Xu et al., 2020).

Optimization of the LAMP conditions to increase the sensitivity and specificity for detecting low concentrations of MTBC DNA in clinical samples. The specificity can be improved by optimizing conditions such as primer concentrations, reaction temperature and reaction time. This is important because specificity helps in the proper detection of 16S rRNA genes specific to MTBC and can decrease false positives due to nonspecific amplification. Optimization enhances the isothermal DNA amplification efficiency (Agel et al., 2020). It is important for enough time to be given between the onset of infection and possible retesting, which proper amplification can accomplish by producing effective DNA as quickly as possible. To improve the robustness and reliability of LAMP target amplification across a spectrum of sample types, it is important to carefully test and optimize reaction components (e.g., buffers, and enzymes) for individual applications to alleviate their inhibitory effects (Srivastava and Prasad, 2023; Agel et al., 2020). In view of the scarcity of commercial kits for LAMP assays in the Indian market, the standardization of in-house kits is the alternative.

Therefore, the aim of this study was to identify target sequences suitable for primer design and determine how LAMP-based detection systems may be improved. This was followed by optimization of the LAMP assay conditions for the H37Rv ATCC® 25177 strain.

Materials and Methods:

Sample collection

Genome samples of M. tuberculosis H37Rv-ATCC® 25177™ were obtained from HiMedia Laboratories Private Limited, "Wagle Industrial Estate, Near Ashar IT Park, Thane West, Maharashtra India". The total amount of total DNA measured by PicoGreen® was approximately 5 µg, and the purity ratio (A260/A280) ranged from 1.6 to 2.0.

LAMP Primer Design Assay

The LAMP primers were designed using Primer Explorer "V3 software (Eiken Chemical, https://primerexplorer.jp/lamp3.0.0/index.html)". For this purpose, the target gene (16S rRNA) of M. tuberculosis H37Rv was developed with six primers used in this work. Three primer pairings specific for the 16S rRNA gene were used in this study: F3-B3, FIP-BIP, and LF-LB. The designated primer sequence was obtained from AgriGenome Labs Pvt. Ltd. (Delhi). Genomic DNA was extracted from bacterial colonies following mechanical disruption, and then in 250 mL of TE buffer containing "10 mM Tris/HCl (pH 8.0), and 1 mM EDTA". A total of 25 µl was used for the LAMP reactions that contained various bacterial DNA contents as well as the "inner primers, FIP and BIP (30 pmol), the outer primers, F3 and B3 (5 pmol), and the loop primers FLP and BLP (20 pmol)". The mixture was incubated separately at various optimized temperatures (Pandey et al., 2008).

Optimization of LAMP

The LAMP assay was optimized in a dry bath (Model: BSH200) according to the manufacturer's instructions. For isothermal temperature optimization, seven different temperatures (0°C, 50°C, 55°C, 60°C, 65°C, 70°C and 75°C) were evaluated via naked-eye visualization after visualization by a smartphone app named color meter- RGB CMYK analysis. The optimal temperature for time optimization was maintained at 0, 30, 35, 40, 45, 50, 55, and 60 minutes (Aulia et al., 2023).

Visualization using a smartphone

The visualization was further confirmed with naked eye observations using a Galaxy A22 5G phone (Android version 13) along with the G/R (green/red) ratio calculated by an RGB HSL CMYK RYB analysis app. The LAMP reaction was visualized with a thermocycler incubation machine. The total reaction size was 25 µl in the complete image captured by the smartphone flash at a distance of 15 cm, and the G/R quotient was also considered positive.

Results:

Primer design

The primer pair targeted the 16S rRNA gene, amplifying a segment of 212 base pairs (bp) ranging from nucleotide bases 31 to 241 within the *M. tuberculosis* H37Rv genome (ATCC® 25177™) (Figure 1 (a) & (b)). The adherence of the designed primers to the target genes is depicted in Figure 1 (c).

Optimization of LAMP

These experiments were conducted to establish the optimum amplification time and temperature for LAMP. LAMP usually works in an isothermal situation where the reaction takes place at a constant temperature. The temperature optimization tests a series of temperatures to determine the optimal conditions that provided amplification with maximal efficiency and specificity. The optimum temperature was determined to be 65^0C following testing at seven different temperatures (Figure 2 (a)). The amplification time is how long the reaction is allowed to continue for the maximum quantity of the library. For each set of samples, the minimum time required for a detectable amplification (Ct value) and the maximum time at which amplification plateaus or reaches an asymptotic length

were also recorded. An assessment of optimal time intervals across eight treatments showed a preference for 55–60 min (Figure 2 (b)).

Visualization using a smartphone

We used a mobile computing program (based on the RGB HSL CMYK RYB) that facilitated visual detection of particularly low-contrast artifacts by calculating ratios in G/R values. The application was employed to store the G/R value-related data of the *M. tuberculosis* H37Rv ATCC-25177 16S rRNA gene for evaluating the temperature optimization results (Figure 2 (a)). The highest G/R value (475) with respect to the optimum temperature was 65°C. In addition, the G/R values calculated during the optimization of the 16S rRNA gene were also documented (Figure 2 (b)). The optimization time had a maximum G/R value (433) at 60 min. The aim was to determine the optimal time conditions and temperature for the LAMP reaction to design a reliable TB diagnostic tool. The goal of time optimization was to determine the minimum increase duration that could be achieved.

Discussion

Due to the simplicity, rapidness, specificity and cost-effectiveness of the LAMP technique, it has become popular in various fields, such as diagnostics and research. Only the Taq polymerase enzyme in the LAMP method, such as Bst, Pfu and Aac, can cause strand displacement. LAMP, developed in 2000 by Notomi and colleagues (Eiken Chemical Co., Ltd.), uses four primers designed to provide six sequences across the DNA or RNA target, which results in amplification at a constant temperature. The LAMP-TB detection method is based on bacterial DNA amplification with high specificity; therefore, the presence of *Mycobacterium tuberculosis* in the test solution was effectively confirmed. Despite its high sensitivity, specificity and proficiency, the LAMP-TB approach

is simpler than other molecular testing strategies. This makes the system convenient to use because it uses a small amount of sample.

In this study, bioinformatics analysis of the primer pair that amplified a 212-bp fragment of the *M. tuberculosis* H37Rv 16S rRNA gene and thus its ability to bind to target genes was also performed. According to Aulia et al., (2023), the genome of *M. tuberculosis* H37Rv was scanned to find suitable primers for the catalase-peroxidase gene and NADH-dependent enoyl-[ACP] reductase gene. Under these conditions, at a minimum length of 18-30 bp, the differences in GC content and Tm ensure that the primers meet the ideal criteria. The Snap gene primer pair was tested in silico, which led to the amplification of a 1,486 bp katG gene segment, while the forward sequence of the inhA gene reached a length of 724 bp. These data were subsequently used with draft primers for LAMP amplification.

In our study, seven different temperatures were tested, and 65°C was determined to be the optimum temperature. Across the eight treatments, 55-60 min was determined to be the best amplification time. Garg et al., (2021) reported that the optimal temperature was 66°C, at which the amplification efficiency was highest, and LAMP reactions were performed between 65–70°C (higher temperatures reduced the detection sensitivity). To minimize the amplification time, a series of LAMP tests for different early incubation periods were performed, and 1-hour minimum reaction time was suggested.

In our study, color visualization via colorimetric methods and, alternatively, confirmation via a mobile computing program were used to detect low-contrast artifacts in the *M. tuberculosis* H37Rv ATCC-25177 16S rRNA gene. The temperature optimization results, showed that the highest G/R value of 475 was achieved at 65°C, and the maximum G/R value of 433 was achieved at 60°C. The goal was to design a reliable TB diagnostic tool. Tamhane et al., (2021) reported that the amplified viral gene was detected by a color

change in Cresol red dye from pink to yellow due to the pH decrease caused by positive amplification. Negative samples will have no color change. The color change was detected by a smartphone with an RGB photo evaluation app and measured on the basis of its spectroscopic absorption properties using a flatbed scanner.

LAMP was used to detect *Mycobacterium tuberculosis* DNA, and its advantageous parameters included constant temperature, multiple primer sets, and the presence of inhibitors in the sample (Neshani et al., 2023). We used this method for straightforward amplification from crude biological samples to minimize time consumption and increase diagnostic accuracy. With its high sensitivity, LAMP enables early diagnosis and detection of infections, making it an important tool in resource-limited settings (Das et al., 2022).

The use of LAMP for the detection of *Mycobacterium tuberculosis* has several limitations: technical challenges in developing multiple QC primers, potential sample interference, and resources for adequate training, personnel, and a sustained desire to deploy/maintain the method (Shete et al., 2019).

Conclusion

LAMP primers for the target 16S rRNA gene were designed using the reference genome of *M. tuberculosis* H37Rv. The target gene 16S rRNA has a length of 212 bp. Three primer pairings specific successfully design for the 16S rRNA gene were used in this study: F3-B3, FIP-BIP, and LF-LB. For LAMP amplification of the 16S rRNA gene, the optimal temperature was found to be 65°C, and the optimal amplification time was also 60 min for the 16S rRNA gene. Whereas PCR is based on a set of primers and a thermal cycler for amplification, LAMP uses multiple sets of primers that function in an isothermal

manner. These divergent approaches to primer design and amplification strategies account for their shared yet distinct utility in molecular biology and diagnostic area.

Acknowledgement:

NA

Conflict Of Interest:

The authors declare no conflicts of interest

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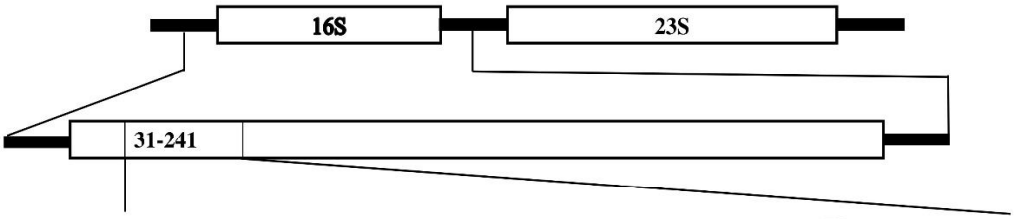
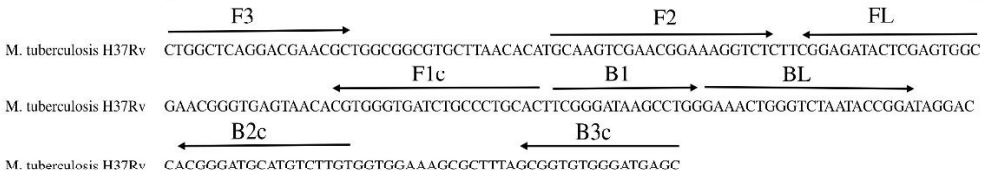
(a)



(b)



(c)

Name	Composition		Sequence
MTB-FIP	F1c	F2	CAACCCACGTGTTACTCATGCAAGTCGAACGGAAGGTCT
MTB-BIP	B1	B2c	TCGGGATAAGCCTGGACCACAAGACATGCATCCCGT
MTB-F3	F3		CTGGCTCAGGACGAACG
MTB-B3	B3c		GCTCATCCCACACCGC
MTB-FLP	FL		GTTCGCCACTCGAGTATCTCCG
MTB-BLP	BL		GAAACTGGGTCTAATACCGG

Figure 1: *M. tuberculosis* complex-targeting primers (a) 16S rRNA gene target within the first variable region: 31–241 of MTB-LAMP. (b) Location of annealing primer site 16S rRNA gene first variable region. (c) Composition and position of the six primers used for MTB-LAMP. The primers in Fig. 1 (b) were combined as follows: F1c and F2 were referred to as MTB-FIP; B1 and B2c were referred to as MTB-BIP; and F3, B3c, FL, and BL were directly used as primers and referred to as MTB-F3, MTB-B3, MTB-FLP and MTB-BLP, respectively.

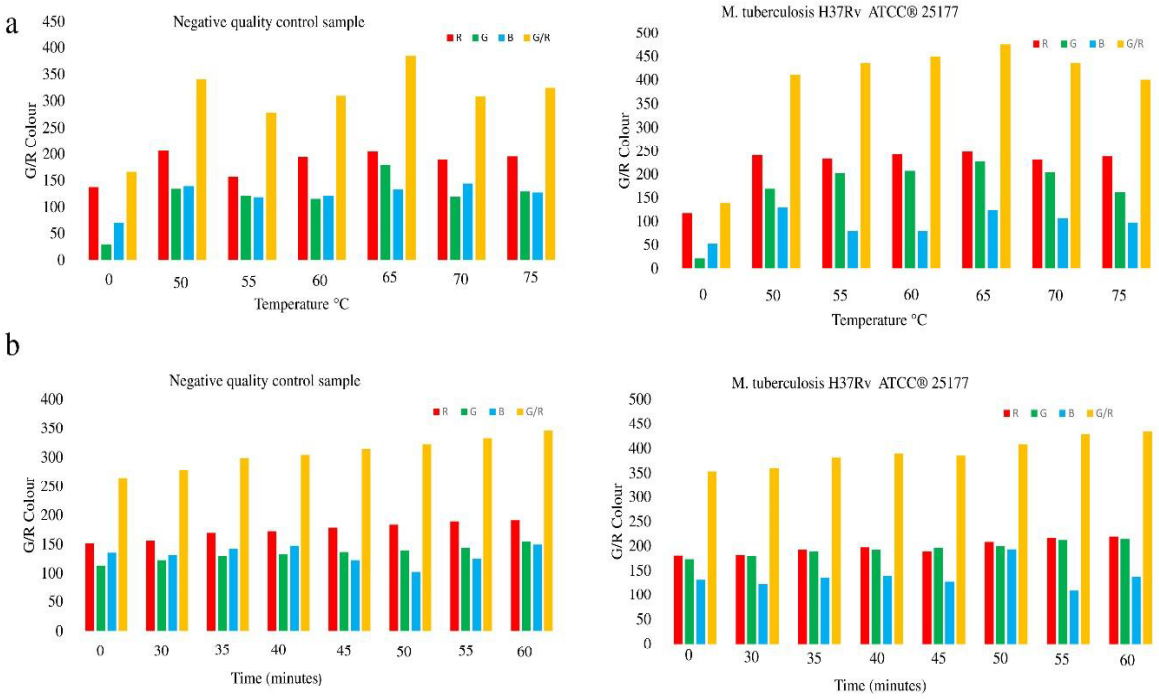


Figure 2: (a) Calculation of temperature optimization through the RGB HSL CMYK RYB application for the 16S rRNA gene (b) Calculation of time optimization through the RGB HSL CMYK RYB application for the 16S rRNA gene

LCMS technique to detect cannabinoids in whole postmortem blood

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Abstract

Introduction

Blood is the matrix for toxicological analysis. One of the major component of *Cannabis* is Delta 9 THC. It retains in body for long time in chronic abusers. Postmortem sample was analyzed for detection of compounds. *Cannabis* belong to plant *Cannabis sativa*. Δ^9 THC is the most psychotropic substance present in the plant. Herbal plant of *Cannabis* is abused mostly around the globe. Various preparations, charas, ganja, bhang, various parts of plant are abused globally.

Materials and Methods

Postmortem arterial blood was collected in sodium fluoride (NaF) vacutainer and stored in freezer at -20^{0}C. 200 µl of blood was taken and fortified with 20 µl of methanol and 20 µl of *Cannabis* extract was added. 500 µl of acetonitrile was added while vortexing and 0.1 % formic acid was added. It was centrifuged at 600 rpm for 10 minute. Supernatants transferred to fresh tube. Solid phase extraction was done. LCMS was done.

Results

Chromatograms were examined for presence of cannabinoids.

Discussion

This work deals with robust, validated method for qualifying cannabinoids in whole blood. Cannabinoids was obtained in a single run and method qualifies THC and other cannabinoid in authentic negative postmortem blood.

Keywords : THC, Cannabis, LCMS, Drug, Toxicology

Introduction

Δ^9 THC is the main psychoactive compound among so many compounds found in Cannabis.[1,2,3] THC disturbs learning and reaction time. Impairment depends on quantity or dose and remains in the body till hours.[4,5]

Blood is the standard matrix for post mortem toxicological analysis.[6] Tetrahydrocannabinol can be detected upto months in the blood of chronic abusers while it detected up to days in the blood of occasional users.

Cannabis is absorbed and reached to different parts of body after its administration. Tetrahydrocannabinol (THC) metabolizes to 11-OH-Δ^9 THC which again oxidized to Δ^9 THCCOOH.[7,8]

In this manuscript, post-mortem authentic sample of blood was taken for analysis and presence of THC, THC-OH, THC-COOH ,etc are examined.

Materials and methods

Leaf extract of Cannabis was procured from nearby forest in Jharkhand state of India.

LCMS grade acetonitrile, methanol, Formic acid, iso-propanol, etc were obtained from Bansa India Corporation.

HLB solid phase extraction cartridges with 15 mg sorbent along with SPE system were obtained from galaxy scientific and a one engineering Works.

Glass bottles with screw caps, auto-sampler vials were obtained from one of the shops of Ranchi.

Deionised water was obtained from Fisher scientific.

Certified negative arterial post mortem blood was obtained from the Department of Forensic medicine and toxicology , RIMS, Ranchi.

This work was performed according to protocol of Hubbard et al. [9]

Preparation of standards

Standard working solution was prepared in solvent methanol and it was kept in freezer at -20^{0}C at BIT Mesra, Ranchi.

Whole post-mortem blood standards

Working standard solutions were mixed to whole blank post mortem blood in a 1:10 dilution to make final concentration.

Cannabis extract preparation

Cannabis leaves were taken. It was washed with tap water and then with distilled water to remove impurities. It was shade dried for 15 days. These dried leaves were made into powder using mortar pestle .5 gram of powder was weighted.

It was mixed with 50 ml of methanol in conical flask. It was kept untouched for 24-48 hours. After that period, this was filtered using filter paper and the extract was used for further analysis. And residues were discarded.

Sample Collection

Post mortem arterial blood was collected in NaF (Sodium Fluoride) vacutainer tubes. This was taken into cryovials and kept in freezer at -20^{0}C.

Sample processing

200 µl of post mortem arterial blood were fortified with 20µl of methanol. 20 µl of *Cannabis* plant extract was added. 500 µl of ice- cold acetonitrile was added while vortexing and 0.1% Formic acid was added and this mixture was centrifuged at 600 rpm for 10 mins. Supernatants were transferred to fresh tube containing 1 ml of deionized water. It was mixed well.

Solid phase extraction

Samples loaded onto SPE cartridge slowly. This method was performed by using positive pressure apparatus. This sample was washed two times using 500 µl of 25% methanol.Now, this sample was eluted two times with 100 µl 90:10 acetonitrile: isopropanol and twice with 100 µl 50:50 acetontrile: methanol. It was completely dried under vacuum . Reconstituted with 200 µl 50% acetonitrile with 0.1% formic acid and cap material was used for its capping. Eppendorf tubes was vortexed and then loaded into auto sampler.

LCMS

Waters Acquity QSM

Solvent A: 5 % Acetonitrile in water

Solvent B : Acetonitrile

Solvent C : Methanol

Solvent D : 5 mM Ammonium acetate

Injection volume (ul): 300

Now, the mass spectrometer was used in positive electrospray ionization.

Parameter source

Source temperature: 150^0C

Method validation

Method validation according to Clinical and Laboratory Standards Institute (CLSI)

C62-A guidelines for LCMS/MS.

Sensitivity and detection criteria:

The peak shape, height, size, determined the compound. Molecular weight determine the compound present in the sample. Retention times were compared between sample and standard to come to a conclusion.

Bias and precision

The bias and precision was measured using controls in triplicate. Imprecision was required to be ≤20%.

Process efficiency and matrix effects

Process efficiency was analyzed by comparing area counts of fortified blank matrix with *cannabis* extract before processing of sample and was compared to area count of sample in elution solvent.

Drug interferences

Lack of interference means ≤ 20 % bias.

Auto sampler stability and carryover

Concentration of control samples during starting run and after weeks.

Results

Chromatograms were examined for the presence of cannabinoids. Various peaks of different heights were obtained. x-axis having retention time and y- axis having relative intensity were examined.

Case- A 25 years old boy was performed suicide be eating poison. His blood was examined , purified and analyzed by LCMS for checking presence of different compounds in his blood.

Retention time	Compound m+h	Compound m-h
12.65	359.2	
15.46	311.2	
22.50	315.3	
8.91	311.1	
9.87	381.2	
22.48	315.3	
23.10	359	
23.82	358.6	
26.91	337.2	
12.66		357.3
13.15		357.3
22.59		313.3
23.38		357.4
12.67		357.3
13.12		357.3

22.60		313.3

Table 1 : Compounds with their retention time

Sample 368 Vial 2:A,4 ID P1.2(SAIF24066275) File LCMS24E13JUN80 Date 13-Jun-2024 Time 18:08:15 Description ACCUCOREC18 150 XS

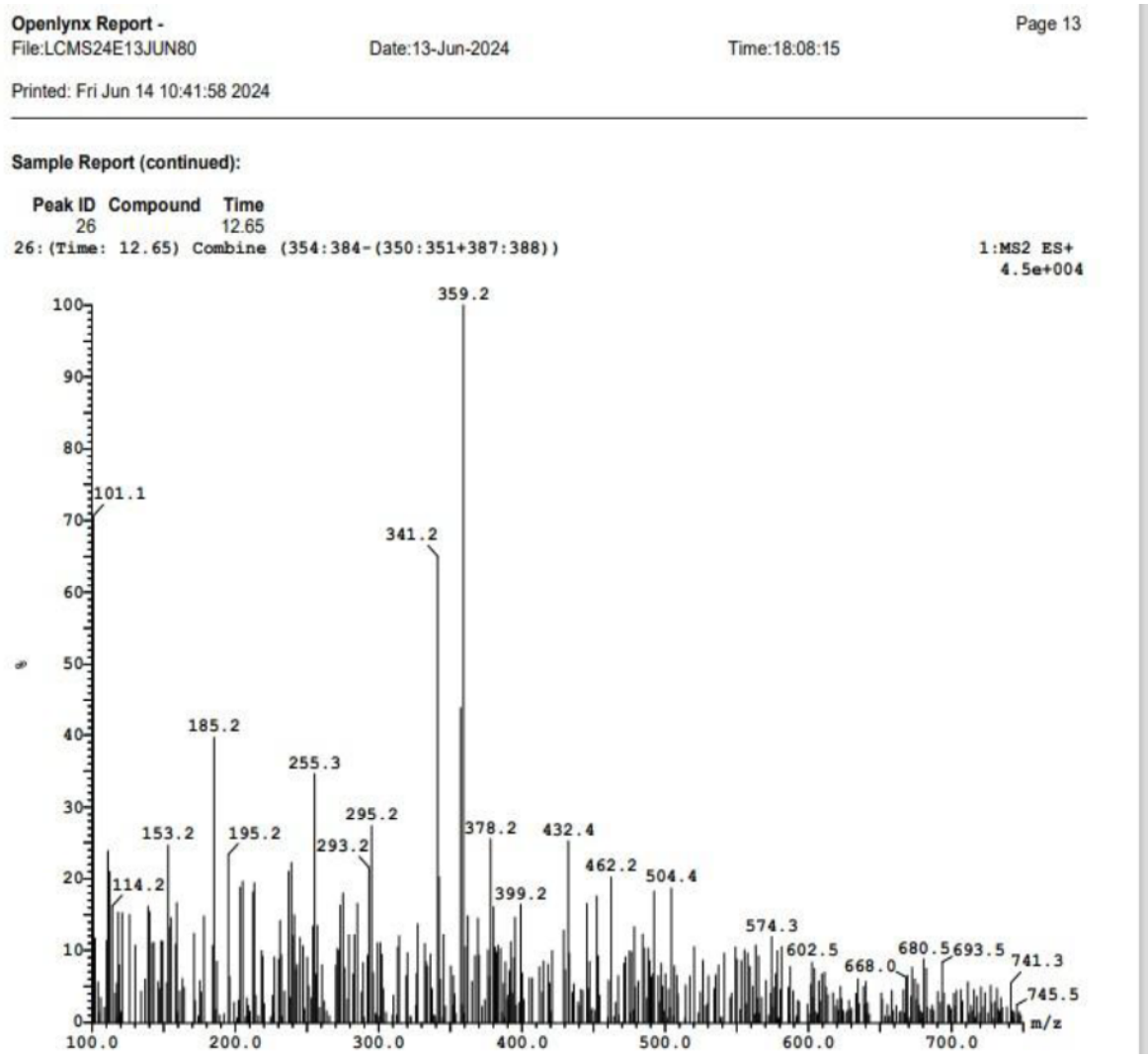

Figure 1 : LCMS report

Openlynx Report - Page 20
File:LCMS24E13JUN80 Date:13-Jun-2024 Time:18:08:15
Printed: Fri Jun 14 10:41:58 2024

Sample Report (continued):

Peak ID	Compound	Time
50		22.50

50:(Time: 22.50) Combine (641:671-(637:638+674:675)) 1:MS2 ES+ 7.7e+004

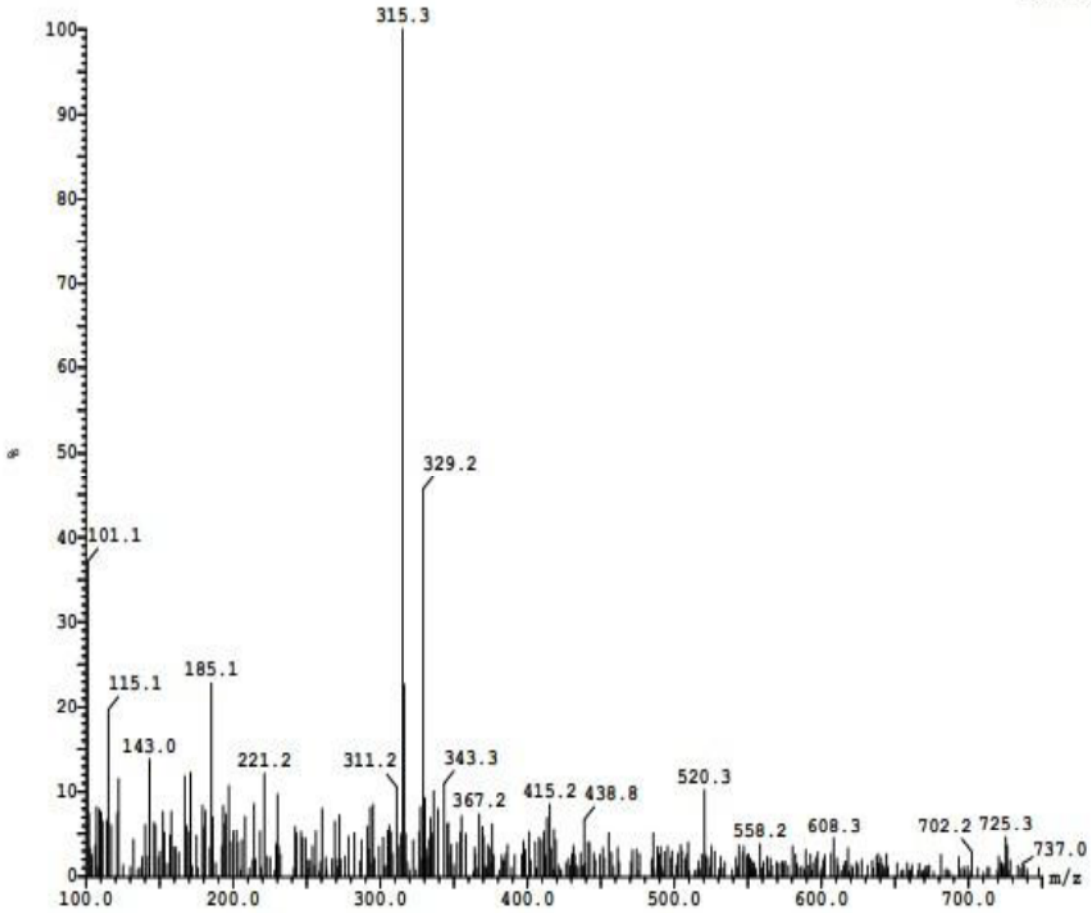

Figure 2 : LCMS report of blood sample having cannabis

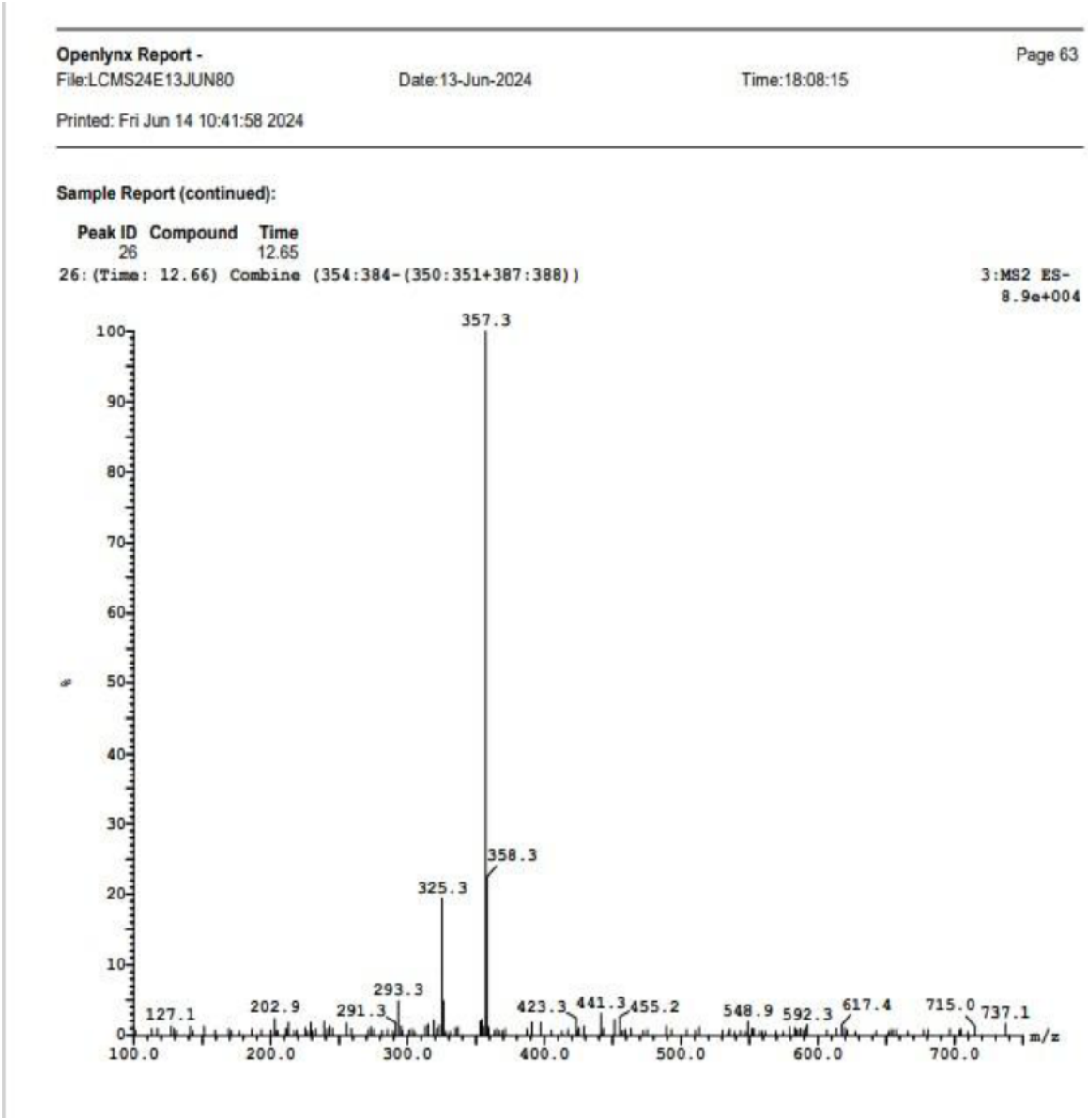

Figure 3 : LCMS report

Discussion

This work deals with robust, validated method for qualifying cannabinoids in whole blood. Cannabinoids were obtained in a single run and method qualifies THC and other cannabinoids in authentic negative post mortem blood.

The goal was to highlight a method with a simple and accurate procedure which qualifies THC in whole blood. This method gives a simple extraction procedure.

Sorensen and collegues deleted interfering phosphor lipids by filtering with a hybrid SPE – phospholipid plate with a stationary phase with bonded zirconia and carefully quantified THCA-A .[10] Disposable pipette was used for extraction and quantification of THC- gluc in whole blood. [11]

Conclusions

This process requires only 1 ml of blood and is analyzed by LCMS/ MS. The post-mortem blood contains *cannabis* as it is the spiked sample.

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Conflict of interest- The authors have no conflict of interest.

Author contribution

We have accepted responsibility for this manuscript .

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Analysis of Original and AI-Generated Voice: A Comparative Study Based on Spectrogram, Gaussian Distribution, and Likelihood Ratio

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Abstract

The voice and the way of speaking are unique to an individual. Hence, it falls under one of the biometric parameters for establishing an individual's identity. Such an individual trait can become vulnerable and a menace to the individual when an exact duplication occurs. Today, AI technologies can create cloned versions of an individual voice. Various applications (free and paid) are available in online stores that serve this purpose and are also easily accessible to the public. If such applications are misused and cause a breach of security measures, they can devastate an individual's identity, reputation, and financial status. Considering such scenarios in the preview, knowing about potential incidents and how to mitigate them is imperative. Thus, it becomes pivotal in identifying techniques that distinguish AI-generated voice from original voice, which is fast, cost-effective, and easy to handle. Therefore, this study is focused on widely accepted, easy-to-handle, and reliable methods through which such AI-generated voice can be distinguished from the original voice of the individual. The methodology utilized spectrographic analysis, gaussian distribution, and likelihood calculation based on probability density to determine the similarities and differences between AI-generated and original voices of individuals. Voice samples of 10 male English-speaking individuals aged 20-30 years were collected, and their text-based AI-generated voice was created using a free AI cloning application. Three words were selected from the text, and spectrographs and features like first formant

frequency, pitch, and intensity were extracted using voice analysis software PRAAT from both Original and AI-generated voices and compared. From the listings of the first formant, pitch, and intensity, the Gaussian distribution was plotted along with the likelihood values based on the ratio of probability density function using R-Programming software. Significant differences were observed in the formant frequencies, Pitch, intensities, and Gaussian distribution between Original and AI-generated voice. The results of the likelihood ratio supported these differences.

Keywords: AI-generated voice, Spectrographic analysis, Gaussian distribution, Likelihood ratio, Formant frequency, Pitch, Intensity.

Introduction:

Recently, the emergence of AI technology (Artificial Intelligence) has increased in every field. Even though it poses many advantages, we have recently witnessed various demerits. Using AI applications or software, several videos and audio resembling different known personalities were spread on social media. Whether it be audio or video, they are very similar to the authentic traits of the personality. A recent case reported in India was regarding the spread of a deepfake video of actress Rashmika Mandanna on social media platforms. Similarly, the famous cricket player Sachin Tendulkar was deepfaked endorsing a gaming app without his consent. In the international arena, a case was reported regarding the unauthorized use of the voice of the celebrity Scarlett Johansson by an AI app developer to advertise their product without her consent using her AI-generated voice.

In all these instances, an explicit impersonation of the individual has taken place. These can also extend to lower levels of society, which are more vulnerable to fraudulent activities. The above-said cases are one side of the coin. On the other hand, there can be scenarios including phishing scams, social engineering attacks, and voice-based authentication

bypasses, creating convincing audio deepfakes, enabling the dissemination of false information, fake news, or manipulated recordings, which can sow confusion, undermine trust in media, and public institutions, and exacerbate societal divisions. Voice cloning technology also introduces new security risks, such as the potential for unauthorized access to voice-activated systems, authentication mechanisms, or sensitive information. Malicious actors could exploit vulnerabilities in voice-based authentication systems to gain unauthorized access to accounts or networks. Voice clones also raise complex legal and ethical questions regarding consent, intellectual property rights, and the authenticity of audio evidence in legal proceedings. Courts may face challenges in determining the admissibility and reliability of voice-based evidence in cases involving AI manipulation.

To mitigate such scenarios, it is necessary first to identify the authenticity of the audio. Authentic audio can be discriminated from AI-generated audio only if ample information is available regarding the characteristics of AI audio over original audio. Hence, it is necessary to develop a simple and cost-effective method for understanding the various features of authentic and AI-generated audio through this study. This study compares the original voice to its AI-generated version. It attempts to utilize spectrographic analysis and analyze features like formants (F1), pitch, and intensity of the voice samples. The statistical method, like Gaussian distribution, determines the distribution of first formant frequencies, pitch, and intensities to compare the original and AI-generated voice versions. The study calculates the likelihood for formants, pitch, and intensity, considering the assertion that the features belong to the original voice. The likelihood ratio is calculated as the ratio between the probability density of a feature occurring in the original and the probability density of a feature occurring in AI.

Aim and Objectives:

This study aims to conduct a comparative study of Original and its AI-generated voices based on features such as spectral patterns, spectral energy, first formant frequencies, pitch, and intensity with the help of spectrogram, gaussian distribution, and likelihood ratio. The main objectives of this study include:

- To collect voice samples from 10 English-speaking males aged 20-40.
- Using an AI cloning application, create a text-dependent AI-generated voice from the collected original voice sample.
- To generate a spectrogram, the first formant plot, pitch plot, and intensity plot for the three words selected from original and AI-generated voices for comparison.
- To create Gaussian distribution comparison plots from the formant, pitch, and intensity listings.
- To calculate the likelihood ratio from the listings to identify the extent of similarity or dissimilarity between Original and AI-generated voice samples.

Materials and Methods:

DEVICES

- Laptop – Dell Inspiron 15 3000 with Windows 10 configuration.
- Wired Microphones – Boat brass heads 122 compatible with a 3.2mm jack and a frequency of 20Hz to 20KHz.

SOFTWARES/APPLICATIONS

- PRAAT Version 6.4.10 – Voice analysis software
- Online AI cloning application
- R 4.3.3 - R Programming software
- Microsoft Excel 2019 edition

METHODOLOGY

SAMPLE COLLECTION

COLLECTION OF ORIGINAL VOICE

Original voices were collected from 10 male individuals between the age group 20-40 years. Male voices were preferred as they possess distinct voice quality compared to females. Hence, the features may appear prominent, which will help analyze and distinguish the features.

The steps followed for collecting samples were as follows:

- First, the participants were told about the study and the importance of collecting their voices. Consent was obtained from them before they headed to the recording process.
- Then, the individuals were seated in a closed room with minimal external noise.
- The voices were recorded using PRAAT software version 6.4.10.
- The PRAAT software was opened after connecting the wired headphones to the laptop. Voices were recorded using the 'Record mono sound' from the 'NEW' option in the PRAAT objects menu.
- The participants were guided to place the headphones at a distance of ~1 inch from the mouth. This is to capture the voice with maximum clarity.
- They were given a paragraph to read in their ordinary tone, which was recorded.
- Every participant was given the same paragraph.
- After recording, the recorded voice was saved in WAV format. Since WAV format preserves the original audio quality with very minimal data loss.
- Each recording was saved by labeling it with the sample number.

GENERATION OF AI VOICE FROM ORIGINAL

For each original voice, its corresponding AI voice needs to be generated. This is done with the help of an online AI cloning app.

The steps followed in generating the AI voice corresponding to the original voice is as follows:

- First, the AI cloning application was logged in. Then, the voice cloning option was selected.
- The original voice to be cloned was uploaded. After the voice clone was ready, the paragraph to be prompted by the AI-cloned voice was loaded, and the regenerate option was selected.
- Then, the application generated the AI-cloned version of the original voice in WAV format.
- This process is repeated for all ten samples, and they were saved with labeling with the sample number.

SAMPLE PREPARATION

WORD SELECTION

The original and AI voices were compared using the text-dependent method. In the text-dependent technique, only comparison is possible with similar words. The text-dependent method was chosen because only comparing features of similar words is possible, as illustrated by the theory that 'only likes can be compared.' Three words were selected from the paragraph the participants prompted to enable this. The only prerequisite while choosing the words was to contain vowels and voiced and voiceless consonants. Based on these criteria, the words selected were 'LIFE,'' CHERISH,' & 'OPEN.'

SPECTROGRAM GENERATION

PRAAT Software was used to generate spectrograms. The steps involved in developing the spectrograms for each of the three words for the original and AI voice are as follows:

- Original and AI voice was opened in the PRAAT objects by selecting 'Read from file' from the 'Open' menu.
- Once the voice samples were added to the PRAAT objects list, each voice was selected, and then the 'View & Edit' option was clicked (Figure 1).
- Two separate windows were opened for the Original and AI voice. This window presented the waveform of the voice in the upper half and the spectrogram in the lower half (Figure 2).
- To capture the spectrum of specific words alone, first, the starting and ending point of the waveform depicting each word was noted for both Original and AI samples.
- Then, the waveform containing the word was selected and enlarged until it fitted the screen. By choosing 'Paint visible spectrogram' from the option 'Spectrogram' in the window (Figure 3), the spectrogram for the selected word will be generated in the PRAAT picture window.
- The generated spectrogram was saved in PNG format (Figure 4).
- These steps were followed for all three words selected to generate their respective spectrogram for original and AI-generated samples.

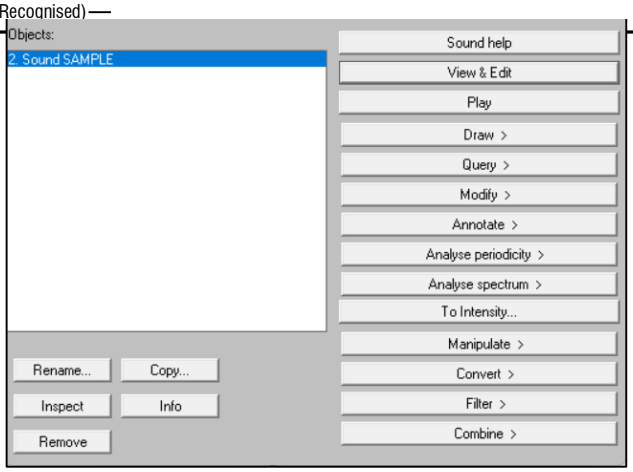

Figure 1:PRAAT Objects Window.

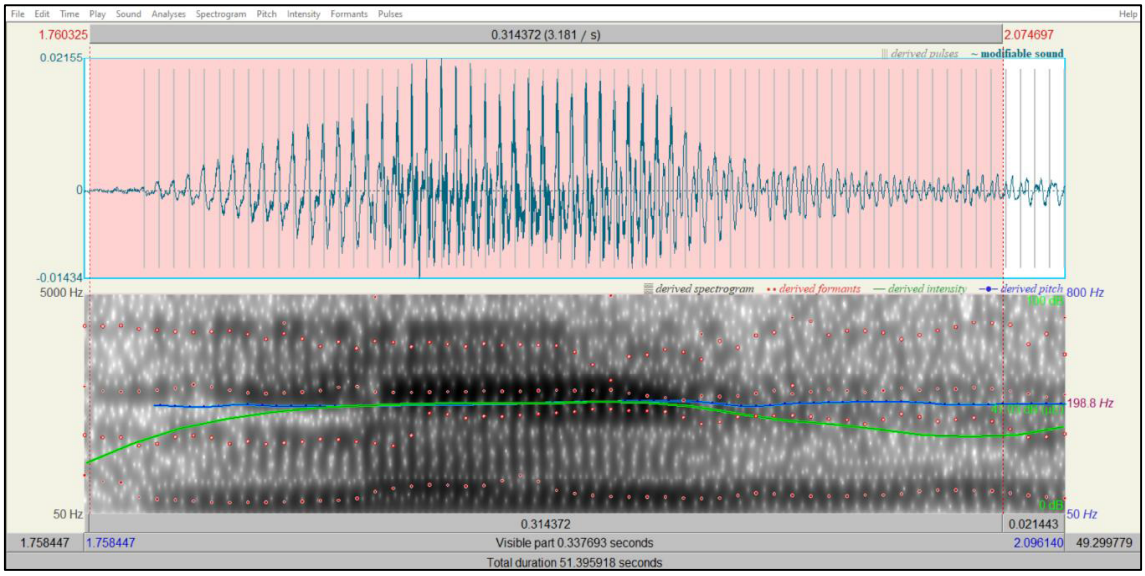

Figure 2: PRAAT Waveform Window.

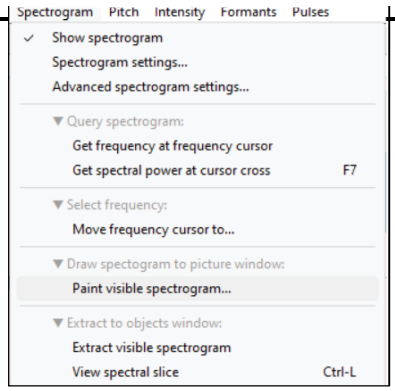

Figure 3: PRAAT Spectrogram selection dropdown.

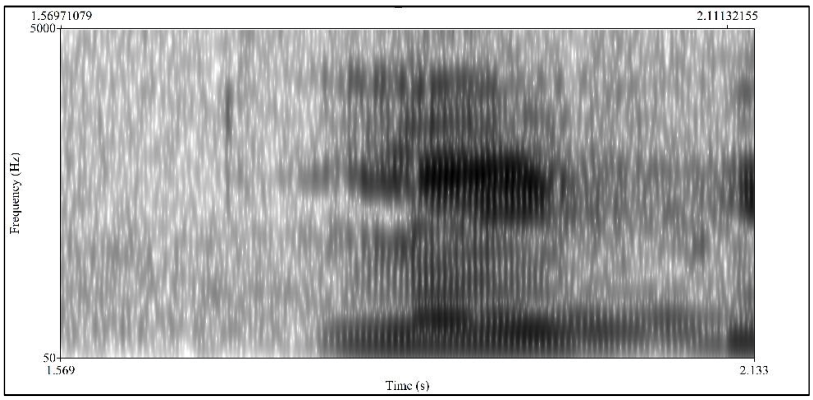

Figure 4: Spectrogram.

PLOT GENERATION FORMANT, PITCH, INTENSITY

After creating the spectrogram, the comparison plots for formants, pitch, and intensity were generated using PRAAT software. For that, first, the waveform of the word needed is selected.

Then the steps involved in creating the plots were as follows:

Formant Comparison Plot

- A Formant plot was generated for the first word in the original voice sample by selecting 'Draw visible formants contour' from the 'Formants' option in the waveform window (Figure 5).
- A dialogue box appeared named 'Draw visible formant contour' (Figure 6); check the option 'Erase first.' and click 'OK.'
- The formant plot for word 1 in the original voice was generated.
- To add the formant plot for word-1 for the AI voice in the same plot, 'Draw visible formants contour' was selected from the 'Formants' option in the waveform window of the AI voice.
- A dialogue box appeared named 'Draw visible formant contour'; uncheck the option 'Erase first.' and click 'OK.'
- The formant plots for word-1 for the original and AI voices were created in the same chart. (Figure 7)
- This formant comparison chart was saved in PNG format with sample labeling.
- These steps were repeated for word 2 and 3 formant comparison plots.

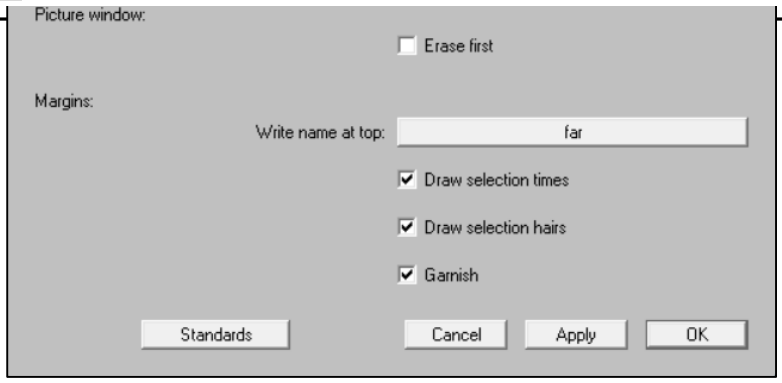

Figure 5: Selection Dropdown.

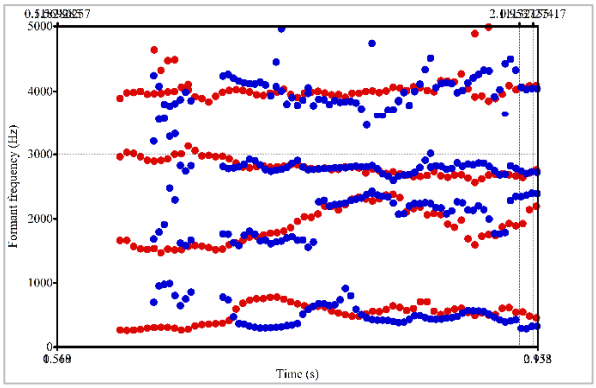

Figure 6: Draw visible formant contour - dialogue box.

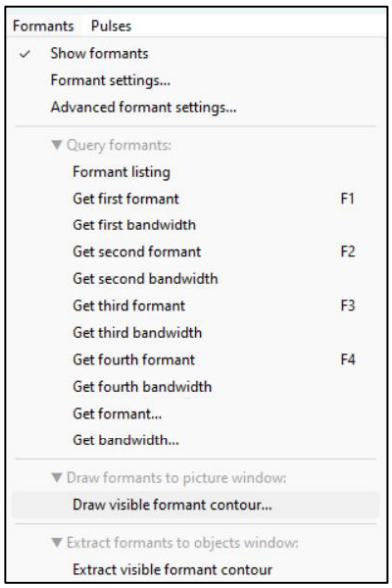

Figure 7: Formants Plot

Pitch Comparison Plot

- The pitch plot was generated for the first word in the original voice sample by selecting 'Draw visible pitch contour' from the 'Pitch' option in the waveform window.
- A dialogue box appeared named 'Draw visible pitch contour'; check the option 'Erase first.' and click 'OK.'
- To add the pitch plot for word-1 for the AI voice in the same plot, 'Draw visible pitch contour' was selected from the 'Pitch' option in the waveform window of the AI voice.
- A dialogue box appeared named 'Draw visible pitch contour'; uncheck the option 'Erase first.' and click 'OK.'
- The original and AI voices' pitch plots for word-1 were created in the same chart. (Figure 8)
- This pitch comparison chart was saved in PNG format with sample labeling.

- These steps were repeated for words 2 and 3 to create pitch comparison plots.

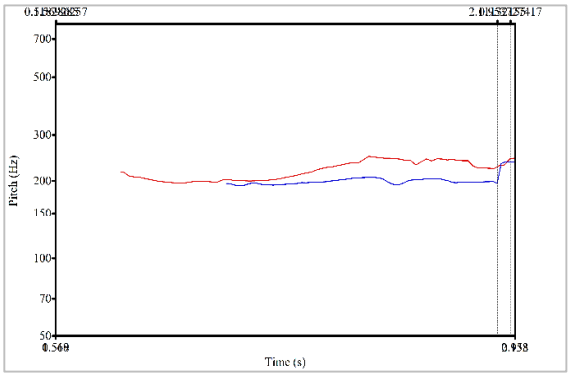

Figure 8: Pitch Plot.

Intensity Comparison Plot

- The intensity plot was generated for the first word in the original voice sample by selecting 'Draw visible intensity contour' from the 'intensity' option in the waveform window.
- A dialogue box appeared named 'Draw visible intensity contour'; check the option 'Erase first.' and click 'OK.'
- To add the intensity plot for word-1 for the AI voice in the same plot, 'Draw visible intensity contour' was selected from the 'intensity' option in the waveform window of the AI voice.
- A dialogue box appeared named 'Draw visible intensity contour'; uncheck the option 'Erase first.' and click 'OK.'
- The original and AI voices' intensity plots for word-1 were created in the same chart. (Figure 9)
- This intensity comparison chart was saved in PNG format with sample labeling.
- These steps were repeated for words 2 and 3 to create intensity comparison plots.

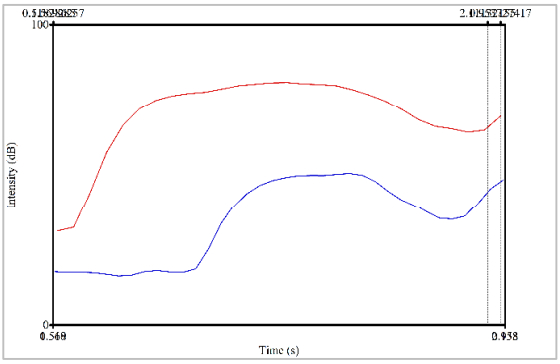

Figure 9: Intensity Plot.

LISTING'S GENERATION – FORMANT, PITCH, INTENSITY

The formants, pitch, and intensity listings were required to generate the Gaussian distribution plot and calculate the likelihood ratio. For that, first, the waveform of the word needed is selected.

Then the steps followed for generating listings are as follows:

Formant Listing

- The formant listings for word-1 of the original voice sample were downloaded by selecting 'Formant listing' under the option 'Formants' in the waveform window. (Figure 10)
- The formant listings were displayed in the 'Praat Info' window (Figure 11). They were saved with the labeling of sample numbers.
- These steps were repeated for words 2 and 3 to download the Original and AI voice sample listings.

Pitch Listing

- The pitch listings for word-1 of the original voice sample were downloaded by selecting 'Pitch listing' under the option 'Pitch' in the waveform window. (Figure 10)
- The pitch listings were displayed in the 'Praat Info' window (Figure 11). They were saved with the labeling of sample numbers.
- These steps were repeated for words 2 and 3 to download the Original and AI voice sample listings.

Intensity Listing

- The intensity listings for word-1 of the original voice sample were downloaded by selecting 'Intensity listing' under the option 'Intensity' in the waveform window. (Figure 10)
- The intensity listings were displayed in the 'Praat Info' window (Figure 11). They were saved with the labeling of sample numbers.
- These steps were repeated for words 2 and 3 to download the Original and AI voice sample listings.

Time_s	F1_Hz	F2_Hz	F3_Hz	F4_Hz
25.504209	316.127622	1397.826242	2730.666816	4191.169204
25.510459	422.808455	1432.036060	2740.869500	4279.917201
25.516709	355.682345	1395.102333	2795.342893	4337.354907
25.522959	324.273394	1285.267868	2813.701761	4163.009685
25.529209	344.309226	1212.066737	2718.643511	3998.012252
25.535459	377.580381	1261.899934	2711.088816	4054.538574
25.541709	370.019204	1365.707293	2764.129139	4043.120758
25.547959	317.417504	1315.838204	2703.186558	3983.553919
25.554209	283.376348	1283.075475	2710.413253	4032.441950
25.560459	301.800670	1054.998976	2664.484764	3962.771781
25.566709	459.915619	858.321395	2638.304776	3817.960312
25.572959	527.671430	1083.311943	2678.861786	4008.717039
25.579209	462.025085	1480.618425	2673.273912	4256.816383
25.585459	473.586928	1678.615791	2653.328558	4261.205456
25.591709	568.450441	1736.551665	2666.406658	4285.711934
25.597959	404.723589	1746.590275	2611.497291	4254.487894
25.604209	415.187614	1754.914023	2587.858219	3963.623980
25.610459	397.482526	1781.913579	2636.938548	3942.675585
25.616709	410.598046	1849.320693	2613.485171	3939.759664
25.622959	452.052674	1933.989180	2595.861037	3942.324884
25.629209	506.134336	2009.490545	2635.581972	3989.404418
25.635459	550.506132	2176.401449	2654.737024	3947.954865

Figure 10: Selection dropdown for downloading formant listing.

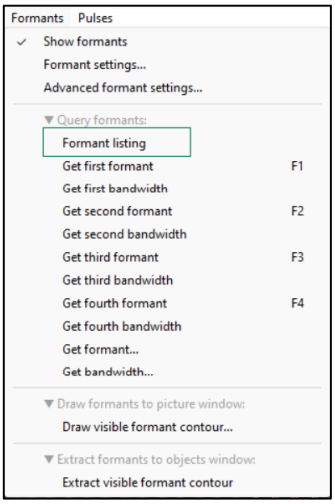

Figure 11 : PRAAT info window displaying formant listings.

GAUSSIAN DISTRIBUTION AND LIKELIHOOD CALCULATION – R PROGRAM

The plotting of Gaussian distribution and the calculation of the likelihood ratio for first formant, pitch, and intensity were calculated using R-programming software.

Probability Density and Gaussian Distribution:

The Gaussian distribution was plotted using the probability density calculated from the listings using R-program software. The mathematical formula to calculate the probability density function or normal distribution is as follows:

$$f(x) = \frac{1}{\sigma\sqrt{2\Pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2}$$

Where, f(x) = Probability density function

x = The listings of the first formant or pitch or intensity

σ = Standard Deviation

μ =Mean

A probability density function (PDF) is a function that defines the probability of a specific random variable to occur in a statistical distribution for a continuous variable. The PDF can be used to determine the likelihood of that observation occurring as a random variable.[41]

The probability density function is calculated for the first formant, Pitch, and Intensity listings for all three words of the Original and AI-generated voice of all ten samples. Then, a comparison Gaussian plot between the Original and AI voice was generated using R-program software for all three words and listing combinations.

Probability Density and Likelihood Ratio:

Since the probability distribution function can be used to determine the likelihood of that observation occurring as a random variable [41], the likelihood ratio between the occurrence of listings of original voice to the occurrence of listings of AI voice is calculated from the probability densities using R-program for all three words and listing combinations.

R program coding for Gaussian distribution and Likelihood ratio calculation:

R-programming software is utilized to plot the comparison Gaussian distribution plot between the Original and AI voice. Comparison plots of the first formant frequency, pitch, and intensity for all three words were created. Using this tool, the likelihood ratio was calculated as the ratio between the original listing's mean of probability density (or normal distribution) and the mean of the probability density of the AI listing; the listing could be of formant frequency, pitch, or intensity. As per Bayes theorem, the likelihood ratio is calculated as follows: [9]

$$\text{Likelihood Ratio} = \frac{P(E|H_0)}{P(E|H_{ai})}$$

Where ,

$P(E|H_o)$ is the probability of occurrence of evidence (any value in the listing) when the assumption is that the sample collected is of the original voice.

$P(E|H_{ai})$ is the probability of occurrence of evidence when the sample collected is not of the original voice. (which analogs the probability of evidence occurrence when the sample collected is of the AI voice).

The likelihood ratios were calculated between the original and AI voice for all three voices and listing combinations of all ten samples by incorporating this statistical relation in R-program coding.

Results:

The bar charts presented here summarize observations from the various comparison plots, such as spectral pattern, energy, formant, pitch, intensity, and Gaussian distribution.

SPECTROGRAM

SPECTRAL PATTERN

When visually comparing the spectrogram patterns between the Original and AI voice samples (Figure 12) for words 1,2 and 3 for all ten samples, it was found that about 80%,50%, and 90% of the spectrograms of words 1,2 and 3, respectively, showed a good similarity between the spectral patterns. The remaining percentage showed only slight or little similarity between the spectrograms of the Original and AI voice samples.

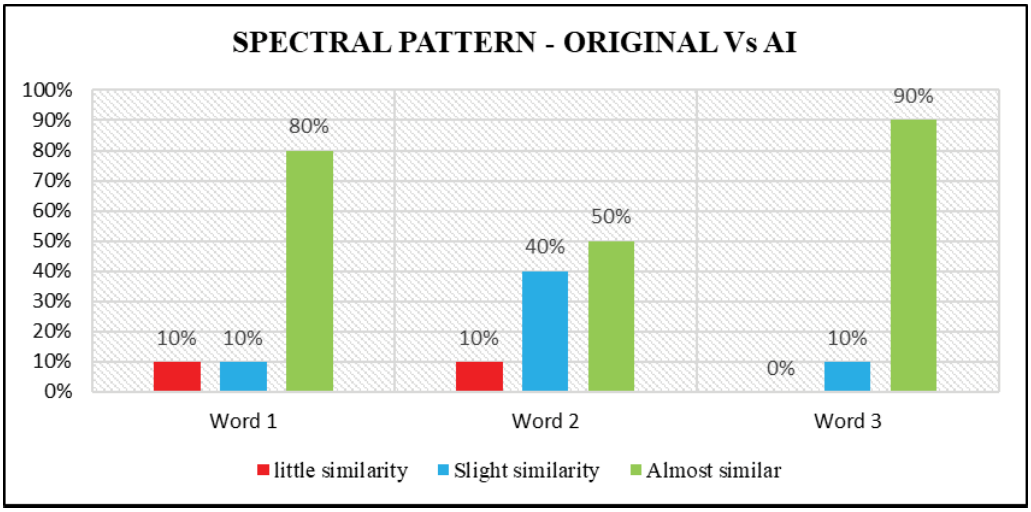

Figure 12 : Spectral pattern evaluation plot.

SPECTRAL ENERGY DISTRIBUTION

Visual examination of the Spectral energy distribution (Figure 13) of each word between original and AI voice samples indicated that a higher distribution of spectral energy was observed for AI voice compared to original voice for samples equal to 60%,50%, and 30% for words 1,2 & 3, respectively. For Original samples, percentages equal to 40%,40%, and

50% for words 1,2 &3, respectively, had a higher energy distribution than its corresponding AI voice spectrogram.

Energy distribution appeared to be similar between the Original and AI voice only for words 1 and 2, with a percentage of 10 and 20, respectively.

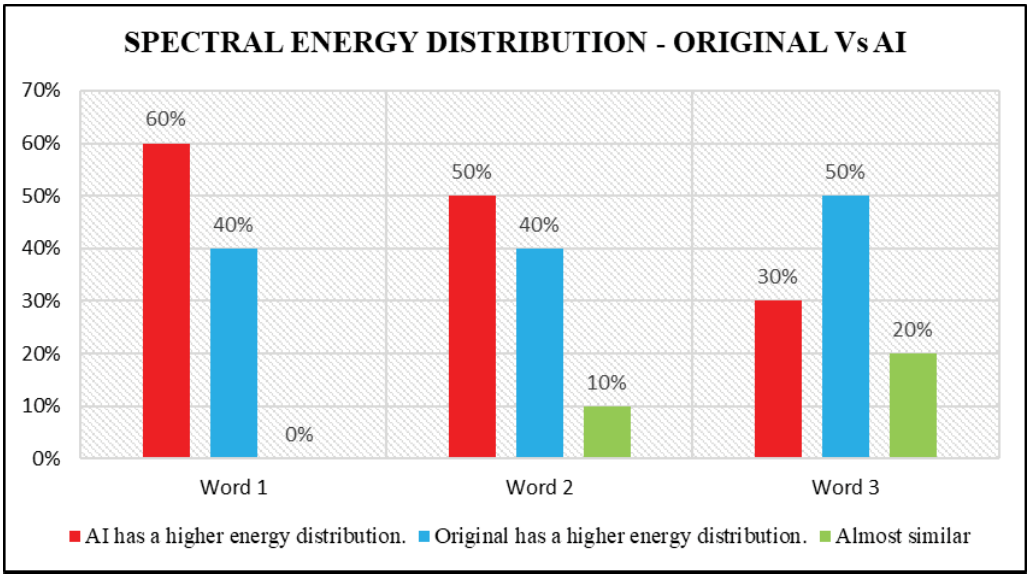

Figure 13: Spectral Energy evaluation plot.

FORMANT COMPARISON PLOT

Visual examination of the comparison plots between the Original and AI Voice (Figure 14) for all three words in all ten samples, the significant observation was that only 10%,30%, and 30% of samples in words 1,2 and 3, respectively, have some similarity in pattern and distribution. In all other scenarios, there is a difference in either pattern, distribution, or both. The plot pattern indicates the shape of the formant value spread across time for the specified word. The distribution of the formant suggests the extent of the formant value spread across time.

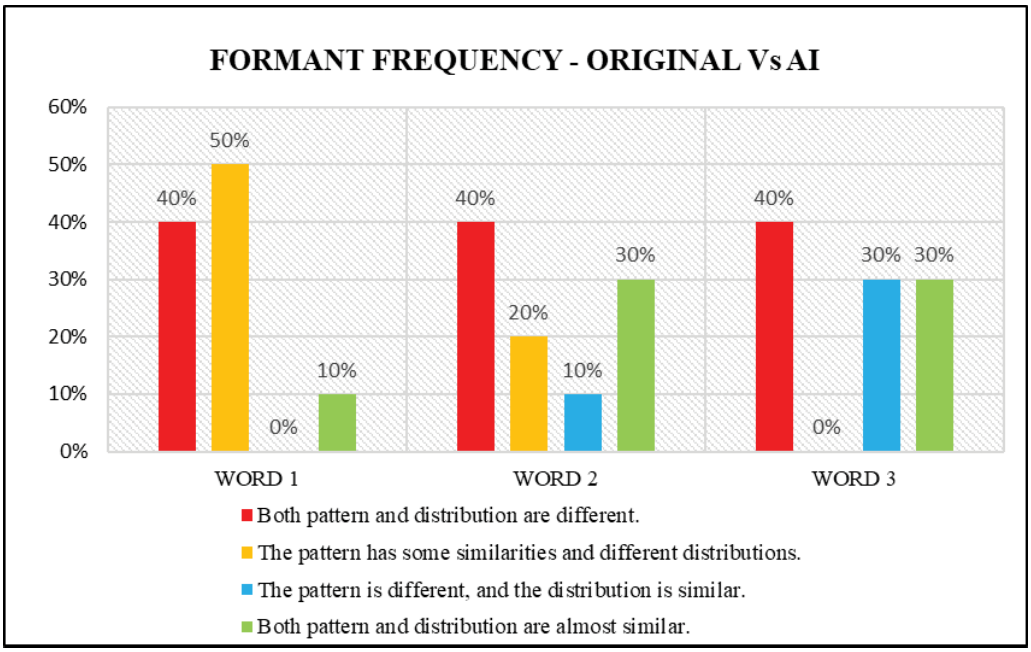

Figure 14: Formant frequency comparison plot.

PITCH COMPARISON PLOT

The visual examination of the pitch comparison plots (Figure 15) indicates a high degree of dissimilarity in the pattern and distribution of pitch between original and AI voices. About 80%,80%, and 100% of the samples in words 1,2 & 3, respectively, exhibit this characteristic. Only 20% and 10% percent samples in words 1 and 2, respectively, show a positive match in both the pattern and distribution of pitch.

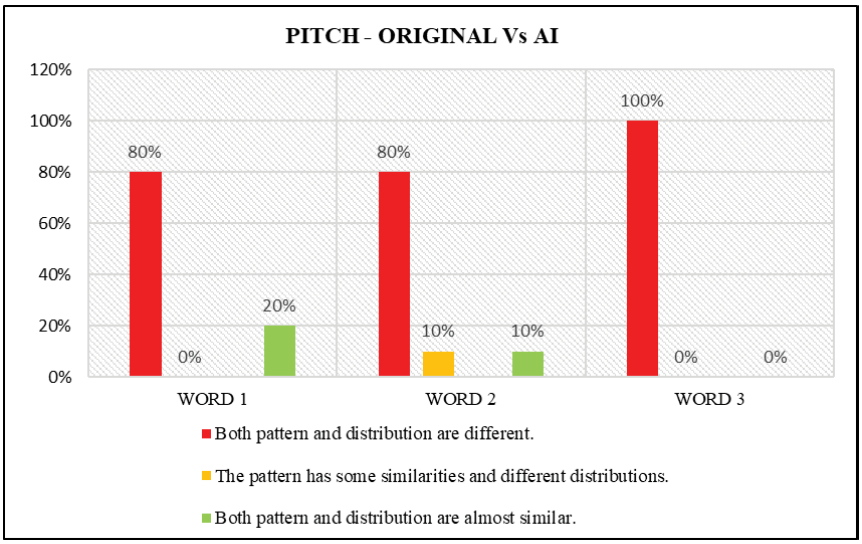

Figure 15: Pitch Comparison plot.

INTENSITY COMPARISON PLOT

The intensity comparison plots (Figure 16) show that none of the words exhibit any pattern and intensity distribution similarity between the Original and AI voice samples. About 50%,50%, and 70% of samples in words 1,2 and 3, respectively, show some similarity in the pattern but no similarity in the intensity distribution. The rest of the samples don't show any similarities in the attributes.

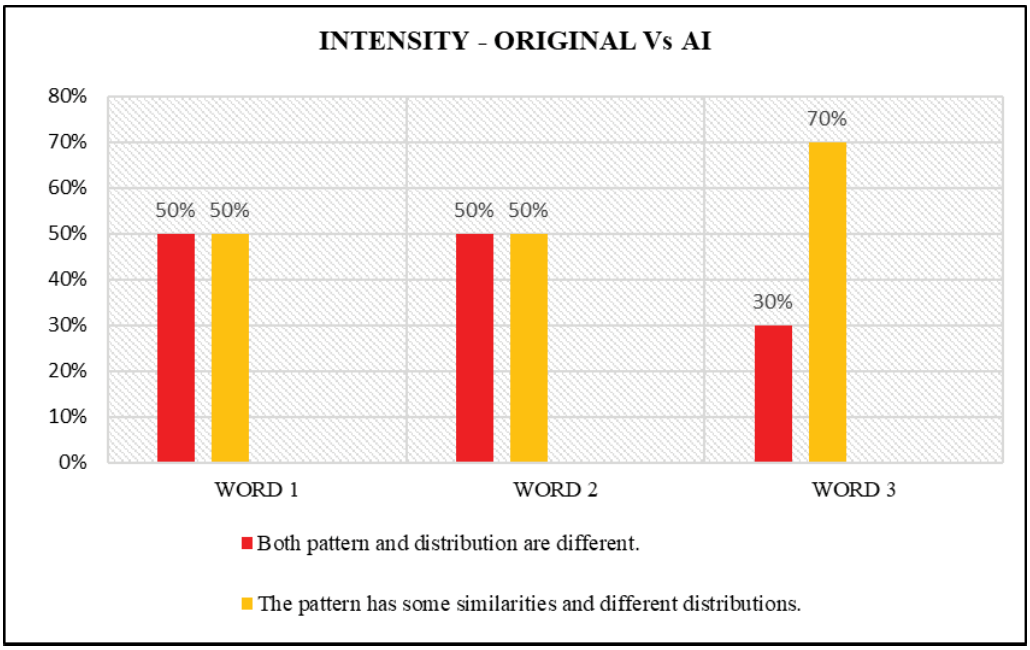

Figure 16 Intensity Comparison plot.

GAUSSIAN DISTRIBUTION

The Gaussian distribution evaluation charts are based on visually examining Gaussian distribution comparison plots between Original and AI voice samples for the first formant, pitch, and intensity listings of words 1,2 and 3 of all samples (Figure 17,18,19, respectively).

The Gaussian distribution of the first formant shows no similarity in 80%,50%, and 90% of the samples in words 1,2 and 3, respectively. Only 10% of the samples show a strong similarity in the distribution of the probability densities of the listings of first formants in words 1 and 2. The remaining percentage indicates only a slight similarity in the Gaussian distribution.

In the case of pitch, only 20% of the samples in word 2 show strong similarity in the Gaussian distribution pattern. The remaining percentage contributes to either slight similarity or no similarity.

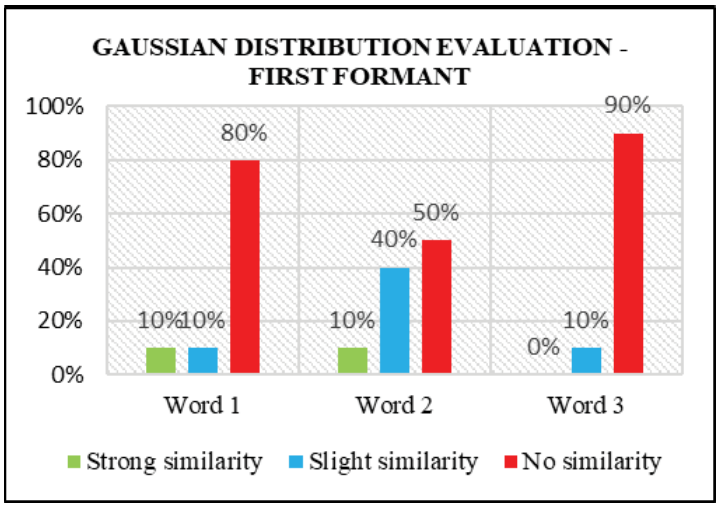

Figure 17: Gaussian disribution evaluation plot for first formant.

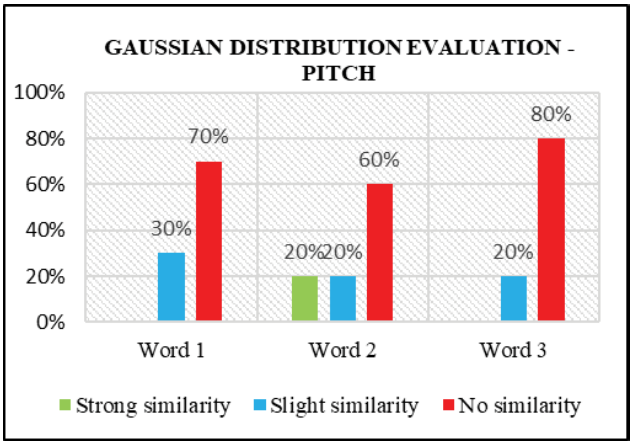

Figure 18: Gaussian distribution evaluation plot for Pitch.

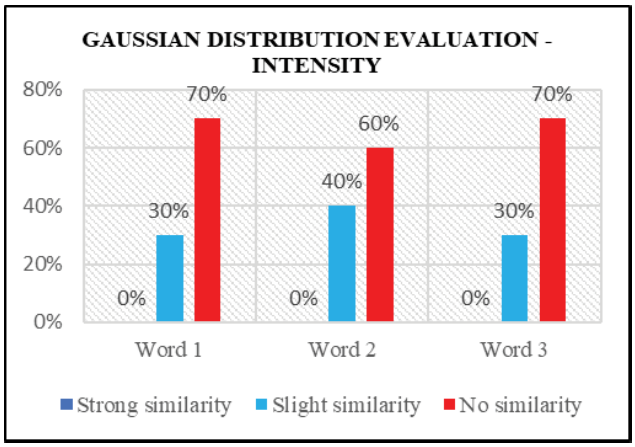

Figure 19: Gaussian distribution evaluation plot for Intensity.

Discussion:

The results of the observations of spectral analysis, Formant comparison, Pitch comparison, intensity comparison, and Gaussian distribution are summarized below:

SPECTROGRAM

The spectral pattern analysis (Figure 20) shows good similarities between original and AI-generated versions in the spectral pattern for 73% of voice samples, irrespective of the words.

However, there is a significant difference in the spectral energy distribution (Figure 21). Only 10 percent of original and AI samples show good similarities in their spectral energy distribution.

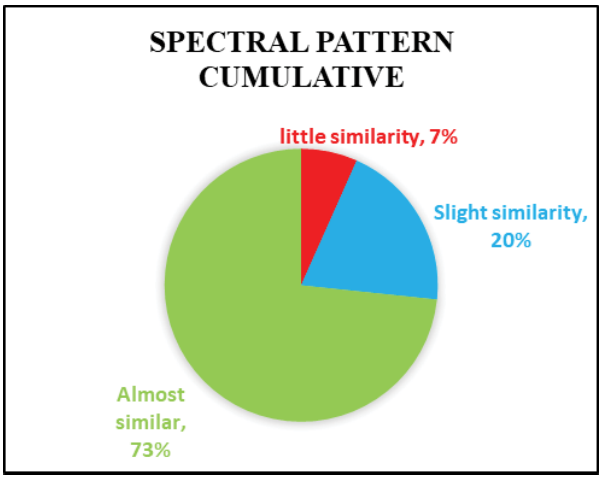

Figure 20:Spectral pattern cumulative chart.

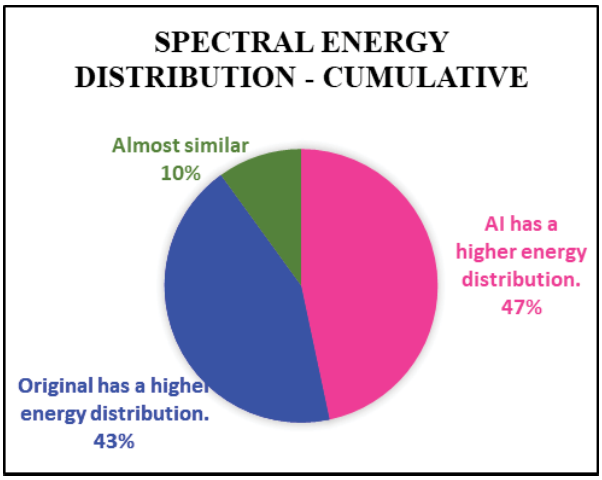

Figure 21: Spectral energy distribution cumulative chart.

So, from the observed results, the comparative study of spectral patterns and energy distribution visually alone could not provide a conclusive result on identifying the original voice from the AI-generated version.

FORMANT, PITCH AND INTENSITY PLOTS

The cumulative result of the observations of the formant, pitch, and intensity plots (Figure 22,23,24 respectively) as discussed below:

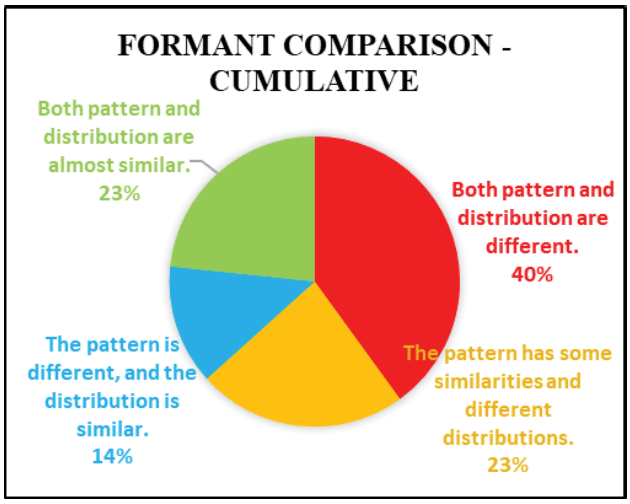

Figure 22: Formant comparison cumulative chart.

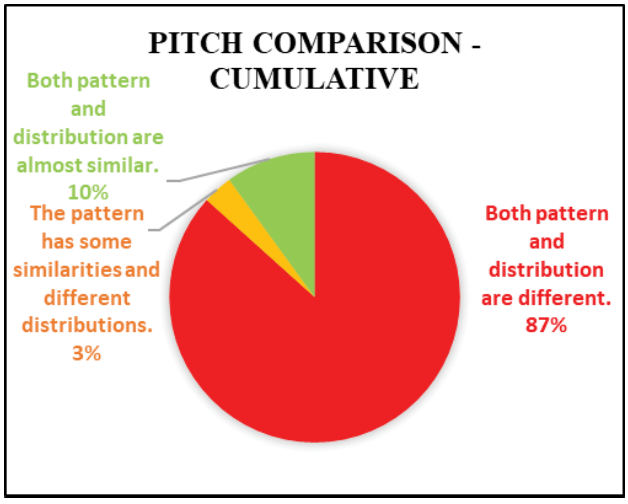

Figure 23: Pitch comparison cumulative chart.

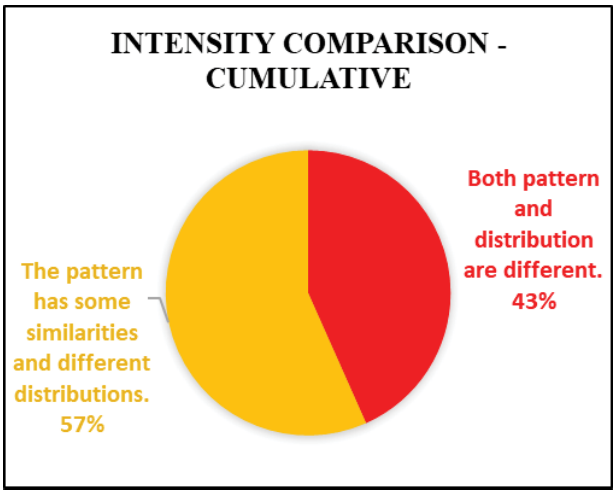

Figure 24 Intensity comparison cumulative chart

Irrespective of the words spoken, there was only a tiny percentage of good similarity present in formant and pitch pattern distributions between Original and AI voice samples, which were 23% and 10%, respectively. In all other scenarios, the remaining percentage indicates a dissimilarity in any attribute, such as pattern, distribution, or both.

These results indicated a much more distinguished identification of the original voice from the AI voice.

GAUSSIAN DISTRIBUTION

The stacked plot (Figure 25) represents the cumulative observations from the Gaussian distribution comparison plots of the first formant, Pitch, and Intensity.

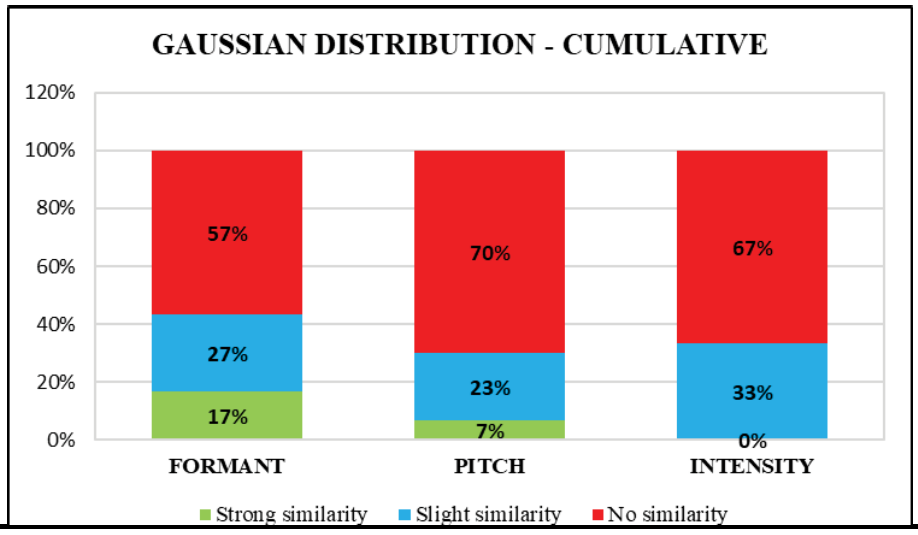

Figure 25 : Gaussian distribution cumulative plot.

The plot shows that the probability distribution of the first formant frequency values of 57% of samples, irrespective of words, doesn't show any similarity. Even the 27% of samples had only very slight similarity with the Gaussian distribution of the original and AI sample's first formant frequency. The variation in the Gaussian distribution differentiates the original voice sample from AI more specifically.

Regarding pitch, the similarity between the original and AI further reduces to 7% of samples. Substantial dissimilarity contributes to 70% of the samples. Again, it supports the differentiation of original from AI voice samples.

The Gaussian distribution for intensity pattern doesn't even show a good similarity with any of the samples. About 60% of the samples show no similarity, and 33% percent show a slight similarity in the Gaussian distribution patterns. These outcomes again reinforce the analysis in discriminating AI-generated voice from original voice

LIKELIHOOD RATIO

Based on the probability density, the likelihood ratio was calculated using R-programming, and the results are tabulated in Table 1.

Table 1: Likelihood Ratio Data

LIKELIHOOD RATIO DATA									
SAMPLE	FORMANT			PITCH			INTENSITY		
	WORD 1	WORD 2	WORD 3	WORD 1	WORD 2	WORD 3	WORD 1	WORD 2	WORD 3
S1	0.9782311	0.9977159	1.368051	0.6611928	1.036727	5.033697	0.8489	0.8648697	1.228568

S2	0.8398547	0.8378328	0.6376015	1.08615	0.9835483	0.6791746	1.528284	0.8344763	0.6798696
S3	0.8624319	1.415927	1.396095	2.428046	1.075643	1.315104	0.8304154	1.258012	1.21954
S4	1.599515	0.9629501	0.4777549	0.6871215	1.145185	1.545867	1.649745	0.5155342	0.6876072
S5	0.97402	4.544061	1.127296	1.267497	1.102124	7.365821	2.013417	0.7909165	0.7696874
S6	0.9534559	0.9154373	1.282355	1.080057	1.141629	2.400488	1.87549	0.8876278	1.240185
S7	1.585784	0.8871605	0.7181881	1.636332	0.8002836	1.098958	1.868894	0.5816832	0.9129216
S8	0.6363433	0.7178183	0.6189196	1.058524	0.5936783	1.42596	1.123594	0.5718159	1.122612
S9	0.4582769	1.037488	0.5698626	4.754793	0.9097266	0.4947676	1.517711	0.7575349	0.7952296
S10	0.434798	1.110859	1.331242	1.703263	2.230873	1.129453	1.463498	1.104466	0.8997316

Since the likelihood ratio is calculated based on the ratio between the probability density of the listings of original and the probability density of the listings of AI voice samples, a value of 1 for the likelihood ratio indicates a complete similarity between the original and AI-generated voice sample listings. Any value greater or lesser than 1 suggests a dissimilarity, either due to a decreased probability density value of AI voice sample (LR>1) or an increased probability density value of AI voice sample (LR<1) compared to the probability density value of original voice sample for features such as first formant frequency, pitch, and intensity.

The distribution of the likelihood ratio for different features is given in the charts below. The black line indicates the required value of ‘1’ for the complete similarity between the original and AI voices.

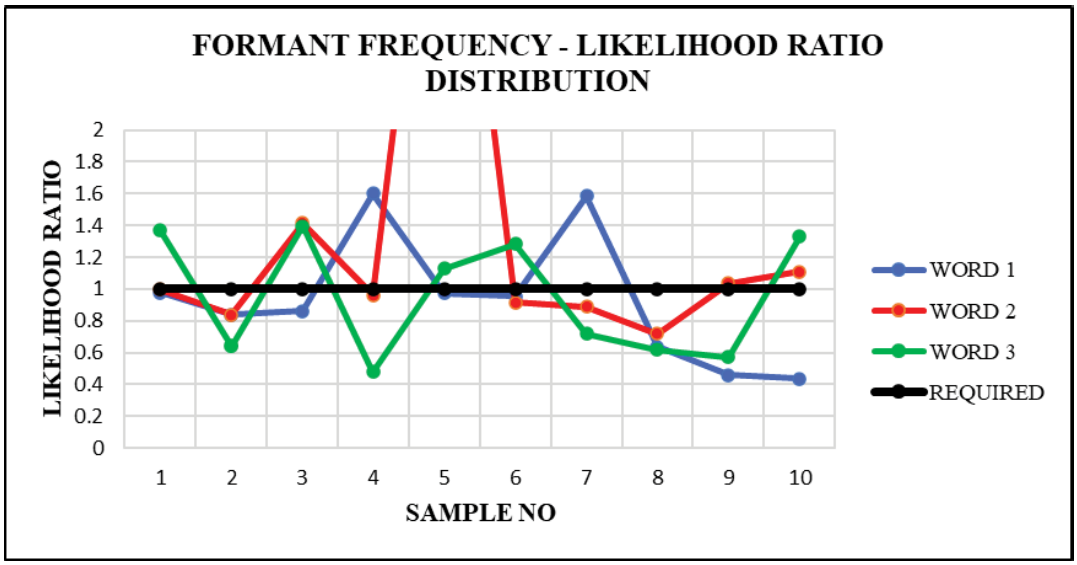

Figure 26: Formant frequency-likelihood ratio distribution.

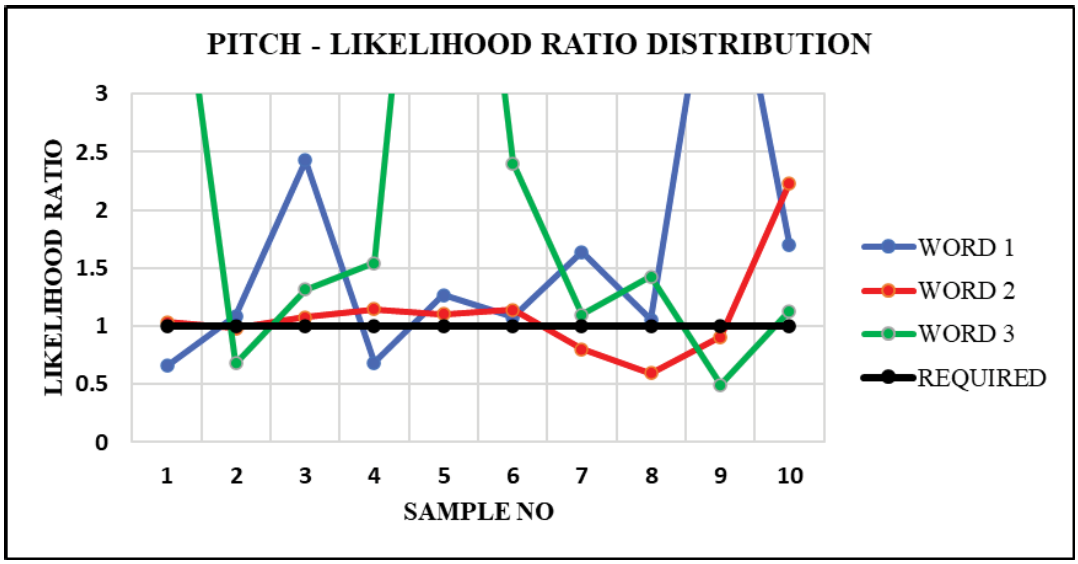

Figure 27 : Pitch-likelihood ratio distribution.

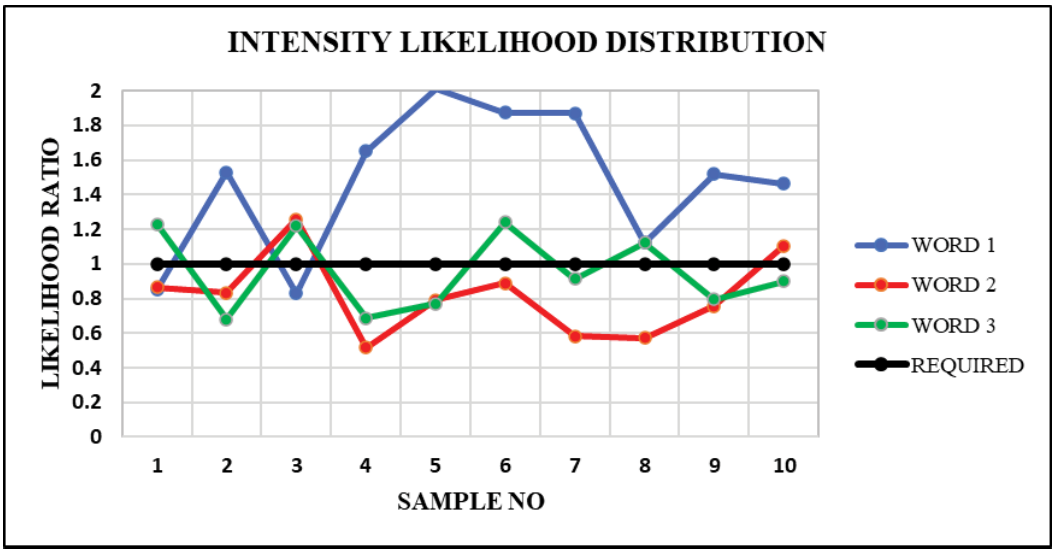

Figure 28: Intensity – likelihood ratio distribution.

The primary observation from all three charts (Figure 26,27,28 respectively) points to a variation in LR away from '1' on the positive and negative sides, showing a dissimilarity in the original and its corresponding AI-generated voice except for a few samples.

LIKELIHOOD RATIO SUMMARY

By analyzing the likelihood ratios for first formants, pitch, and intensity from Table 1, the outcome in Figure 29 was obtained, which throws light on how far there is similarity or dissimilarity between the original and AI voice samples.

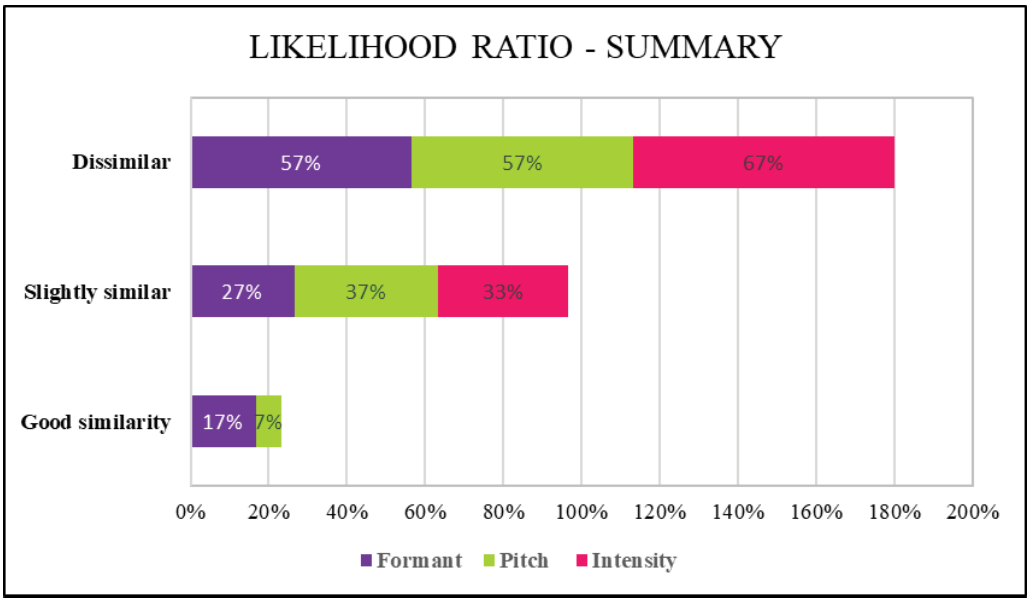

Figure 29: Likelihood Ratio summary.

Good similarity is obtained in first formant frequency between original and AI samples, which accounts for only 17% of the sample irrespective of the words and only 7% in terms of pitch.

The likelihood ratio for most samples reveals that the original and AI voices are dissimilar. The percentage amounts to 57 for formants,57 for pitch, and 67 for Intensity.

The slightly similar category has a higher chance of becoming dissimilar than good similarity, which is evident from their Gaussian distribution.

Conclusion:

Since there is a surge in cases involving AI-cloned voices, which are used to deceive innocents for financial benefit by impersonation, it is high time that we find out various simple techniques to discriminate such cloned voices to aid in the investigation process. This study gains relevancy as it utilizes traditional, widely accepted techniques with statistical backup in discriminating AI-generated voices, which are easy to use and cost-effective. The study compared the original voice with its AI-generated version in terms

of its spectrogram, formant frequency, pitch, and intensity with the help of statistical tools such as Gaussian distribution and likelihood ratio.

The spectrographic analysis compared the spectrogram's spectral pattern and energy distribution for the three selected words for all ten original voice samples and their corresponding AI-generated voice. Most of the samples showed similarity in the spectral pattern but a variation in the energy distribution. The comparative study of the formant, pitch, and intensity plots revealed a lesser similarity between the Original and AI voice in those features, with some exceptions in a few samples. The study of Gaussian distribution plots gave much more clarity on the distribution of values of these features, which pointed out the dissimilarities and similarities. Most samples showed a dissimilarity between Original and AI-generated voices concerning their Gaussian distribution.

Based on various analyses of the original and its AI-generated version of voice samples, it was evident that spectrograms alone will not distinguish AI-generated voice samples from the original ones. Formant, pitch, and intensity patterns and distributions must be analyzed along with spectrograms to clarify the distinguishing. To strengthen the identification process, Gaussian distribution plots were supported to a more significant extent. The calculation of likelihood ratios helped determine the extent of similarity and dissimilarity between features such as first formants, pitch, and intensity, adding strength to the differentiation. The study possesses an accuracy of 92.2% (Refer to Appendix 7) in positively differentiating the original voice sample from its AI-generated version. Hence, the methods above can be utilized as an assessment tool to distinguish a suspected AI voice sample from the original.

Acknowledgement:

I want to express my sincere gratitude to my mentors, peers, and everyone who contributed directly or indirectly to make this study fruitful.

Conflict Of Interest: No conflict of interest exists.

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Single Nucleotide Polymorphism

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Abstract

A Single Nucleotide polymorphism, also known as simple nucleotide polymorphism, Each SNP represents a difference in a single DNA building block called as a nucleotide. SNPs are genetic markers category then can be apply for making genetic profile information that may outcome in new investigative shows and human recognise determination. And also SNPs have been witness to useful in the forensic field which often feature cheapen samples. SNPs are used for individualizations. SNPs occur normally all over a person's DNA. They occur nearly once in every 1,000 nucleotides on mean, which means there are around 4 to 5 million SNPs in a person's genome. SNPs are patrimonial variations at defined zones and occur in at least 1 % of the citizens. DNA extraction from demanding forensic samples, such as bones or meat, own technical trouble. SNPs allow treasures information on geographical origin and individual recognitions of unknown humans, plants and microorganisms samples. The most common form of a SNP is via a transition mutation, where a purine is return with a purine (e.g., A to G or G to A) or a pyrimidine is return with a pyrimidine (e.g., C to T to T to C). Single- nucleotide polymorphism are polymorphisms of a DNA sequence led to a single nucleotide variation at the genomic level between humans. SNP act as a chromosomal tag to certain zones of DNA, and these zones can be study for variant that may be connected with a human illness or disorder. SNPs found to be connected with illness may be useful for features purposes. SNPs can be

used to recognise the site of genes on chromosomes. SNPs occur in both coding and non-coding zones of the genome. SNPs are not spread uniformly over the genome. A vast number of SNPs are spread all over the non-coding region of the genome. SNPs can act as biological markers, helping scientists find genes that are connected with illness. single nucleotide polymorphisms are soon known to as base substitutions which were contracted in the molecular community as SNPs, these types of polymorphisms have always a newer authority in molecular biology too.

Keywords: Single nucleotide polymorphism; DNA; Forensic; Markers;

T

Introduction:

Short-tandem repeat (STR) figures are generally used in forensic investigations and have been used for decapods in the forensic field. STRs are well settled with civil databases of STR figures based on basis STR markers. Another character of genetic marker, single-nucleotide polymorphisms (SNPs) have been authorized advantageous in the forensic field, which regularly aspect depraved samples. Although a bigger number of SNPs are appropriate to meet the same favouritism power as STRs, SNPs give Electrophoresis several conveniences over STR profiles. There are an enormous number of SNPs (e.g., in millions) in each chromosome in the human genome, much bigger than the number of STRs. SNPs grant for lineage model ahead first- degree relatives with acutely low mutation rates (i.e., 10−8). Single nucleotide polymorphisms (SNPs) are the maximum accepted form of genetic changes in the human genome. Occurring almost one in every 1000 bases, SNPs are actually agreeable to analysis by next generation sequencing NGS. certainly, with whole genome sequencing (WGS), counting microarray technologies, hundreds of thousands to millions of SNPs can be assumed per sample in a single investigation. Microhaplotypes, combined of various closely related single nucleotide polymorphisms (SNPs) commonly inside a genomic area of 300 base pairs, offer a beneficial access for characterizing mixtures. In comparison to single SNP markers, microhaplotypes are multi-allelic loci, by that they are less concerned by allele sharing. The coding region SNP (cSNP) based on these mRNAs afford more instructions than mere body fluid identification. These

cSNPs have been enforced in deconvoluting alloy using both the capillary electrophoresis platform and MPS. SNP inquiry is a more conscious and auspicious method than STR inquiry. The shorter SNP PCR products (150 bp) and a low amount of DNA can give an entire figure for identification. SNPs are treated the third-generation forensic genetic marker as a result of their low mutation rate, which is especially significant in such as paternity testing and family searching. SNPs also attempt asset in the inquiry of degraded and mixture samples, as they have less amplicons and gives biallelic results (Sobrino et al. 2005). moreover, SNPs display the maximum level of polymorphism amid genetic markers due to their across- the- board circumstance in the human genome, best to a growing application of SNPs in forensic applications. It is no longer amazing that single nucleotide polymorphisms (SNPs) are being used in kinship testing along with STRs (Lareu et al. 2012; Grandell et al. 2016; Mo et al. 2016; Li et al. 2019). They are generally assigned and mean the most common genetic variation in the human genome. The small polymorphism of SNPs that is generally biallelic can be affected by high-throughput inquiry using microarrays or massively parallel sequencing (MPS) systems (Laframboise 2009; Davey et al. 2011), compassionate the use of a bigger number of SNPs in kinship testing analysis (Skare et al. 2009; Kling et al. 2012; Mo et al. 2018). Over the years, economically applicable or in-house SNP panels advanced for human identification have been calculated for their convenience in kinship testing. A set of 94 SNPs from an economical forensic panel showed an interest when both STRs and SNPs were connected in calculating the possibility ratio for kinship (Li et al. 2019).

Materials and Methods:

Characteristics of SNP Markers:

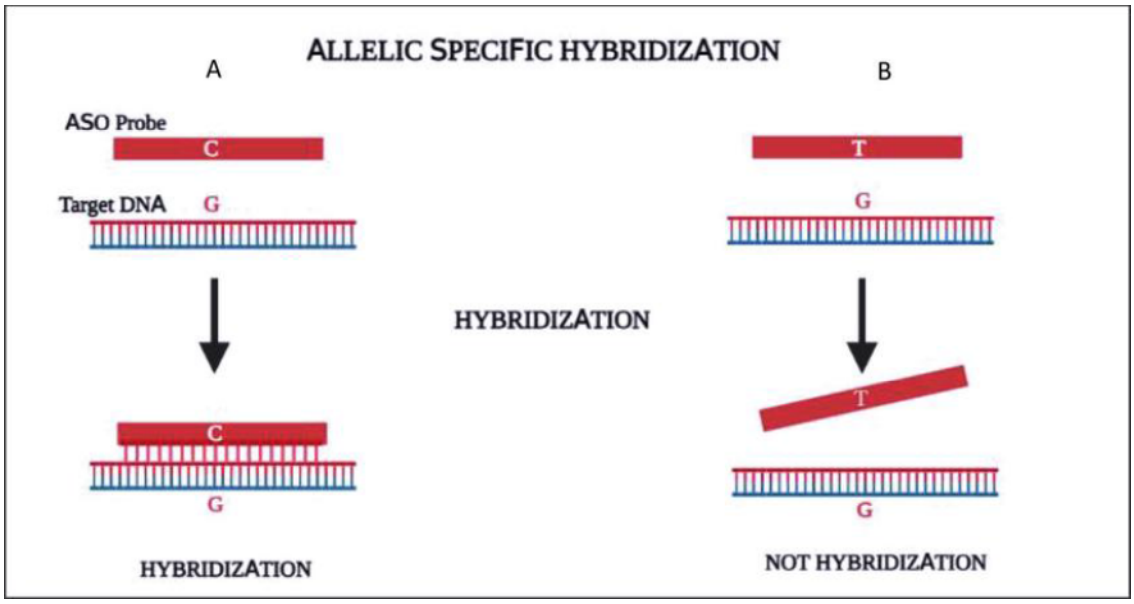
In a biallelic SNP marker, two available alleles are current in a population. Out of these two current alleles, one allele is called as minor allele and another one is called a major allele. Those alleles having a density of more than 95% are called major alleles, whereas alleles having density of <5%

are called minor alleles. Through biallelic SNP markers are commonly found in the human genome, tri allelic SNPs are rare in nature (Phillips et al.2020)

Techniques:

Allele-Specific Hybridization

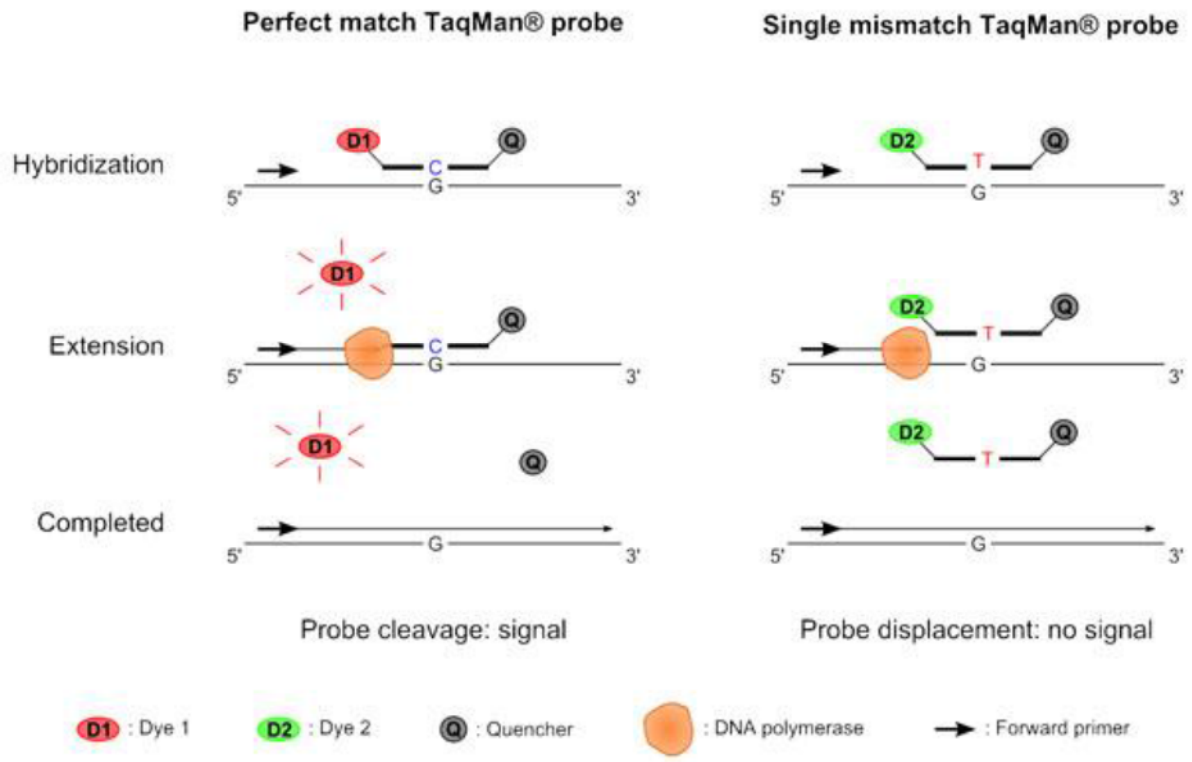
Allele-specific hybridization or allele-specific oligonucleotide hybridization (ASO) is the most accepted SNP typing method used in forensics exclusively in suspect identification at crime scene. The allele-specific oligonucleotide hybridization probe analyses SNPs on specific genetic loci. In this method, two probes are planned; each is specific to an SNP site and only the completely identical probe will hybridize to the target and is stable. Different detection methods have been popularized since the inception of allele-specific oligonucleotide hybridization method.



FRET-Based Methods

Fluorescence resonance energy transfer (FRET) is a circumstance in which the energy emitted by one fluorophore is consumed by another fluorophore in the closeness and the second flourophore is

excited by this energy transfer. In FRET based methods of SNP genotyping, allele-specific probes are directely associated to two different flourophores. The detection is done in real time on qPCR platform. There are certain abnormalities of the FRET-based chemistry. Frequently used TaqMan probe assay (ABI) is based on the 50- nuclease action of Taq polymerase all along PCR (Livak et al. 1995). Two TaqMan probes are designed for each SNP locus, one probe is integral to the wild-type allele and the other to the mutant allele. Each probe has different dyes conjugated to their 50 OH ends along with a quencher at the 30 OH end. When the probes are not conclusive to the internal DNA, the quencher quenches the fluorescence of the flourophore. During the PCR, only specific integral probe anneals to the template DNA and during extension step, Taq polymerase cleaves the 50 fluorescent dyes ensuing in rise in its fluorescence. Discrepancy probes which do not anneal to the template are not sundered by Taq polymerase offering no fluorescence. The genotype of the sample is disclosed by aligning the fluorescence intensity of dyes used for each probe. Other method based on FRET chemistry was advanced by Roche, the LightCyclar® method. In this process, two probes are calculated in such a way that they are established adjacent to each other on target SNP site. Probe 1 has fluorescein label at its 3 -end and probe 2 is associated to LC Red at its 50 -end. the probes find exact integral match, they hybridize adjoining to each other in closeness. First the fluorescein dye is agitated and the wavelength emitted is seized by LC Red for its own excitation. This energy transfer is only available when both probes are placed close to each other. When the PCR cycles development, the florescence intensity increases and is proportional to the extent of DNA coincidental during PCR. One of the main benefits of FRET methods is their real-time SNP detection and accordingly no post-PCR steps are compulsory. This allows ease of SNP genotyping with rigor but a main limitation of FRET-based methods is lack of multiplexing which makes this procedure expensive.



Array Hybridization

In this access, primer sequences are immovable on a matrix to form a microarray. These primers are SNP specific, and also the hybridization ability with the integral PCR product depends upon the oblique sequence at the (a) TaqMan assay technology (b) Schematic representation of LightCyclar chemistry 348 R. Saluja et al. SNP site. The target sample DNA is PCR coincidental and a fluorescent label (e.g. SYBR Green) is added along with PCR amplification. The characterized PCR products are then hybridized to the oligo microarray. The hybridization is probe-specific and the fluorescence is examined. This method gives parallel disclosure of many SNPs on a single array. One condition of this process is complicated in designing the excellent conditions for large multiplexing. The test probes include all possible sequences at the polymorphic site penultimate nucleotides

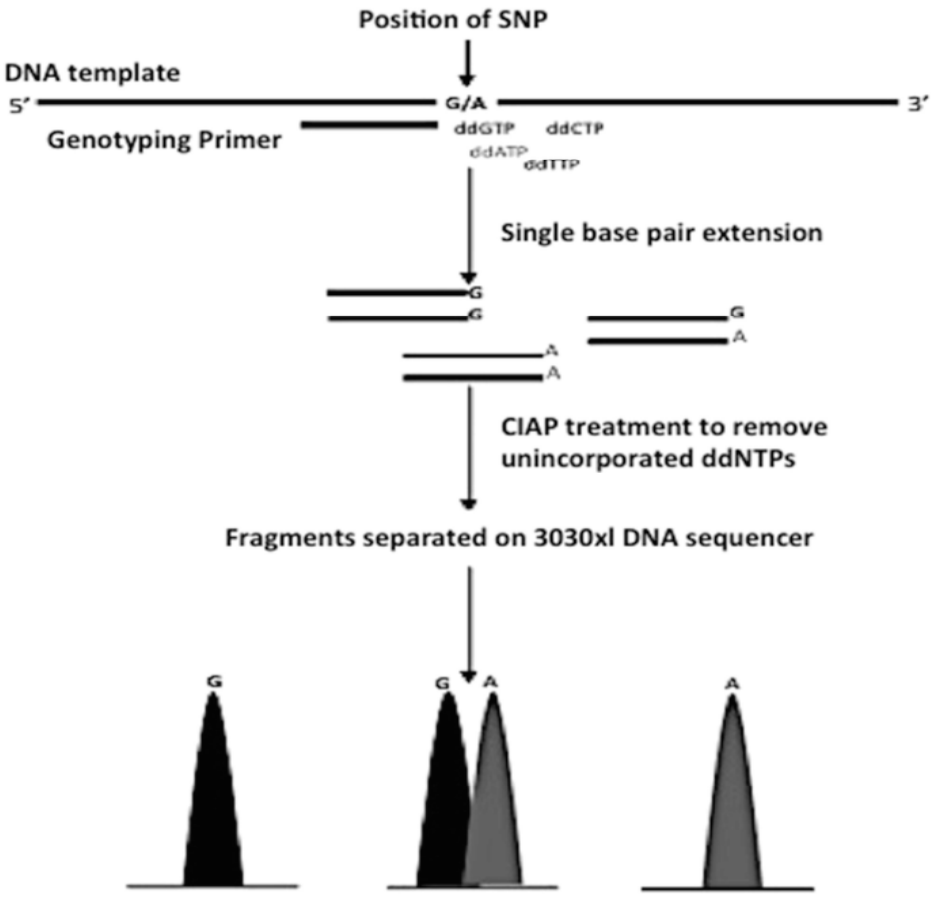
oblique the SNP, related to tiling array (Fodor et al. 1991; Pease et al. 1994). This approach can be used for typing large number of SNPs, effective current forensic demands.

MALDI-TOF Mass Spectroscopy

This matrix-assisted laser desorption ionization-time of flight method is a single base extension method in which the mass of the single based extension DNA fragment is consistent. The single based extension is done by DNA polymerase where an integral ddNTP and the continued DNA fragment is immovable onto a matrix over a chip or plate. The radiation are then fired on the matrix in a process called desorption which impact in the vaporization (ionization) of the matrix particles counting attached DNA. The DNA goes through the flight tube, where it goes to the detector under the influence of electric field. The TOF and joining the detector is directly proportional to the mass to charge ratio of the DNA fragment. The mass of single base continued is measured in a very rigor manner and the detection of nucleotide added is completed. The resolution of this method is very big as it is fully based on mass of the individual nucleotide added at the end of the DNA target. There are many accesses SNP typing based on MALDI-TOF chemistry.

SNaPshotTM Method by Applied Biosystem

The SNaPshot method is the equity of ABI and is the most accepted SNP detection method in Forensics. This is a single base extension PCR method in which the primer ends just penultimate to the SNP site. Only ddNTPs are used in the reaction, and each ddNTP is fluorescently labelled with exclusive dyes. When DNA polymerase performs PCR, the fluorescently labelled integral ddNTP is added and further extension is delayed. The single base extended fragments are removed and imaged in the capillary electrophoresis sequencer and the presence of homozygous/heterozygous allele is detected. SNaPshot can be multiplexed to 30-plex with ease conditional resilience and rigor to the process. Freshly Daniel et al. have connected SNaPshot with next-generation sequencing using Ion Torrent PGM (Life Technologies) system genotyping 136 SNPs in a single run.



Results & Discussion :

This review defined common methodologies for detecting SNPs promptly, while ideal SNP detecting methods must build on a combination of advances in biochemistry, engineering and analytical software. Such as, the third-generation-sequencing has been engaged in SNP analysis in many schemes as its increasingly practical technology and pyramidally certainty. By improving some specific gene fragments or assimilation existing methods, large-scale high-precision SNP detection methods can be settled. The physical and chemical properties of SNPs and their by-product, such as optical, electrical and magnetic properties, can be used to analyse new methods. It is hoped that an ideal SNP detection method will be advanced. certainly, the eventual goal of genomic studies is almost never detecting SNPs or effective SNP genotypes but rather to detect SNPs or genes that are identical with phenotypic trait(s) of interest. Accordingly, it is attractive to have statistical methods

that can incorporate concern in genotype calls, for consequent imputation and finally for corporation mapping. There is a rich recent article for testing rare variants detected in sequencing-based studies. In addition, population achievement, a potential confounder for corporation analysis, warrants further research in the new sequencing context It is unclear whether common genetic variants alone suffice for population infrastructure inference, or whether rare variants detected through sequencing can improve the rigor of ancestry inference, which would finally lead to decorated power in corporation analysis.

Conclusion

The aim of this study was to establish a detection techniques of SNP assay for mixture deconvolution. Most samples analysed in this study allowed allele and loci detection rates pasturing from 90% to above 99%. SNP typing over DNA inputs pasturing from 50 pg to 5 ng was highly fortunate with ostensible stochastic effects, and with intra-locus balance often much larger than the delinquency 50% intra-locus balance quality flag set in the UAS. The results from large input quantities of DNA were not over assorted on the flow cell because of normalization of the libraries previous to pooling. Samples with less than 1 ng DNA provided consequential genetic knowledge for human identification purposes. Snedecor, et al. The forensic application potential of the database was marked based on the polymorphism of shared loci in global and global areas, AIM selection, genetic distance inquiry, and mapping with a genome glossary file. Information gain was calculated for the different allele formats; results proved the advantages offered by using sequence-based alleles, related to common length-based nomenclature. In the molecular scheme, single nucleotide polymorphisms are previous known to as base substitutions which were brief in the molecular association as SNPs, these types of polymorphisms have always an advanced concern in molecular biology too. Absolutely, in appropriate cases the facts arising from the bi, tri or tetra-allelic character of SNPs is limited; there seem to be detail whereby they can determine major facts and information that accomplice relating exact genes or population genetic structures or genetic organization and

phenotypes. The high region/segment of SNPs in genome structure allows advancing some of these bp in an appropriate locus of a few hundreds of base pairs.

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Conflict Of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tracing Identity: Cheiloscopy Examination in Maratha's Population

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Abstract

In a crime scenario, traces of the lip prints are often observed at the crime scene. The study of lip prints is also known as Cheiloscopy, an emerging and valuable tool in forensic science. This method involves analysing the unique patterns of the lips, such as vertical lines, intersected lines, branched lines, and reticular lines. Lip prints also have some individualistic characteristics such as fingerprints. This study aims to explore the hereditary nature of lip print patterns which are passed from generation to generation. In this study, we have collected samples of 100 families, a family must include a father, mother and a child of the Maratha Population in Maharashtra. The lip prints were collected categorised and analyzed according to Suzuki and Tsuchihashi's classification. The study emphasized the non-invasive and cost-effective features of Cheiloscopy, making it an accessible means of identification verification. Specific patterns were observed in the Maratha population, underscoring the potential for creating a specialized database of lip prints. This study helped to reveal the unique characteristics of patterns of the Lip prints of the Maratha Population, as well as helped in analyzing the data and revealed the common patterns present on the lips of the family. The results will include the analyzed data of the most prominent pattern type in mothers, fathers and children, and the data of the most prominent common pattern type observed in Mother and Female Child; Father and Female Child; Mother and Male Child; Father and Male Child.

Keywords - Cheiloscopy; Hereditary; Maratha Population; Lip Prints.

Introduction

"Cheiloscopy is the study of the distinctive pattern known as the "lip prints," which are formed by the wrinkling grooves on the labial mucosa (also known as sulci-labrum)." Human identification via lip prints is the focus of a very new and unrecognised forensic investigative method called as Cheiloscopy[3]. Much like fingerprints, lip prints are distinctive to each person and can be impacted by ageing, trauma, surgery, cosmetic procedures, and way of life. The appearance and texture of the lips can change with age, and this might eventually change the pattern of the lip print. Lip surgeries or injuries can also affect the lip print by changing the curve of the lip or leaving scars. Furthermore, aesthetic operations like tattoos and fillers can change the natural lip curves and patterns. Lip prints can also vary because of the lifestyle choices like smoking, tanning, and biting your lips. Overall, lip prints are thought to be quite stable for identifying purposes; nevertheless, several events may cause changes to the lip print pattern. The texture and colour of the lips can be changed by skin disorders such as vitiligo, eczema, or psoriasis, which may also affect the pattern of the lip prints. Lip prints may be affected by inflammatory diseases like cheilitis or oral ulcers, which can also affect how the lips look-the lip print pattern. Although lip prints can be uniquely identified, differences in the print may result from underlying medical issues affecting the lips. Lip prints can be indirectly impacted by some disorders, so it is important to take lip health condition into account when analysing lip prints. The major principle of the lip prints is:- individuality, uniqueness, persistent. The basic principles of the lip prints are its unique characteristics, they are not similar in any other lip prints, have the persistence characteristics which makes it unique. The lip prints can be classified in many different types which can be based on characteristics such as vertical lines, the branched grooves, the intersecting lines, and the reticular patterns. The lip prints can persist on the surfaces like glass, fabric, or paper. It may also vary in terms like shape, size, fullness, and features (characteristics). The lip print patterns of monozygotic and dizygotic twins were compared, it was found that the former had greater similarities with the latter, concerning lip prints, except for monozygotic twins, which are innate, immutable even after death, and specific to each individual, they play a crucial role in

Cheiloscopy [3]. Forensic examination of lip prints involves analysis of the characteristics of lip prints. There are some types of characteristics analysed, class characteristics and individual characteristics. The lip prints characteristics which are common within the group of people and not encountered in the examination of a single person's lip prints are called class characteristics[7]. The lip print characteristics which are unique to a person are called individual Characteristics". Lip print characteristics most used today is given by two Japanese scientists, Y Tsuchihashi and T Suzuki, they gave the lip print classification on the basis of its basic characteristics and the arrangement of the grooves and the patterns formed on the lips. Overview of the Cheiloscopy, dates to the early twentieth century, when the study of the lip prints started for the identification of the individual. Being the first to recognise the uniqueness of lip prints for individual identification, French anthropologist Edmond Locard is credited with introducing the use of lip prints in forensic investigations in the 1930s. In the intervening period, scientists have worked to categorise lip print patterns, prove their accuracy, and investigate their potential uses in forensic investigations. In a variety of criminal cases—including those involving missing persons, sexual assaults, and other offences for which lip prints can yield important evidence—Cheiloscopy has been used. Cheiloscopy's accuracy and effectiveness in forensic practice have increased over time due to developments in research methodology and technology. Currently acknowledged as a useful forensic instrument, Cheiloscopy advances the discipline by providing extra methods of identification. Le Moyne Snyder was the first to describe and identify the furrows found on human lips in 1902. Yasuo Tsuchihashi and Kazuo Suzuki, two Japanese scientists, conducted research in Europe in 1961 and discovered individualistic lines. Between 1985 and 1997, Cheiloscopic procedures were applied in 85 cases, of which 34 had an identification. examinations conducted in India and other nations have validated the efficacy of Cheiloscopic examinations as a supplemental technique [2]. In the context of legal investigations into criminal activities, it is imperative to underscore the significance of uniqueness in facilitating the accurate identification of individuals. Recognizing people

caught up in civil, criminal, or mass disaster proceedings can be challenging. The most often utilised methods in this context, among the many others suggested, are most likely the comparisons of dental records, DNA, and fingerprints[4]. A key component of forensic science is accurately identifying live or deceased individuals based on the distinctive features of their teeth and jaws. Odontology, anthropometry, fingerprints, and other methods that aid in determining factors like gender, approximate age, and height are frequently used by investigators to gather data and evidence (odontology, forensic odontology) [23]. But one of the most fascinating new studies that have its roots in forensic and criminal investigations is “human lip recognition,” or Cheiloscopy (Caldas et al., 2007; Sharma et al., 2009; Reddy and Reddy, 2011) [4]. In a variety of criminal cases-including those involving missing persons, sexual assaults, and other offences for which lip prints can yield important evidence–Cheiloscopy has been used. Cheiloscopy’s accuracy and effectiveness in forensic practice have increased over time due to developments in research methodology and technology, currently acknowledged as a useful forensic instrument. Cheiloscopy advances the discipline by providing extra methods of identification. Since Cheiloscopy is the least invasive and most accessible mode for research purposes, most researchers have been quite interested in this field [12]. In cases of homicide where victims lack teeth or easily accessible dental records, lip prints can also be used to corroborate comparisons between dental records [16]. The following research question was formulated in accordance with the patient/population, intervention, comparison, and outcome (PICO) framework: "Is it possible to establish a familial relationship (outcome) between individuals (population) from the analysis of their lip prints (intervention)?" This led to the definition of a more focused review question: "Is the superficial framework of lip prints hereditary?" [1]. From the sixth week of intrauterine life onwards, lip patterns can be recognized. Lip prints are highly developed and recognizable during six weeks of fetal intrauterine life. When kept in a closed container with a temperature maintained at about 25 C, the longevity of a lip print on paper can last up to 12 weeks, even in the presence of ambient conditions. However, when it comes to glass, the 9 reliability of the print can last up to 12

weeks in the presence of ambient conditions. If taken within twenty-four hours after the death, clear and distinguishable lip prints can be acquired. A 1972 study involving two identical twins found that while their lip prints were different, the twins could not be distinguished from one another by any other way. Family members' lip prints can be analyzed to reveal that while children do inherit some of their parents' lip print characteristics, the locations of these lines vary, and no two prints—not even twins'—are exactly alike. The lip prints vary among all people depending upon the characteristics and the patterns observed in the Kleins region. The previous study conducted on the lip prints says that the female has Type I was mostly seen pattern in males and Type III was seen mostly in females; whereas Type I' was found commonly in both males and females. Studying this factors in Indian families shown the patterns which were most prevalently seen; Type I, Type I' and Type III. Studying these factors on the Maratha Population based on our knowledge of different patterns on the red part of the lips, Kleins area, enhances our knowledge on inheritance. The study of arrangement of patterns and grooves on the lips and the inheritance, this study gives light to the hereditary features of lip print patterns and grooves and their significant application in the contemporary technology. These study explains the hereditary factors observed in the lip print patterns in the family (mother, father and child).

Materials and Methods:

The lips' mucosal surface, known as the Klein's zone, is covered in wrinkles and grooves that create the distinctive lip prints and patterns [1]. The Federal University of Rio de Janeiro, Brazil's Clauco Martin Santos, a professor of forensic dentistry, initially categorized lip grooves into four types in 1967 [2,15]. A different lip print classification system was proposed by Suzuki and Tsuchihashi in 1970 [2,15]. Renaud, a French biologist, categorized and examined 4,000 lip prints. The groove organization method was used in 1979 to classify Afchar-Bayat lip prints [15]. Lip patterns were divided into 23 categories of distinct features by Kasprzak. In Cheiloscopy, lip

architecture, thickness, and location are also examined. Based on lip thickness, four categories of lips exist [15].

Simple types (formed by single element)	Composite types
Straight line	Bifurcated
Curved line	Trifurcated
Angled line	Irregular
Sine shaped line	---------

Table 1: Lip print classification by Clauco Martin Santos

Types	Characteristics
Type a	Complete vertical
Type b	Incomplete vertical
Type c	Complete bifurcated
Type e	Incomplete bifurcated
Type f	Incomplete intersecting
Type g	Reticular
Type h	In the form of sword
Type i	Horizontal
Type j	Other types

Table 2: Lip print classification by Renaud

A1: Vertical and straight grooves, covering the whole lip
A2: Like the former, but not covering the whole lip
B1: Straight branched grooves
B2: Angulated branched grooves
C: Converging grooves
D: Reticular pattern grooves
E: Other grooves

Table 3: Lip print classification by Afchar-Bayat

The basic classification characteristics of the lip prints are-

i) Grooves – The lines or the pattern formed on the lips are the distinct and unique patterns on the lips. These grooves or patterns can be classified into different types as– the straight lines, the branched lines, the intersecting lines.

Figure 1: Grooves

ii) Shape – The shape of the lips may vary from the person to person, which may influence the overall appearance of the lip print. These shape of the lip prints can be classified as thin, thick or full lips.

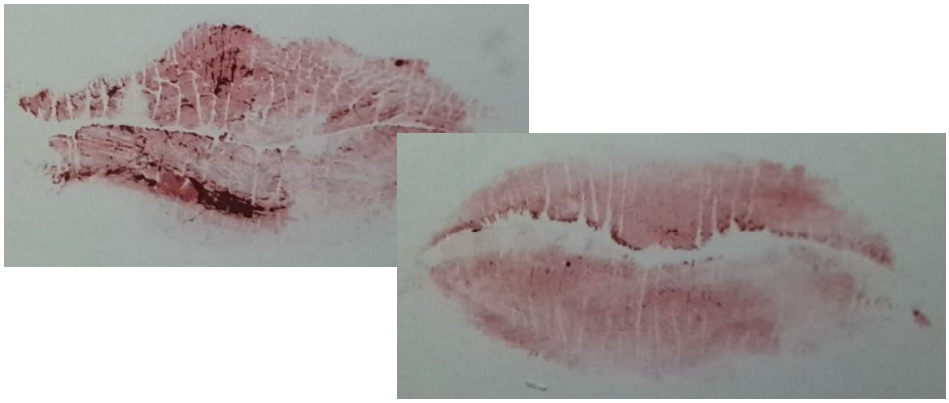

Figure 2: Shape

iii) Fullness – The fullness of the lips may affect the depth and the clarity of the lip print. An individual with the fuller lips may leave the detailed and defined lip prints compared to the lips which are thinner in shape.

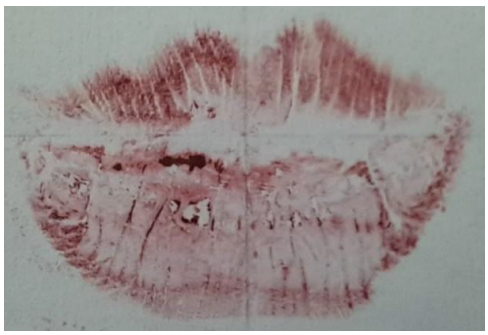

Figure 3: Fullness

The main classification of the lip prints was given by Y. Tsuchihashi and T, Suzuki as – Class characteristics. The individual classification characteristics we given by Kasprzak [10].

Type I	Complete Vertical lines
Type I'	Incomplete Vertical lines
Type II	Branched Groove
Type III	Intersected Groove
Type IV	Reticular Groove
Type V	Other Patterns

Table 4: Tsuchihashi and T Suzuki,

Class characteristics classification

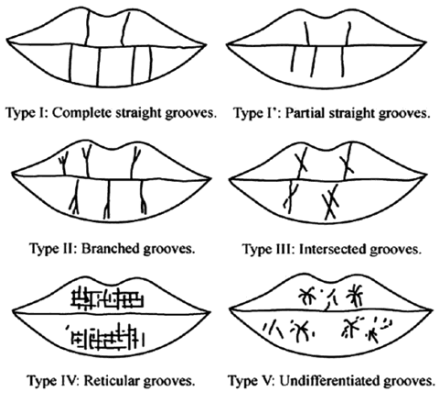

Figure 4: Tsuchihashi and T Suzuki,

Class characteristics classification

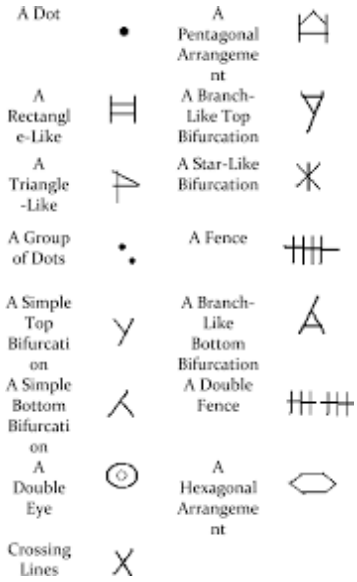

Figure 5: Individual characteristics features by Kasprzak

Materials Required-

i) Lip-stick

ii) Lip-stick remover

iii) Questionnaire

iv) Consent form

v) Tissue paper/cotton

Methodology-

This is the study conducted in Maharashtra among the Maratha population, all the subjects involved in the study were recruited after taking written informed consent from them. A total of 100 families were recruited in the study. A family includes mother, father and a child. Inclusion criteria for the study, subjects without any disease related to lips, with normal lip mucosa were included. Excluded criteria for the study, subjects having any diseases like congenital deformities of lips, and those with any inflammation, allergic to the lip stick used

and with any other kind of diseases were all excluded from the study. During the collection of lip prints, all the participants were informed about the study, its method and objectives were explained thoroughly to the subjects in details and they were made comfortable. Steps of collection of samples –

1. The consent paper was explained thoroughly and participants were asked to give signature on it, followed by the questionnaires.

2. The lips were cleaned and a thin-layer of the lipstick was applied on the lips, and they were asked to spread it evenly on the lips.

3. A blank page was placed on the lips and they were instructed to press their lips by applying pressure evenly on the page. All the lip prints collected were taken carefully without any damage in prints.

4. The prints collected were studied individually by using the Suzuki and Tsuchihashi classification, by using the magnifying glass lens and double check was done before the data entry.

Results:

The data collected was used for the generation of the profile, all the pattern types of the lips were noted down of all three members of the family; mother, father, and child. The most prominent pattern type was noted down of the family members. Each pattern type is denoted as a score. If a child has one, two and three similar pattern that of the parents, then it will be denoted as 1, 2 and 3 score card, respectively. Given below is the tabular chart representing the pattern types of child, mother, and father.

	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V
F1	1		1	1				1	1	1				1	1	1								
F2	1		1					1						1	1	1								
F3	1		1	1				1	1					1	1									
F4	1		1					1	1	1				1	1	1								
F5	1		1	1				1			1			1	1	1								
F6	1			1				1			1									1	1			
F7	1		1	1				1	1	1										1	1			
F8	1		1	1				1	1	1				1	1									
F9	1			1				1	1	1				1	1	1								
F10	1			1				1		1										1	1			
F11	1		1					1	1	1	1									1	1	1		
F12	1				1			1		1				1		1								
F13	1				1			1	1					1		1								
F14	1		1					1			1			1	1	1								
F15	1		1					1	1	1				1	1									
F16	1		1					1	1	1				1	1									
F17	1		1					1	1					1	1									
F18	1		1					1	1	1										1	1	1		
F19	1		1					1	1					1	1		1							
F20	1		1	1				1	1											1	1	1		
F21	1			1				1	1					1	1									
F22	1		1	1				1			1									1	1	1		
F23	1		1	1				1	1											1	1	1		
F24	1		1	1				1	1											1	1	1		
F25	1		1	1				1	1											1	1	1		
F26	1		1					1	1					1	1									
F27	1		1					1	1					1	1									
F28	1			1				1	1											1	1	1		
F29	1		1	1				1	1	1										1	1		1	
F30	1		1	1				1	1											1	1	1		
F31	1		1	1				1	1											1	1		1	
F32	1		1	1				1	1											1	1	1		
F33	1		1	1				1		1				1	1									
F34	1		1					1	1											1	1			
F35	1		1					1	1					1	1		1							
F36	1		1	1				1	1											1	1	1		
F37	1		1	1				1	1	1				1	1									
F38	1		1	1				1	1					1	1									
F39	1		1		1			1	1					1	1		1							
F40	1		1	1	1			1	1	1										1	1	1		
F41	1		1	1	1			1	1	1				1	1									
F42	1		1	1				1	1	1				1	1	1								
F43	1		1	1				1	1		1			1	1									
F44	1		1	1				1	1		1			1	1									
F45	1		1	1				1		1				1	1									
F46	1		1	1				1	1	1				1	1		1							
F47	1		1	1				1	1	1				1	1	1								
F48	1			1				1	1	1	1									1	1	1		
F49	1			1				1	1					1	1									
F50	1		1	1				1		1										1	1			
F51	1		1	1				1	1					1	1	1								
F52	1		1	1				1	1											1	1	1		
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F57	1		1		1			1	1					1	1									
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F59	1		1	1				1	1					1	1									
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F61	1		1					1			1									1	1	1	1	
F62	1		1					1	1		1									1	1	1		
F63	1		1		1			1	1											1	1		1	
F64	1		1	1				1	1											1	1	1		
F65	1		1	1				1	1					1	1		1							
F66	1		1		1			1	1		1			1	1									
F67	1		1					1	1											1	1	1		
F68	1		1					1	1					1	1									
F69	1		1	1				1	1					1	1									
F70	1		1	1				1	1					1	1									
F71	1		1	1				1	1											1	1	1		
F72	1		1	1				1	1											1	1	1		
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F74	1		1	1				1	1					1	1									
F75	1		1					1	1	1				1	1									
F76	1		1					1	1											1	1	1		
F77	1		1	1				1	1											1	1	1		
F78	1		1	1				1	1											1	1	1	1	
F79	1		1	1				1	1	1										1	1	1		
F80	1		1	1				1	1	1										1	1	1		
F81	1		1	1				1	1					1	1									
F82	1		1		1			1	1											1	1		1	
F83	1		1	1	1			1	1											1	1	1		
F84	1		1	1				1	1					1	1	1								
F85	1		1	1				1	1											1	1			
F86	1		1					1	1	1										1	1	1		
F87	1		1					1	1	1										1	1	1		
F88	1		1					1	1	1										1	1			
F89	1		1	1				1	1	1										1	1	1		
F90	1		1					1	1	1										1	1	1		
F91	1			1				1	1	1										1	1	1		
F92	1		1	1				1			1			1	1				1					
F93	1			1				1		1				1	1									
F94	1		1	1				1	1											1		1		
F95	1		1	1				1	1											1	1	1		
F96	1			1				1	1											1	1			
F97	1		1	1				1	1	1										1		1		
F98	1		1	1				1		1				1	1									
F99	1			1				1	1	1										1	1			
F100	1			1				1		1				1	1									

Figure 6: Prominent pattern Type of Mother, Father, and Child

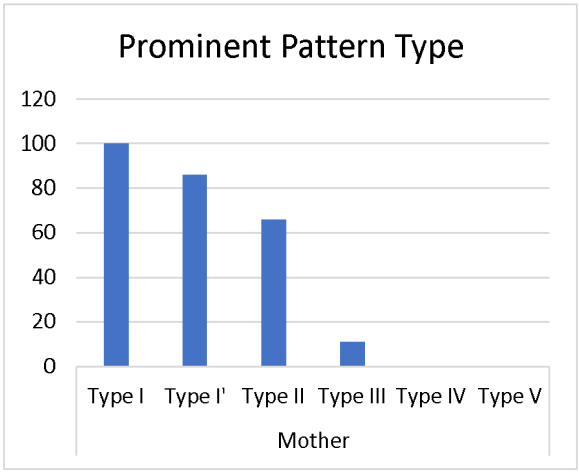


Figure 7: Prominent Pattern Type of Mother

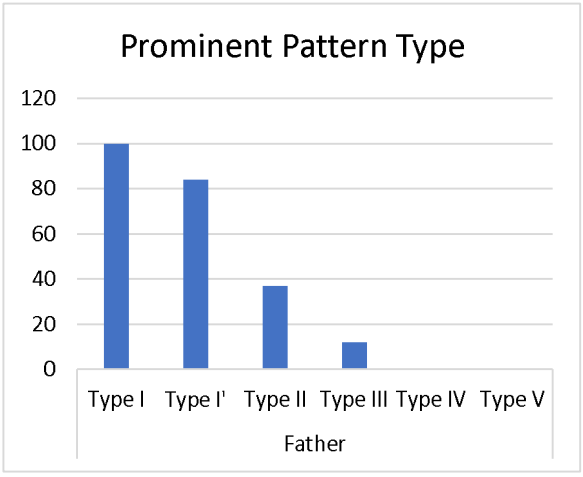


Figure 8: Prominent Pattern Type of Father

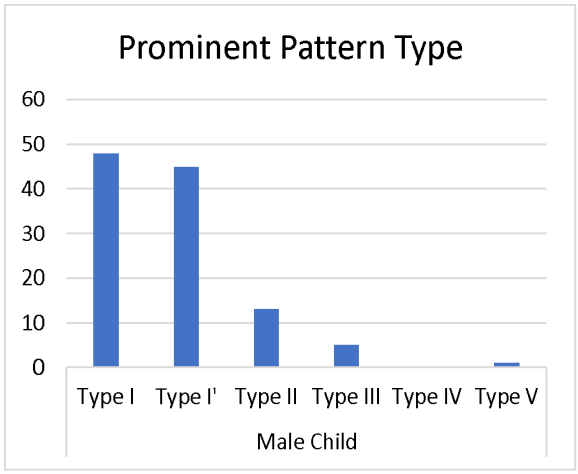


Figure 9: Prominent Pattern Type of Female Child

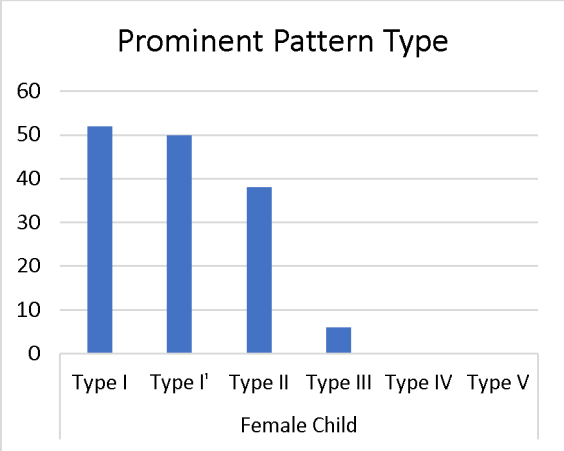
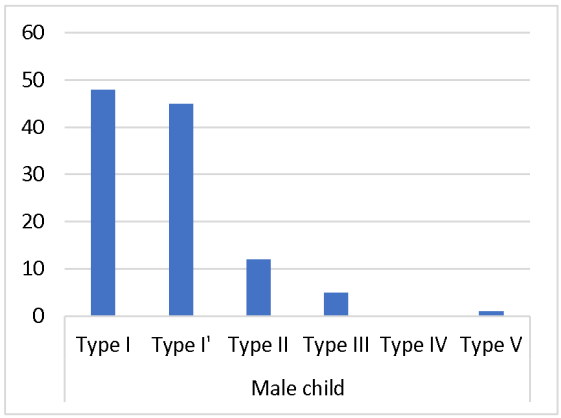
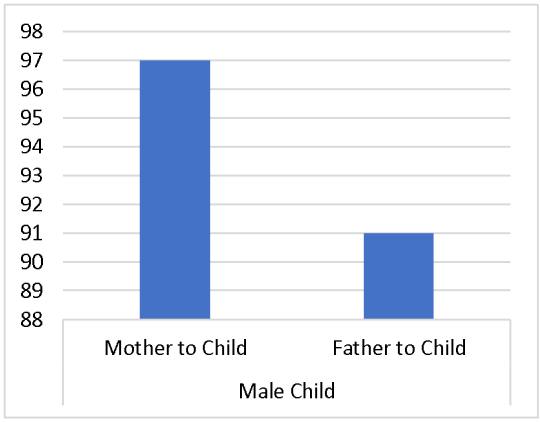


Figure 10: Prominent Pattern Type

of Male Child

According to the study Type I is seen to be most prominent in both Mothers and Fathers participants, whereas Type II was seen more in Mothers, and Type I' and Type III was seen slightly more in Fathers. The male child has Type I most prominent and Type V as the least prominent, whereas the female child has Type I' and Type II as the most prominent and Type III as the least prominent pattern type. The prominent patterns observed in the prints were compared with the mother and father of both male and female child for the association of the hereditary factors.





Family	Male child					
	Type I	Type I'	Type II	Type III	Type IV	Type V
F1	1	1	1			
F2	1	1	1			
F3	1	1				
F4	1	1	1			
F5	1	1				
F8	1	1				
F9	1	1	1			
F12	1		1			
F13	1		1			
F14	1	1	1			
F15	1	1				
F16	1	1				
F17	1	1				
F19	1	1		1		
F21	1	1				
F26	1	1				
F27	1	1				
F33	1	1				
F35	1	1		1		
F37	1	1				
F38	1	1				
F39	1	1		1		
F41	1	1				
F42	1	1	1			
F43	1	1				
F44	1	1				
F45	1	1				
F46	1	1		1		
F47	1	1	1			
F49	1	1				
F51	1	1	1			
F53	1	1				
F55	1		1			
F57	1	1				
F59	1	1				
F65	1	1		1		
F66	1	1				
F68	1	1				
F69	1	1				
F70	1	1				
F74	1	1				
F75	1	1				
F81	1	1				
F84	1	1	1			
F92	1	1				
F93	1	1				
F98	1	1				
F100	1	1				

Figure 11: Most prominent patterns type in Male Child

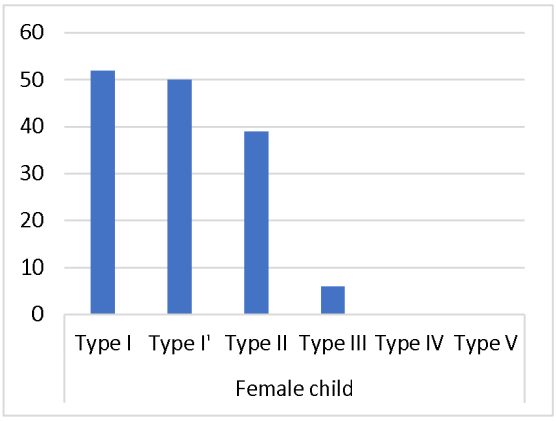


Figure 15: Most prominent pattern type in female Child

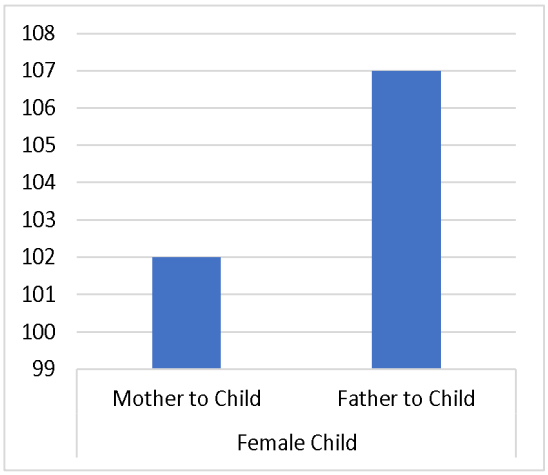


Figure 16: Most prominent patterns type in Female child with the

Sr. no.	Family	Female child					
		Type I	Type I'	Type II	Type III	Type IV	Type V
1	F6	1	1				
2	F7	1	1				
3	F10	1	1				
4	F11	1	1	1			
5	F18	1	1	1			
6	F20	1	1	1			
7	F22	1	1	1			
8	F23	1	1	1			
9	F24	1	1	1			
10	F25	1	1	1			
11	F28	1	1	1			
12	F29	1	1		1		
13	F30	1	1	1			
14	F31	1	1		1		
15	F32	1	1	1			
16	F34	1	1				
17	F36	1	1	1			
18	F40	1	1	1			
19	F48	1	1	1			
20	F50	1	1				
21	F52	1	1	1			
22	F54	1	1	1			
23	F56	1	1	1			
24	F58	1	1	1			
25	F60	1	1				
26	F61	1	1	1	1		
27	F62	1	1	1			
28	F63	1	1		1		
29	F64	1	1	1			
30	F67	1	1	1			
31	F71	1	1	1			
32	F72	1	1	1			
33	F73	1	1	1			
34	F76	1	1	1			
35	F77	1	1	1			
36	F78	1	1	1	1		
37	F79	1	1	1			
38	F80	1	1	1			
39	F82	1	1		1		
40	F83	1	1	1			
41	F85	1	1				
42	F86	1	1	1			
43	F87	1	1	1			
44	F88	1	1				
45	F89	1	1	1			
46	F90	1	1	1			
47	F91	1	1	1			
48	F94	1		1			
49	F95	1	1	1			
50	F96	1	1				
51	F97	1		1			
52	F99	1	1	1			

Figure 14: Most prominent patterns type in

Female child

According to the observation of the print of the participants, it is observed that the Type I is most prominent in both Male and Female child, Type II is more prominent in Female child. The mother and father association observed in the Male and Female child showed that the most prominent pattern types of the male child associates more with the Mother than that of to their Father, whereas female child associates more with Father than that of to their Mother.

Table 17: Percentage associations of the parents with male and female child.

Percentage of Association s	Parents	Percentage of Associations of lip print patterns in Female child	Percentage of Associations of lip print patterns in Male child
	Father	35.66	30.33
	Mother	40.66	32.33

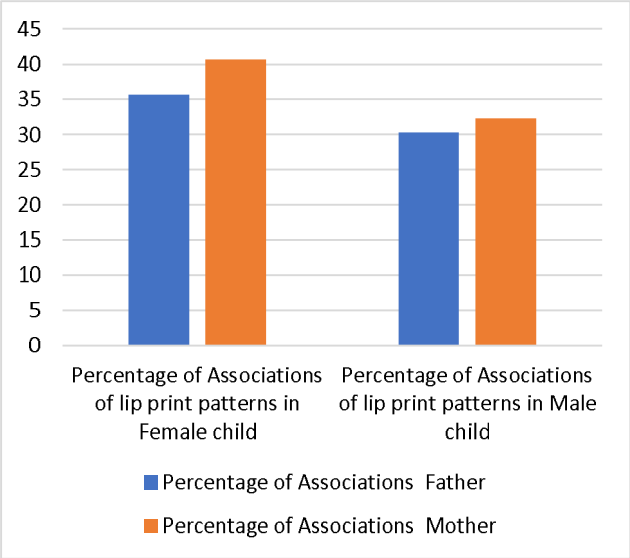


Figure 17: Percentage association of lip print patterns in both Male and Female child

On comparison of association of lip print patterns in both Male and Female child showed that among the both Male and Female child, Mothers pattern types associates more with the children as compared to the Fathers pattern types.

Conclusion

The study of lip prints is known as Cheiloscopy. The lip prints have distinct patterns and grooves. The lips' mucosal surface, known as the Klein's zone, is covered in wrinkles and grooves that create the distinctive lip prints and patterns [1]. The present study was conducted on the Maratha Population to know the hereditary resemblance between mother, father, and child. The result found from the study was Type I was found to be more prominent in both Mothers and Fathers, as well as it was more prominent in their Children. Whereas Type II was seen more in Mothers, and Type III was seen mostly in Fathers and Type IV and V was the least in both Mothers and Fathers. The male and female child has Type I most prominent and Type V as the least prominent in male child. The prominent patterns observed in the prints were compared with the mother and father of the child for the association of the hereditary factors. it is observed that the Type I is most prominent in both Male and Female child, Type II and III is more prominent in Female child. On comparison of association of lip print patterns in both Male and Female child showed that among the both Male and Female child, Mothers pattern types associate more with the children as compared to the Fathers pattern types. To resemble more about the hereditary factors a greater number of sampling is required for the resemblance of the hereditary factors between Mothers, Fathers, and Child.

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I am also very much thankful to my parents, friends and villagers for giving time and help for collection of samples. And my whole family for their support and help.

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Table 1: Prominent types of patterns in all population

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV	Type V
Male	148	148	129	50	17	-	1
Female	152	152	136	104	17	-	-

Table 2: Prominent types of patterns in Mother and Father

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV
Mother	100	100	86	66	11	-
Father	100	100	84	37	12	-

Table 3: Prominent types of patterns in Male and Female child

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV
Male child	48	48	45	13	5	-
Female child	52	52	50	38	6	-

Table 4: : Percentage of Associations of lip print patterns in Female child and male child .

Percentage of Associations	Parents	Percentage of Associations of lip print patterns in Female child	Percentage of Associations of lip print patterns in Male child
	Father	35.66	30.33
	Mother	40.66	32.33

Appendixes

CONSENT FORM

TITLE OF STUDY: Tracing Identity: Cheiloscopy Examination in Maratha's Population

PLACE OF STUDY: Govt. Institute of Forensic Science, Chhatrapati Sambhajinagar

ABOUT THE STUDY:

Lip print examination, also known as cheiloscopy, is a forensic technique used to analyze the patterns, grooves, and characteristics of lip prints left on surfaces such as glass, paper, or other objects. Lip prints are unique to individuals, similar to fingerprints, and can be used for identification purposes in forensic investigations. Lip print examination involves studying the shape, size, and arrangement of lines and grooves on the lips to identify individuals.

In simple terms, lip prints refer to the unique patterns and lines that are naturally present on a person's lips. Just like fingerprints, lip prints are specific to each individual and can be used for identification purposes. Lip print examination involves studying these patterns and characteristics to help identify a person, especially in forensic investigations.

Note: Your identity will be completely confidential and it will not be revealed at any point in this research.

CONSENT: I hereby give you my consent for collection of my handwriting samples. The project work was explained to me and I have understood all the information. I agree that my participation is voluntary for the study.

NAME OF PARTICIPANT: _____

SIGNATURE:

DATE AND TIME:

SUBJECT INFORMATION FORM

NAME: _____

First name Middle name Last name

DATE OF BIRTH: | | | |

Day Month Year

AGE:_____ **GENDER:** **MALE** ☐ **FEMALE** ☐

ADDRESS: _____

QUALIFICATION: _____

OCCUPATION: _____

HABIT:

SMOKING ☐ **CHEWING TOBACCO** ☐

LIP BITING ☐ **LIP LICKING** ☐

LIP CARE HABITS:

LIP BALM ☐ **LIPSTICKS** ☐

LIP GLOSS ☐ **HYDRATION (MOISTURISER)** ☐

ANY OTHER _____

ARE YOU SUFFERING FROM ANY DISEASE REALTED TO LIPS?

YES ☐ **NO** ☐

IF YES, THEN WHAT DESEASE YOU ARE SUFFERING?

_____

DO YOU HAVE ANY OTHER HEALTH ISSUE?

_____

FAMILY HISTORY IF ANY (related to lips):

_____

Quantification Kinetics of Touch DNA in Forensic Case Work

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Abstract

Touch DNA (Deoxyribonucleic acid) is a DNA that is derived from shed skin cells when friction occurs between objects. In forensic casework use of Touch DNA is increased due to its high degree of potential to connect perpetrator with the crime. Quantification of Touch DNA can narrow down the investigation by giving adequate amount of DNA which is helpful for profiling. In this study six different fabrics samples has been taken to replicate common items encountered in crime scene and DNA has been extracted using automated DNA extraction procedure under different time interval. Further quantification of DNA samples has been done using Real-Time PCR Systems, which can be used for profiling of perpetrator. In most cases DNA leaves sufficient quantity and quality of DNA through which profile can be generated. However, quantification of touch DNA samples depend on different factors such as time intervals, environmental condition, extraction technique, collection area and type of sampling methods.

Keywords: Forensics, Touch DNA, RT-PCR, DNA Quantification.

Introduction:

Deoxyribonucleic acid (DNA) is a long polymer of nucleic acid found in a living system. DNA acts as a genetic material in most organisms. DNA constitutes of genetic material which is transferred from parents to progeny. Every individual has somehow unique genetic material due to variation in code. DNA is made up of double helical structure of sugar and phosphate and the bases are projected inside. These bases are adenine, guanine, cytosine, and thymine. Genetic code is made of

combination of these three bases which leads to protein synthesis. Every individual is unique due to the difference in only two percent of this protein.

Touch DNA or Trace DNA or low copy number DNA is a DNA derived from shed skin cells when friction occurs between objects. Humans shed thousands of skin cells every day, and these cells transferred to the surfaces where it comes in contact while rubbing. Shed skin cells contain corneocytes cells, dead cells, and epithelial cells. Those cells that contain nucleus will be helpful for extraction of DNA which will be further helpful for profiling. Touch DNA is an essential tool in Forensics to recover DNA that was left behind at the crime scene and matches it with someone who might have committed a crime. Touch DNA provides a new sight that not only body fluids, but small number of cells can be helpful to generate a full profile of either accused or victim if examined. The quality and quantity of DNA deposited on an object determines the ability to recover the DNA evidence.

Methodology

2.1 Experimental set up and deposition

A selection of six different fabrics and non-porous surfaces were chosen to replicate common items encountered in crime scene. All non- porous surfaces were cleaned by viricidal disinfectants (2%Virkon) and ultraviolet radiation (UV) for 15min.

For DNA deposition, a participant was asked to wear different cloth material for different time intervals and washing was restricted. All the samples were packed in brown paper.

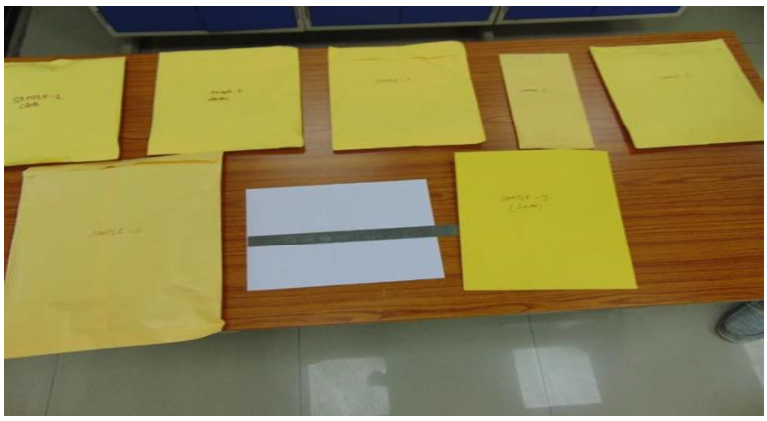

Fig:2.1.1 Collected samples

2.2 Conditions

To assess the effect of time in different environmental conditions, the selected fabrics and non – porous surface were left for eight different time periods (Immediate, 24h, 48h, 1 week, 2 week, 3week, 4 week) in two different environmental condition a) Room temperature b) Moderate humidity (20℃- 25℃). Ovens were used to maintain humid condition, but it has very low humidity, so a plastic container of water was kept inside the oven to moderate the humidity.

2.3 Sample preparation

Two different sampling techniques were used for collection, one is cutting, and another is swabbing. For cutting of sample (A to F) firstly disinfectant (25% ethanol and 75% water) were prepared and used to stain cover tip forceps, Brophy (to hold fabric) and measuring scale to reduce the chances of contamination. Each fabric piece is cut into pieces having same length and breadth (3cm,1cm). Mostly in case of t-shirt cutting done from armpit and collar but in case of socks cutting of sample done from heel and big toe. All the cut pieces are put into dip drop for further extraction process. For sample (G and H) wet swabs were taken through gauge pieces. Gauge piece having equal length and breadth (2cm,2cm) moistened with 100µL of sterile distilled water applied using a plastic spray bottle technique. Each cut piece is put into dip drop.

Water was added when touch DNA was collected at room temperature.

Table:2.3.1

S.NO.	Sample Marked	Description of sample	Dissolved in TE (µL)
1	A	Cotton T- Shirt	20
2	B	Nylon T-Shirt	20
3	C	Cotton socks	20
4	D	Wool socks	20
5	E	Nylon Socks	20
6	F	Polyester socks	20
7	G	Spectacles	20
8	H	Earbuds	20

Fig:2.3.2 Sample measurement and cutting

2.4. DNA Extraction

Total eight sample were collected and before taking it to Automated DNA extraction process there were addition of Buffer G2 (290µL) to relieve inhibitory nucleic acid particle complex and Protease kinase (PK) 10µL to catalyze the breakdown of contaminating proteins present in the solution to its component amino acids. All the content in a dip drop were mixed properly with the help of vertex (eppendof Mix Mate), paraffin film (wax paper) was used to cover the dip drop. Then the sample were heated at 56℃ for 2hr with the help of Thermostart C (eppendrof 12mm). After 2hr the sample were centrifuge (Remi) for 1min. Then the liquid part of the sample was transferred to the next tube and the rest of the settled part discarded. Now samples are ready for Automated DNA extraction using Qiagen (EZ2 Connect Fx).

Fig: -2.4.1 – Automated DNA extractor (Qiagen EZ2 Connect Fx serial no.-P0222009F, Cartridge

2.5 DNA Quantification

Quantification of Touch DNA was done by using QuantStudio 3 and 5 Real-Time PCR Systems. Quantification of Touch DNA is necessary to know the amount of DNA that is present when carrying out functions like profiling of DNA. These systems detect differences in target quality as

low as 1.5-fold, allow remote monitoring, and feature optional cloud- based applied Biosystems analysis modules and data sharing.

Fig: - 2.5.1- Quant studio 5 by applied biosystem Serial no.- 2725210405

Conclusion

Touch DNA is DNA derived from shed skin cells and other biological material that is transferred from a donor to an object or person by physical contact. Although the origin of human DNA in contact samples had not been definitively determined, much evidence suggests sloughed corneocytes, endogenous or transfected nucleated epithelial cells, fragmented cells, and nuclei, and acellular suggesting that it is likely to be DNA. Based on Locard's exchange principle that every contact leaves a trace', the collection of contact DNA to obtain meaningful profiles of different surfaces remains an important technique in forensic investigations. Touch DNA is an essential tool in Forensics to recover DNA that was left behind at the scene and matches it with someone who might have committed a crime. More accurate recoveries and testing of not just body fluids but DNA that has been deposited in a variety of specimens was made possible by improved science techniques. In this research work included different variable factors that influence quantity of touch DNA like different time interval, environmental factors like humidity and different cloth material. Result included that time had a significant impact on the quantity of touch DNA, so it's important to collect

Touch sample as soon as possible to get a good amount of DNA. There was not much variability in the amount of touch DNA because of room temperature and humidity. The amount of touch DNA in the case of different cloth materials depends upon the area of collection of touch samples like collar, underarm, heel, etc.

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A RESEARCH STUDY ON OCCUPATIONAL FINGERPRINT MARKS OF BRICKMAKERS FROM THE GEOGRAPHIC AREA OF JOGA VILLAGE OF MANSA DISTRICT IN PUNJAB, INDIA

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ABSTRACT

Fingerprints are the most crucial evidence in forensic science with Dactyloscopy as the field dedicated to their study. In the occupational marks, some ridges are observed. These ridges exhibit minutiae characteristics, also known as ridges details or Galton's details. The fingerprints are unique for every individual but extensive work experience exceeding ten years in fields such as brick maker can result in occupational marks like blisters, cuts, scars, and most importantly rupture of fingers skin. This study on occupational marks aims to know about the minutiae characteristics in the occupational fingerprints of brick makers. Such insights prove invaluable for forensic science investigations involving fingerprints.

Keywords – occupational, fingerprints, minutiae, forensic science, ridges identifications.

Introduction: The concept of fingerprint was first introduced by Sir Francis Galton. He defined 5 types of ridges known as Galton details. Galton introduced an identification system on the work done by Sir William J. Herschel, whose collection began in 1857 [1].

Fingerprints are different for every individual and even identical twins have different fingerprints. Fingerprints have unique ridges and valleys on the skin giving different patterns of loops, whorls, and ridges on thumbs thus providing each finger with its distinctive identifications [2-3].

The fabrication or duplication of fingerprints is difficult; it plays a crucial role in criminal and biometrics identifications [2]. Occupational marks serve as indicators of an individual's job status, revealing details through features such as ridge tracing, ridge count, minutiae, calluses, abrasions, scars, & cuts because of instruments used for work which indicate activities and work-related conditions person is exposed to their occupation [7].

Consider the case of a brickmaker working with sand which faces physical challenges, stacking and feeding of clay into wire bow-box (brick-making tool), as well as arrangement of bricks.

In dusty & high environmental conditions brickmakers are exposed to airborne particles. Due to loading trucks or tractors for delivery and handling of bricks, can leave distinctive occupational marks on their fingers such as ridge tracing, ridge count, minutiae, blisters, burns, and calluses as well as rupture of finger skin. Consequently, the process of brick manufacturing leaves detectable and analyzable occupational marks on fingers [8].

Materials and Methods:

Materials:

Oil paint Winsor & Newton oil colour ivory black (with almost the same consistency as fingerprint ink), Roller (Size - small), Square glass plate (Size -20.5x20.5 cm), Magnifier glass (Type - Sonex 75mm), A4 Sheet white paper, Black makers, Pencil/pen, Phone camera (Product Name – Samsung Galaxy M32 5G).

Sample Collection:

Fifty-two samples were gathered from the right-hand index fingers of brickmakers. Fingerprints were collected in Joga village, located in Mansa tehsil of Mansa district in Punjab, India. Joga village is positioned 20km away from Mansa, serving as both district and sub-district headquarters, covering a total geographic area of 3559 hectares. Each sample was associated with the individual's name, age, and experience. Clear instructions were provided to the brickmakers to ensure a clean impression and prevent smudging of fingerprints. To achieve this, workers were directed to wash their hands with liquid soap and dry them with a towel, minimizing the impact of the oil paint spills on the collected specimens.

Methodology:

Sample collection and identification involved the use of a hand magnifier and lens to scrutinize occupational marks and a rupture of finger's skin was identified. The specimens were gathered from brickmakers for examination. The sampling period spanned from December 2023 to January 2024, encompassing individuals aged 18 to 50 years. Before fingerprinting, brickmakers were instructed to cleanse their hands with liquid soap and dry them with a towel to minimize dust particles. Then, the rolled fingerprints were taken by rolling the index finger of the right hand from one nail edge to another, ensuring a continuous motion to capture intricate minutiae details and prevent smudging of fingerprint impression.

Results:

Analysis:

A total of 52 samples were gathered and subjected to analysis using a magnifier and hand lens. The examination focused on recording ridge count, ridge tracing, and minutiae, categorized based on the years of work experience. Additionally, the analysis included the evaluation of skin ruptures and pores specifically in the right-hand index finger.

Result:

The analysis of the 52 samples unveiled significant observations and detailed analyses. The ridge tracing, ridge count, minutiae details, and the identification of pores were conducted for every fingerprint specimen. Upon examination of the fingerprints from the brickmaker, it was observed that the ridges were partially damaged. While ridge tracing, ridge count, and minutiae analyses were successfully carried out in many samples, a subset presented challenges due to partial damage and rupture of finger skin. Additionally, the presence of small blisters was observed. These issues were linked to the prolonged manual engagement with brick tools and sand, spanning 8 to 9 hours daily, leading to skin problems that had previously gone unnoticed. The continuous friction and pressure on fingerprints over time resulted in the gradual wearing down of ridge prints. The detailed observations from the specimens are presented in the table and figures below. As a result, it plays a vital role in forensic investigation with the help of occupational marks. It is easy to identify the occupation of the individual because those who work hard and rough in companies or industries like brickmaking, wooden workers, and carpenters their fingerprints and occupational marks can be observed.

Discussion: From the 52 samples taken by the rolling right-hand index finger from brickmakers with their name, age, and work of experience. These 3 types of occupational marks were observed ridge count, ridge tracing, and minutiae. The purpose of this research study was to provide insights into the use of occupational marks in forensic investigation [8]. Where the occupational marks affect the fingerprints and skin as well. The study aims to find out the occupational marks. As seen the workers who work hardened or rash, who play instrumental music with guitars or flutes their fingerprints have occupational marks. The brickmaker who work with tools manually have their skin fingers and as well as palms skin damaged because of dust particles [13]. The occupational marks help in the forensic investigation to find the occupation of the worker. There are different occupational marks for ridge tracing, ridge count, and minutiae samples that are tabulated independently in observations [17].

Conclusion: Fingerprints play a vital role in crime and the study of this to know about the brickmakers, right-hand index finger occupational marks which is potential for forensic investigation. Occupational marks help to find out the person's field of work. The occupational marks showed a rupture of finger skin and tiny pocket-like blisters on the hands.

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Tables

Table 1: [Shows the observations of occupational fingerprint marks along with work experience]

Sample number	AGE	WORK EXPERIENCE	OBSERVATIONS
1.	58/M	10 YEARS	Rupture of tissue, ridge count is difficult and minutia are partially present.
2.	35/M	7 YEARS	Minutia are partially present, ridge tracing is present
3.	24/M	6 YEARS	No clear visibility and fingerprints could not be studied.
4.	23/M	4 TO 5 MONTHS	No proper fingerprint could be seen, ridge tracing could be seen.
5.	25/M	5 YEARS	Completely damaged of the fingerprint.
6.	36/M	10 YEARS	Multiple pores are present, minutia is damaged.

7.	45/M	10 YEARS	Right side minutiae could be seen, ridge tracing is not seen.
8.	44/M	10 YEARS	Ridge tracing is not possible, minutiae are damaged.
9.	22/M	2-3 MONTHS	Partial study only.
10.	35/M	2 MONTHS	A complete study was done.
11.	60/M	1YEAR	Partial study.
12.	35/M	3 YEARS	Partial rupture of tissue and partial ridge tracing could be done, minutiae could be partially studied.
13.	60/M	5 YEARS	Rupture of tissue, ridge tracing, ridge count & minutia are damaged.
14.	22/M	6 YEARS	Clear visibility in all 3 could be studied.
15.	22/M	3 YEARS	Partial ridge count and ridge tracing could be done, and

			minutia studied.
16.	40/M	3 YEARS	Tracing could be done; minutia could be identified.
17.	35/M	4 MONTHS	Clear visibility in all 3 could be studied.
18.	23/M	2-3 MONTHS	Clear visibility in all 3 could be studied.
19.	28/M	4 MONTHS	Clear visibility in all 3 could be studied. But the tip of the fingerprint was damaged.
20.	22/M	2 MONTHS	Complete study.
21.	20/M	2 MONTHS	Complete study.
22.	24/M	3 MONTHS	Complete study.
23.	23/M	3YEARS	Ridge tracing is difficult, and partial identification of minutia could be seen.
24.	26/M	2 YEARS	Minutia and ridge count both could be identified.

25.	23/M	1YEAR	Slight rupture of tissue, easy ridge count, and minutia could be seen.
26.	42/M	3 YEARS	Partial rupture of tissue, and minutia are a little difficult & ridge tracing could be seen.
27.	38/M	4 YEARS	Partial Minutia & ridge count could be studied.
28.	29/M	3 YEARS	Minutia could be studied but ridge count partially studied.
29.	40/M	6 YEARS	Rupture of tissue so difficult to identify minutia, ridge tracing, & ridge count.
30.	18/M	4 YEARS	Rupture of tissue so difficult to identify minutia, ridge tracing, & ridge count.
31.	28/M	2 YEARS	Pores could be seen, but difficult to

			identify minutia, ridge tracing, & ridge count.
32.	51/M	10 YEARS	Minutia are partially identified, and ridge count is possible.
33.	45/M	10 YEARS	Pores are present, multiple cuts could be seen, but difficult to identify minutia, ridge tracing, & ridge count.
34.	30/M	1 YEAR	Clear visibility in all 3 could be studied.
35.	38/M	4 YEARS	Minutia could be studied.
36.	35/M	7YEARS	Ridge counting is difficult, partially minutia could be studied.
37.	26/M	5 YEARS	Minutia are present, partial ridge count could be studied.
38.	38/M	10 YEARS	Minutia present but ridge count and ridge tracing could not be studied.

39.	34/M	10 YEARS	All 3 are difficult to identify.
40.	24/M	1YEAR	Clear visibility in all 3 could be studied.
41.	35/M	10YEAR	Completely ruptured skin.
42.	25/M	5 YEARS	Minutia present, ridge count & ridge tracing partially could be studied.
43.	22/M	3YEARS	Completely ruptured skin.
44.	40/M	7 MONTHS	All 3 could be studied.
45.	30/M	3 MONTHS	All 3 could be studied.
46.	32/M	10 YEARS	Minutia is difficult to study, and ridge count & ridge tracing are not possible.
47.	35/M	10 YEARS	All 3 are difficult to identify.
48.	30/M	7YEARS	Minutia could be

			studied, but ridge count & ridge tracing are not possible.
49.	26/M	5 YEARS	All 3 are difficult to identify.
50.	25/M	5 YEARS	Minutia are partially studied, but difficult to identify ridge count & ridge tracing.
51.	23/M	2YEARS	Partially studied.
52.	20/M	2YEARS	Partially studied.

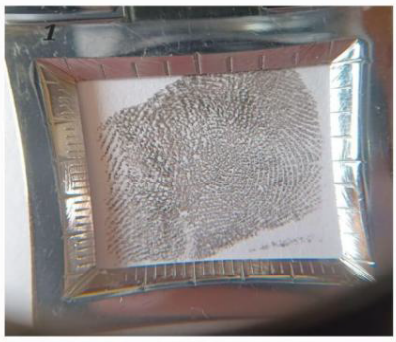
Figure Legends

Figure 1: [Represents the hands of brickmakers.]

FIGURE 1: Image of the left-hand fingers

Figure 2: [shows the rupture of skin on the surface of their fingerprints.]

FIGURE 2: Image of right-hand fingers



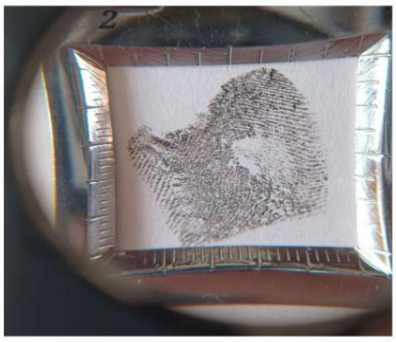


Figure 3 shows pores in a fingerprint. Figure 4 shows damage to fingerprints.

Figure 5 shows the complete study of fingerprints and Figure 6 Shows only minutiae could be studied respectively.

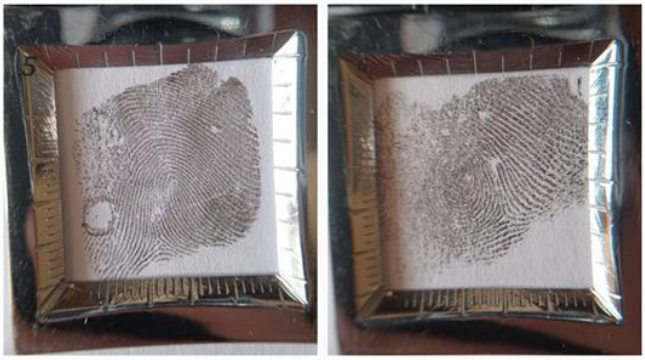


Figure 7 and Figure 8 show that ridge count and ridge tracing could be partially studied respectively.

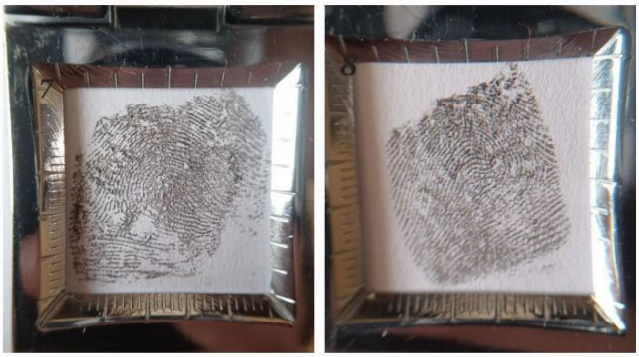


Figure 9 and Figure 10 show the rupture of tissue and partial study of fingerprints respectively.

Identification Of 2 D Latent Footwear Impression By Powder Method (Charcoal, Turmeric, Talcum powder)

Malti saini

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ABSTRACT

The Forensic Science contains a variety of sciences like serology, dermatoglyphics etc that are applied in order to assist and answer questions of interest to the legal and judiciary system. By examining the characteristics of a footwear impression, the forensic scientist can provide the investigator with valuable information about the footwear and sometimes even about the wearer by examining individual and class characteristics. Ultimately, the footwear impression is so unique that it can be individualized and identified to a specific shoe. Powder dusting is the most suitable method to develop latent prints on a wide range of nonporous surfaces like wood, tile, aluminium foil etc. In today's work, a commercially available talcum powder (white colour) generally used as a common beauty product, charcoal (coal power) and Turmeric (roots of flowering plant) a common ingredient in Indian food has been used to decipher latent prints on non-porous surfaces commonly encountered in daily life. These powders are economic and harmless in nature and easily available in market and the best part they are cost effective. The results showed that the powder developed good quality of prints on most of the surfaces and can be a good substitute of conventional powders. We performed the experiments to see the results and the results are really amazing. The power method did give the results and the best result came from charcoal powder.

Keywords: Decipherment, Latent fingerprints, Powder dusting, Talcum powder, Non-porous surface, flowering plants

INTRODUCTION

2D impression footwear by powder method is a technique utilized in forensic science to create a two-dimensional image of a shoe or boot print left at a crime scene. The technique involves applying a fine powder, to the impression left by the footwear. The powder adheres and follows the impression, making it visible and allowing for detailed and point to point analysis. Due to cautious and deliberate tendency of perpetrator/culprit to escape, most often only latent prints are left at the crime scene.(1). Latent footwear impressions are formed when the sole of a shoe leaves a barely visible or undetectable mark on a surface through the transfer of residue like dirt, dust, or moisture/dampness.

Discovering and developing a latent print is an exceptionally difficult task in a crime scene investigation due to enormous diversity of surfaces on which prints may be found. Generally, three methods i.e., powder dusting, fuming and chemical development are used to develop latent fingerprints on a wide range of surfaces which may be commonly classified as non porous/non permeable, porous/permeable and semi porous surfaces/ semi permeable surfaces.

The powder technique is specially useful for capturing impressions on non-porous surfaces, such as tile, hardwood floors etc, where conventional casting methods may not be effective and successful. The resulting image developed from power method can provide important evidence in criminal investigations, such as identifying a suspect or linking a suspect to a crime scene.

The 2D impression footwear by powder method is a technique utilized in forensic science to recover two-dimensional pictures of shoeprints from a crime scene. The method is used to identify suspects, determine the number of perpetrators, and link suspects to the crime scene. It can also be used to determine the sequence of events at a crime scene.

There is variability in the quality of footwear impressions because of the variety of surfaces on which the impressions are made. Details retained in a shoe mark may be insufficient to interestingly identify an individual shoe but is still exceptionally very valuable. Due to the wide variety of shoes available on the market, with most having particular outsole patterns, this infers that any specific model of shoe will be possessed by exceptionally a very small fraction of the general population. Moreover the same outsole pattern can be found on several different footwear brands and models. If the outsole pattern of a shoe can be determined from its mark, then this can altogether narrow the search for a particular suspect

Overall, the 2D impression footwear by powder method is a profitable tool for forensic/ legal investigators/examiners in analysing shoeprints and gathering evidence to help solve crimes. It is

a non-destructive method that can be utilized in a wide run of crime scenes and can give valuable important data to help solve complex criminal cases.

Class Characteristics of footwear

Characteristics that repeat during the manufacturing Process and are shared or participated by one or further shoes. These include: size, design/pattern, brand style, material, colour and mold Characteristics. Size refers to length, width of the shoe while shape represents the overall form like whether the shoe is pointed or rounded. Also there are different style of shoes like if they are athletic shoes or formal shoes. Class characteristics narrow down the large number of shoes to a smaller group of similar shoes for comparison.

Individual Characteristics of footwear

Unique, accidental, random damage on the outsole that distinguish one specific shoe from another on the basis of it's usage and wear. These cuts, scraps, embedded objects in the shoe like glass, stone, holes and repair or modifications that has been done to the shoe, deformities caused by the person's foot shape or his/her walking style called gait patterns are in the outsole accidentally and in a totally arbitrary shape, Orientation, exposure and position.

I am reviewing the decipherment of latent footwear impressions by powder method. The powder I chose is talcum powder, Turmeric powder and charcoal powder. I have performed the experiments by myself and results are amazing.

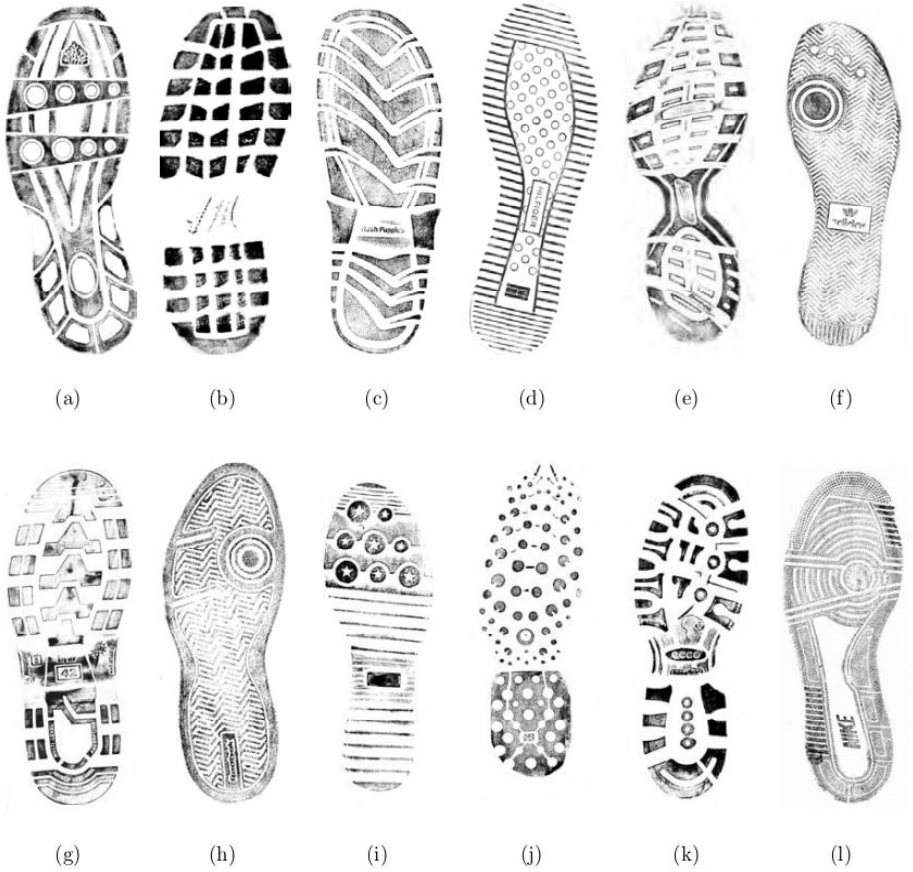


Fig. Different types of footwear patterns from different brands.

CHARCOAL POWDER/ BLACK POWDER

Charcoal powder is a fine, black substance made from charcoal. It is formed by grinding charcoal into a powder after it has been created through the process of pyrolysis, where wood or other organic materials are burned in a low-oxygen environment to remove water and volatile compounds. When wood or other organic materials are burned in a low-oxygen environment, such as a charcoal kiln or oven. This process removes water and volatile compounds, leaving behind the carbon-rich material known as charcoal and the process called pyrolysis.After pyrolysis the charcoal is allowed to cool down.The cooled charcoal is then grind into a fine powder using various methods, such as crushing or milling.

Charcoal powder has magnetic properties that help it adhere to the oils and debris in the print,highlighting the unique characteristics of the shoe.

Fig 1. Black/Charcoal powder

TURMERIC POWDER

Turmeric is a flowering plant of ginger family , zingiberaceae and its roots are used in cooking. This plant grows in warm, rainy regions of India and Southeast Asia. Farmers collect the roots (called rhizomes) each year; some are saved for planting next season, and others are used as food.

The roots can be used fresh or boiled and dried, then ground into a yellow-orange powder. This powder is used to add color and flavor to Asian dishes, especially curries, and also as a dye. The main ingredient in turmeric that gives it these properties is called curcumin, which is bright yellow and approved as a food additive by health organizations worldwide.

Turmeric powder tastes warm and bitter, similar to black pepper, with an earthy, mustard-like smell. While turmeric has been used in traditional Ayurvedic medicine, there is no strong scientific evidence that it effectively treats any diseases.

The yellow colour of turmeric powder contrasts well with darker surfaces, making it ideal for certain types of footwear impressions. Turmeric powder has adhesive properties that help it stick to the impression, revealing intricate details of the shoe pattern.

Fig 2. Turmeric powder

TALCUM POWDER/ WHITE POWDER

Talc is a soft mineral made of hydrated magnesium silicate with the formula Mg3Si4O10(OH)2. In its powdered form, often mixed with corn starch, it is used as baby powder. Talc acts as a thickening agent and lubricant and is found in ceramics, paints, roofing materials, and many cosmetics. It usually appears in sheet-like or fibrous forms and rarely as crystals. Talc is the softest mineral, rated 1 on the Mohs scale of hardness, and produces a white streak when scratched on a plate. Its colours range from whitish grey to green, with a shiny, pearly appearance. Talc does not dissolve in water and only slightly in weak acids.

Fig 3. Talcum powder

METHODOLOGY TO PERFORM EXPERIMENTS

DEVELOPMENT OF 2D FOOTWEAR IMPRESSIONS

Once a footwear impression is detected at the crime scene it should be treated and recovered according to these guidelines.

- Choose the appropriate powder:
  Select the appropriate powder depending on the surface on which the footwear impression was made. For example, black powder can be used on light coloured surfaces, while white powder is suitable for dark surfaces.
- Apply the powder:
  Use a brush to apply the powder evenly over the footwear impression. Be careful not to apply too much pressure, as it can damage the impression.
- Photograph the impression:

Once the powder is applied, take a photograph of the impression using a camera with a scale or ruler for reference. When investigating a crime scene, it's crucial to take photos of the evidence to document its location and serve as a backup in case any evidence is lost later. For instance, with a footprint, the examiner might need these photos for a closer study. The process involves starting with wide shots to capture the overall scene, followed by medium shots to show the relationships between different pieces of evidence, and ending with close-up shots for detailed images of each item. This systematic approach ensures a thorough visual record of the crime scene.The image can then be analyzed by forensic experts to identify characteristics of the shoe, such as the size, make, and model. In some cases, the image can also be used to identify specific wear patterns on the sole of the shoe, which can be matched to a suspect's footwear. (11)

- Document the lifted impression by taking photographs of the impression before packaging it for transportation to the forensic laboratory.
- Package the impression: The lifted impression should be carefully packaged to prevent any damage during transportation to the laboratory.

Due to variety of footwear impressions there are several recovery methods available, of which some are best suited for crime scenes, others for laboratory work , and a few for both. The most powerful tool in the process of recovering evidence is photography, a non-destructive method that is primarily applied at all crime scenes. Most of the times a photogenic enhancement provides sufficient details of the impression and, consequently, additional methods are not necessary (Bodziak, 2000).(12)

Types of 2 Dimensional Impressions

There are two types of 2D impressions, positive and negative in which the positive is the most common one as negative impressions are visible through naked eyes so they can be removed by the perpetrator. Generally,a positive two-Dimensional impression is made on a hard plane and clean surface and consist of static energy and dust particles that creates an image of the outsole. A negative impression is the opposite of a positive impression as it is made on a dirty surface by a clean outsole of shoe that removes particles from the surface and creates an impression. Two dimensional impression may be either visible to the eye, latent or partly latent and by applying different methods all three types can be recovered. (Bodziak,2000)

Detecting latent impressions by light.

In some cases photography solely can emerge a latent impression but more often additional processing is required. One useful method to detect any latent footwear impression is to illuminate from low angle. The light will reflect the print (i.e. the dust and residues it constitutes) which becomes more visible enabling for a subsequent lift or further enhancement. An adequate lightening at the crime scene is probably the most crucial parameter in detecting both latent and visible impressions.(Bodziak,2000)

RESULTS AND DISCUSSIONS

- Observations Of 2D Footwear Impressions Developed By Black Powder (Negative impression).

SAMPLE 1

Fig 11. 2D right footwear impression developed by black/charcoal powder.

SAMPLE 2

Fig 14. 2D left footwear impresssion developed by turmeric powder.

SAMPLE 3

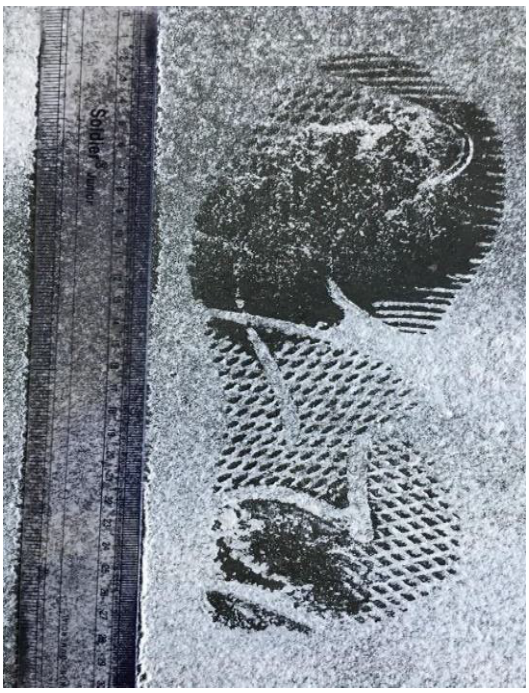


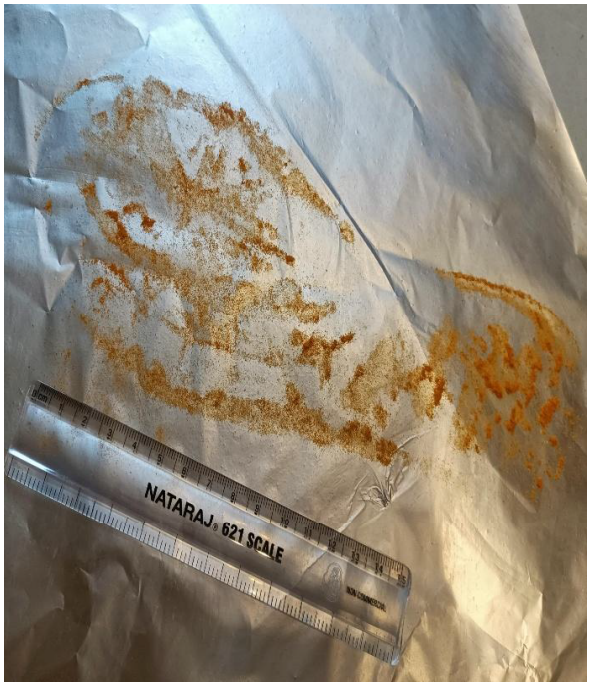
Fig 20. 2D left footwear impresssion developed by talcum powder.

Observations Of 2D Footwear Impressions Developed By Turmeric powder (Positive impression).

SAMPLE 4

Footwear impression developed on wooden surface

SAMPLE 5



footwear impression developed on Aluminium foil

SAMPLE 6

Fig footwear impression developed on white tile

SAMPLE 7

Observations Of 2D Footwear Impressions Developed By Talcum powder (Positive impression)

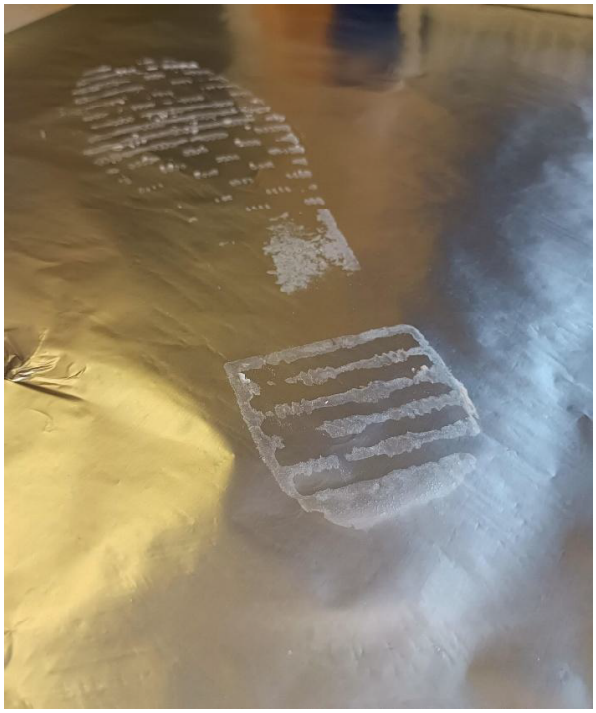
Footwear impression developed on wooden surface

SAMPLE 8

SAMPLE 9

footwear impression developed on steel

SAMPLE 10



footwear impression developed on Aluminium foil

Observations Of 2D Footwear Impressions Developed By Charcoal powder (Positive impression).

SAMPLE 11

SAMPLE 12

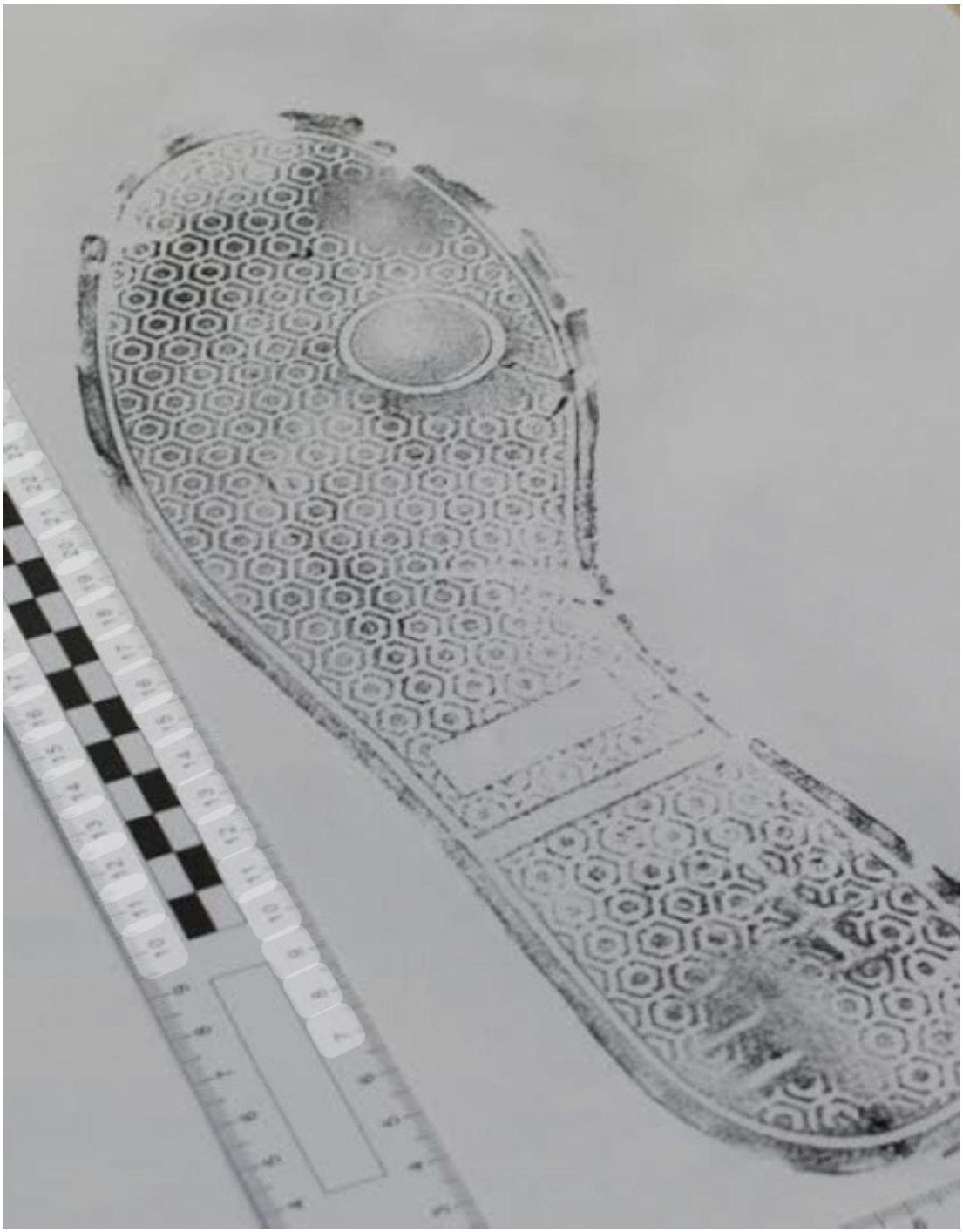


Fig Footwear impression developed on tile

RESULTS

The study contains analysis of 12 samples on different surfaces using black, talcum & turmeric powder. The results obtained from samples lifted by black powder gave the best results. The 2D footwear impressions thus obtained can provide valuable information for investigators. The impressions can be analysed to determine the manufacturer and model of the shoe as well as any unique characteristics or wear patterns. This information can be compared to known shoe prints or database to help identify the suspect or provide additional evidence in a criminal investigation. The black powder method involves applying a fine powder, such as carbon or graphite, to the impression. The powder sticks to the moisture left behind by the shoe, highlighting the edges and design patterns in the footwear impression. This creates a 2D image of the impression that can be photographed, measured, and compared to shoe prints taken from suspects.

DISCUSSION

The black powder method is typically used on hard surfaces, such as concrete or asphalt, where the footwear impression is well-defined and can be easily captured. However, it may not be effective on softer surfaces, such as soil or sand, where the impression may be less distinct.

In order to obtain accurate and reliable results, it is important that the black powder method is performed carefully and accurately. This includes using appropriate lighting, ensuring the surface is clean and dry, and using a suitable powder and brush to apply the powder evenly. In addition, the technique used to lift the impression, such as tape or casting, must also be chosen carefully depending on the surface being analysed.

Overall, the black powder method is a valuable tool in forensic science for the analysis of footwear impressions. It provides important information that can help investigators link a suspect to a crime scene or track the movements of an individual. However, it should be used in conjunction with other evidence and techniques to build a comprehensive picture of the crime scene and the individuals involved.

CONCLUSION

The 2D impression footwear by powder method is a forensic technique used to recover two dimensional images of shoeprints left at a crime scene. It involves applying a fine powder to the impression, capturing the image using a camera, and analysing the image to identify characteristics of the shoe, such as size and wear patterns. The method is non-destructive, can be used on non-porous surfaces, and can provide valuable evidence in criminal investigations. The powder method is a commonly used technique in forensic investigations for analyzing 2D

footwear impressions left at a crime scene. The process involves applying a fine powder, such as aluminium or magnetic powder, onto the impression, which adheres to the ridges and contours of the impression.

The resulting powder pattern can then be examined and compared to known shoe sole patterns to identify potential matches or exclusions. By examining the design and pattern, forensic investigators can determine the make and model of the shoe, as well as any unique interesting characteristics such as wear patterns or damage.

Overall, the powder method is a valuable tool in forensic investigations and can provide important evidence to help solve crimes. However, the accuracy and reliability of the analysis depend on the quality of the impression and the expertise of the investigator.

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Tracing Identity: Cheiloscopy Examination in Maratha's Population

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Abstract

In a crime scenario, traces of the lip prints are often observed at the crime scene. The study of lip prints is also known as Cheiloscopy, an emerging and valuable tool in forensic science. This method involves analysing the unique patterns of the lips, such as vertical lines, intersected lines, branched lines, and reticular lines. Lip prints also have some individualistic characteristics such as fingerprints. This study aims to explore the hereditary nature of lip print patterns which are passed from generation to generation. In this study, we have collected samples of 100 families, a family must include a father, mother and a child of the Maratha Population in Maharashtra. The lip prints were collected categorised and analyzed according to Suzuki and Tsuchihashi's classification. The study emphasized the non-invasive and cost-effective features of Cheiloscopy, making it an accessible means of identification verification. Specific patterns were observed in the Maratha

population, underscoring the potential for creating a specialized database of lip prints. This study helped to reveal the unique characteristics of patterns of the Lip prints of the Maratha Population, as well as helped in analyzing the data and revealed the common patterns present on the lips of the family. The results will include the analyzed data of the most prominent pattern type in mothers, fathers and children, and the data of the most prominent common pattern type observed in Mother and Female Child; Father and Female Child; Mother and Male Child; Father and Male Child.

Keywords - Cheiloscopy; Hereditary; Maratha Population; Lip Prints.

Introduction

"Cheiloscopy is the study of the distinctive pattern known as the "lip prints," which are formed by the wrinkling grooves on the labial mucosa (also known as sulci-labrum)." Human identification via lip prints is the focus of a very new and unrecognised forensic investigative method called as Cheiloscopy[3]. Much like fingerprints, lip prints are distinctive to each person and can be impacted by ageing, trauma, surgery, cosmetic procedures, and way of life. The appearance and texture of the lips can change with age, and this might eventually change the pattern of the lip print. Lip surgeries or injuries can also affect the lip print by changing the curve of the lip or leaving scars. Furthermore, aesthetic operations like tattoos and fillers can change the natural lip curves and patterns. Lip prints can also vary because of the lifestyle choices like smoking, tanning, and biting your lips. Overall, lip prints are thought to be quite stable for identifying purposes; nevertheless, several events may cause changes to the lip print pattern. The texture and colour of the lips can be changed by skin disorders such as vitiligo, eczema, or psoriasis, which may also affect the pattern of the lip prints. Lip prints may be affected by inflammatory diseases like cheilitis or oral ulcers, which can also affect how the lips look-the lip print pattern. Although lip prints can be uniquely identified, differences in the print may result from underlying medical issues affecting the lips. Lip prints can be indirectly impacted by some disorders, so it is important to take lip health condition into account when analysing lip prints. The major principle of the lip prints is:- individuality, uniqueness, persistent. The basic principles of the lip prints are its unique characteristics, they are not similar in any other lip prints, have the persistence characteristics which makes it unique. The lip prints can be classified in many different types which can be based on characteristics such as vertical lines, the branched grooves, the intersecting lines, and the reticular patterns. The lip prints can persist on the surfaces like glass, fabric, or paper. It may also vary in terms like shape, size, fullness, and features (characteristics). The lip print patterns of monozygotic and dizygotic twins were compared, it was found that the former had greater similarities with the latter, concerning lip

prints, except for monozygotic twins, which are innate, immutable even after death, and specific to each individual, they play a crucial role in Cheiloscopy [3]. Forensic examination of lip prints involves analysis of the characteristics of lip prints. There are some types of characteristics analysed, class characteristics and individual characteristics. The lip prints characteristics which are common within the group of people and not encountered in the examination of a single person's lip prints are called class characteristics[7]. The lip print characteristics which are unique to a person are called individual Characteristics". Lip print characteristics most used today is given by two Japanese scientists, Y Tsuchihashi and T Suzuki, they gave the lip print classification on the basis of its basic characteristics and the arrangement of the grooves and the patterns formed on the lips. Overview of the Cheiloscopy, dates to the early twentieth century, when the study of the lip prints started for the identification of the individual. Being the first to recognise the uniqueness of lip prints for individual identification, French anthropologist Edmond Locard is credited with introducing the use of lip prints in forensic investigations in the 1930s. In the intervening period, scientists have worked to categorise lip print patterns, prove their accuracy, and investigate their potential uses in forensic investigations. In a variety of criminal cases—including those involving missing persons, sexual assaults, and other offences for which lip prints can yield important evidence—Cheiloscopy has been used. Cheiloscopy's accuracy and effectiveness in forensic practice have increased over time due to developments in research methodology and technology. Currently acknowledged as a useful forensic instrument, Cheiloscopy advances the discipline by providing extra methods of identification. Le Moyne Snyder was the first to describe and identify the furrows found on human lips in 1902. Yasuo Tsuchihashi and Kazuo Suzuki, two Japanese scientists, conducted research in Europe in 1961 and discovered individualistic lines. Between 1985 and 1997, Cheiloscopic procedures were applied in 85 cases, of which 34 had an identification. examinations conducted in India and other nations have validated the efficacy of Cheiloscopic examinations as a supplemental technique [2]. In the context of legal investigations into criminal activities, it is imperative to underscore the significance of uniqueness in facilitating the accurate identification of individuals. Recognizing people

caught up in civil, criminal, or mass disaster proceedings can be challenging. The most often utilised methods in this context, among the many others suggested, are most likely the comparisons of dental records, DNA, and fingerprints[4]. A key component of forensic science is accurately identifying live or deceased individuals based on the distinctive features of their teeth and jaws. Odontology, anthropometry, fingerprints, and other methods that aid in determining factors like gender, approximate age, and height are frequently used by investigators to gather data and evidence (odontology, forensic odontology) [23]. But one of the most fascinating new studies that

have its roots in forensic and criminal investigations is “human lip recognition,” or Cheiloscopy (Caldas et al., 2007; Sharma et al., 2009; Reddy and Reddy, 2011) [4]. In a variety of criminal cases-including those involving missing persons, sexual assaults, and other offences for which lip prints can yield important evidence–Cheiloscopy has been used. Cheiloscopy’s accuracy and effectiveness in forensic practice have increased over time due to developments in research methodology and technology, currently acknowledged as a useful forensic instrument. Cheiloscopy advances the discipline by providing extra methods of identification. Since Cheiloscopy is the least invasive and most accessible mode for research purposes, most researchers have been quite interested in this field [12]. In cases of homicide where victims lack teeth or easily accessible dental records, lip prints can also be used to corroborate comparisons between dental records [16]. The following research question was formulated in accordance with the patient/population, intervention, comparison, and outcome (PICO) framework: "Is it possible to establish a familial relationship (outcome) between individuals (population) from the analysis of their lip prints (intervention)?" This led to the definition of a more focused review question: "Is the superficial framework of lip prints hereditary?" [1]. From the sixth week of intrauterine life onwards, lip patterns can be recognized. Lip prints are highly developed and recognizable during six weeks of fetal intrauterine life. When kept in a closed container with a temperature maintained at about 25 C, the longevity of a lip print on paper can last up to 12 weeks, even in the presence of ambient conditions. However, when it comes to glass, the 9 reliability of the print can last up to 12 weeks in the presence of ambient conditions. If taken within twenty-four hours after the death, clear and distinguishable lip prints can be acquired. A 1972 study involving two identical twins found that while their lip prints were different, the twins could not be distinguished from one another by any other way. Family members' lip prints can be analyzed to reveal that while children do inherit some of their parents' lip print characteristics, the locations of these lines vary, and no two prints—not even twins'—are exactly alike. The lip prints vary among all people depending upon the characteristics and the patterns observed in the Kleins region. The previous study conducted on the lip prints says that the female has Type I was mostly seen pattern in males and Type III was seen mostly in females; whereas Type I’ was found commonly in both males and females. Studying this factors in Indian families shown the patterns which were most prevalently seen; Type I, Type I’ and Type III. Studying these factors on the Maratha Population based on our knowledge of different patterns on the red part of the lips, Kleins area, enhances our knowledge on inheritance. The study of arrangement of patterns and grooves on the lips and the inheritance, this study gives light to the hereditary features of lip print patterns and grooves and their significant application in the

contemporary technology. These study explains the hereditary factors observed in the lip print patterns in the family (mother, father and child).

Materials and Methods:

The lips' mucosal surface, known as the Klein's zone, is covered in wrinkles and grooves that create the distinctive lip prints and patterns [1]. The Federal University of Rio de Janeiro, Brazil's Clauco Martin Santos, a professor of forensic dentistry, initially categorized lip grooves into four types in 1967 [2,15]. A different lip print classification system was proposed by Suzuki and Tsuchihashi in 1970 [2,15]. Renaud, a French biologist, categorized and examined 4,000 lip prints. The groove organization method was used in 1979 to classify Afchar-Bayat lip prints [15]. Lip patterns were divided into 23 categories of distinct features by Kasprzak. In Cheiloscopy, lip architecture, thickness, and location are also examined. Based on lip thickness, four categories of lips exist [15].

Simple types (formed by single element)	Composite types
Straight line	Bifurcated
Curved line	Trifurcated
Angled line	Irregular
Sine shaped line	---------

Table 1: Lip print classification by Clauco Martin Santos

Types	Characteristics
Type a	Complete vertical
Type b	Incomplete vertical
Type c	Complete bifurcated

Type e	Incomplete bifurcated
Type f	Incomplete intersecting
Type g	Reticular
Type h	In the form of sword
Type i	Horizontal
Type j	Other types

Table 2: Lip print classification by Renaud

A1: Vertical and straight grooves, covering the whole lip
A2: Like the former, but not covering the whole lip
B1: Straight branched grooves
B2: Angulated branched grooves
C: Converging grooves
D: Reticular pattern grooves
E: Other grooves

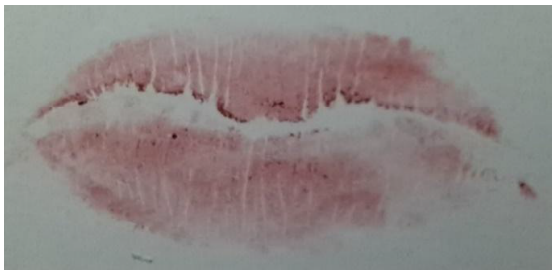
Table 3: Lip print classification by Afchar-Bayat

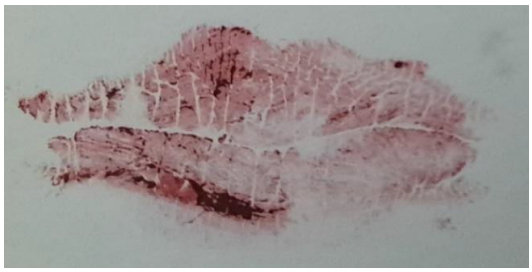
The basic classification characteristics of the lip prints are-

iv) Grooves – The lines or the pattern formed on the lips are the distinct and unique patterns on the lips. These grooves or patterns can be classified into different types as– the straight lines, the branched lines, the intersecting lines.

Figure 1: Grooves

v) Shape – The shape of the lips may vary from the person to person, which may influencethe overall appearance of the lip print. These shape of the lip prints can be classified as thin, thick or full lips.





vi) Fullness – The fullness of the lips may affect the depth and the clarity of the lip print. An individual with the fuller lips may leave the detailed and defined lip prints compared to the lips which are thinner in shape.

Figure 3: Fullness

The main classification of the lip prints was given by Y. Tsuchihashi and T, Suzuki as – Class characteristics. The individual classification characteristics we given by Kasprzak [10].

Type I	Complete Vertical lines
Type I'	Incomplete Vertical lines
Type II	Branched Groove
Type III	Intersected Groove
Type IV	Reticular Groove
Type V	Other Patterns

Table 4: Tsuchihashi and T Suzuki, Class characteristics classification

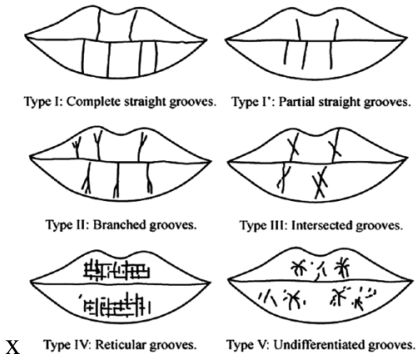


Figure 4: Tsuchihashi and T Suzuki,

Class characteristics classification

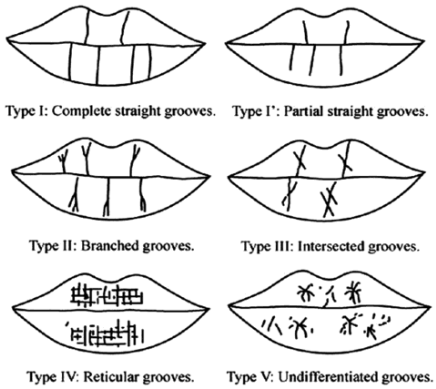


Figure 4: Tsuchihashi and T Suzuki,

Class characteristics classification

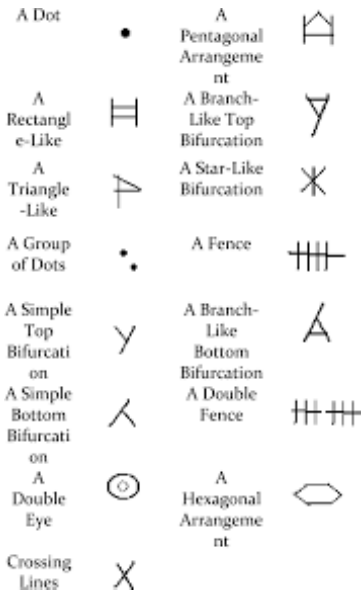


Figure 5: Individual characteristics features by Kasprzak

Materials Required-

vi) Lip-stick

vii) Lip-stick remover

viii) Questionnaire

ix) Consent form

x) Tissue paper/cotton

Methodology-

This is the study conducted in Maharashtra among the Maratha population, all the subjects involved in the study were recruited after taking written informed consent from them. A total of 100 families were recruited in the study. A family includes mother, father and a child. Inclusion criteria for the study, subjects without any disease related to lips, with normal lip mucosa were included. Excluded criteria for the study, subjects having any diseases like congenital deformities of lips, and those with any inflammation, allergic to the lip stick used and with any other kind of diseases were all excluded from the study. During the collection of lip prints, all the participants were informed about the study, its method and objectives were explained thoroughly to the subjects in details and they were made comfortable. Steps of collection of samples –

1. The consent paper was explained thoroughly and participants were asked to give signature on it, followed by the questionnaires.
2. The lips were cleaned and a thin-layer of the lipstick was applied on the lips, and they were asked to spread it evenly on the lips.
3. A blank page was placed on the lips and they were instructed to press their lips by applying pressure evenly on the page. All the lip prints collected were taken carefully without any damage in prints.
4. The prints collected were studied individually by using the Suzuki and Tsuchihashi classification, by using the magnifying glass lens and double check was done before the

data entry.

Results:

The data collected was used for the generation of the profile, all the pattern types of the lips were noted down of all three members of the family; mother, father, and child. The most prominent pattern type was noted down of the family members. Each pattern type is denoted as a score. If a child has one, two and three similar pattern that of the parents, then it will be denoted as 1, 2 and 3 score card, respectively. Given below is the tabular chart representing the pattern types of child, mother, and father.

	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V	Type I	Type I'	Type II	Type III	Type IV	Type V
F1	1	1	1				1	1	1				1	1	1									
F2	1	1					1						1	1	1									
F3	1	1	1				1	1					1	1										
F4	1	1					1	1	1				1	1	1									
F5	1	1	1				1			1			1	1	1									
F6	1		1				1			1									1	1				
F7	1	1	1				1	1	1										1	1				
F8	1	1	1				1	1	1				1	1										
F9	1		1				1	1	1				1	1	1									
F10	1		1				1		1										1	1				
F11	1	1					1	1	1	1									1	1	1			
F12	1			1			1		1				1		1									
F13	1			1			1	1					1		1									
F14	1	1					1			1			1	1	1									
F15	1	1					1	1	1				1	1										
F16	1	1					1	1	1				1	1										
F17	1	1					1	1					1	1										
F18	1	1					1	1	1										1	1	1			
F19	1	1					1	1					1	1		1								
F20	1	1	1				1	1											1	1	1			
F21	1		1				1	1					1	1										
F22	1	1	1				1			1									1	1	1			
F23	1	1	1				1	1											1	1	1			
F24	1	1	1				1	1											1	1	1			
F25	1	1	1				1	1											1	1	1			
F26	1	1					1	1					1	1										
F27	1	1					1	1					1	1										
F28	1		1				1	1											1	1	1			
F29	1	1	1				1	1	1										1	1		1		
F30	1	1	1				1	1											1	1	1			
F31	1	1	1				1	1											1	1		1		
F32	1	1	1				1	1											1	1	1			
F33	1	1	1				1		1				1	1										

F67	1	1					1	1											1	1	1			
F68	1	1					1	1					1	1										
F69	1	1	1				1	1					1	1										
F70	1	1	1				1	1					1	1										
F71	1	1	1				1	1											1	1	1			
F72	1	1	1				1	1											1	1	1			
F73	1	1					1	1											1	1	1			
F74	1	1	1				1	1					1	1										
F75	1	1					1	1	1				1	1										
F76	1	1					1	1											1	1	1			
F77	1	1	1				1	1											1	1	1			
F78	1	1	1				1	1											1	1	1	1		
F79	1	1	1				1	1	1										1	1	1			
F80	1	1	1				1	1	1										1	1	1			
F81	1	1	1				1	1					1	1										
F82	1	1		1			1	1											1	1		1		
F83	1	1	1	1			1	1											1	1	1			
F84	1	1	1				1	1					1	1	1									
F85	1	1	1				1	1											1	1				
F86	1	1					1	1	1										1	1	1			
F87	1	1					1	1	1										1	1	1			
F88	1	1					1	1	1										1	1				
F89	1	1	1				1	1	1										1	1	1			
F90	1	1					1	1	1										1	1	1			
F91	1		1				1	1	1										1	1	1			
F92	1	1	1				1			1			1	1				1						
F93	1		1				1		1				1	1										
F94	1	1	1				1	1											1		1			
F95	1	1	1				1	1											1	1	1			
F96	1		1				1	1											1	1				
F97	1	1	1				1	1	1										1		1			
F98	1	1	1				1		1				1	1										
F99	1		1				1	1	1										1	1				
F100	1		1				1		1				1	1										

Figure 6: Prominent pattern Type of Mother, Father, and Child

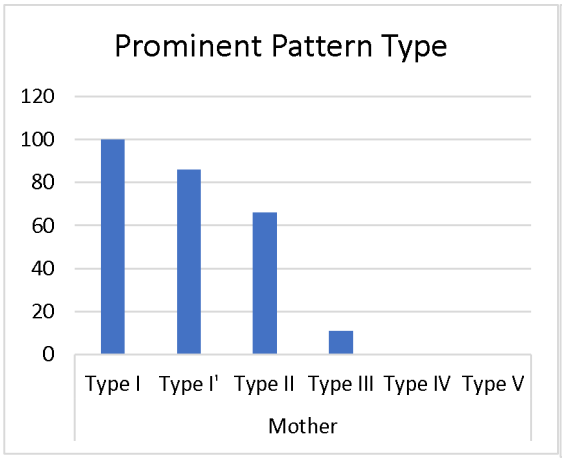


Figure 7: Prominent Pattern Type of Mother

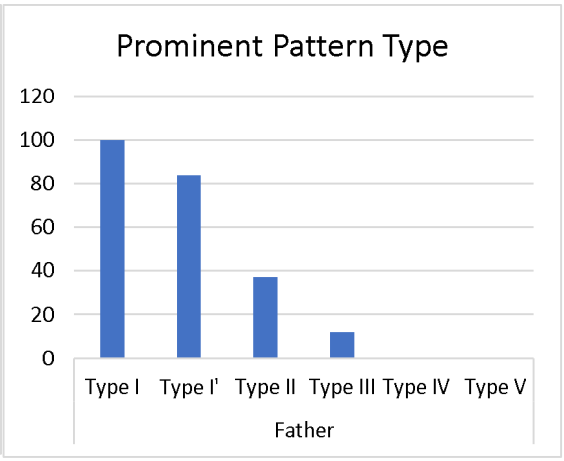


Figure 8: Prominent Pattern Type of Father

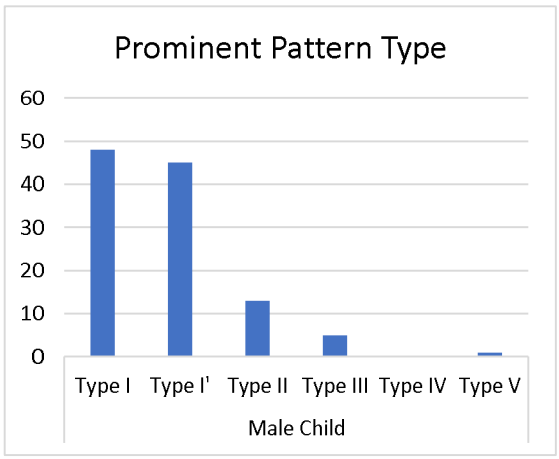


Figure 9: Prominent Pattern Type of Male Child

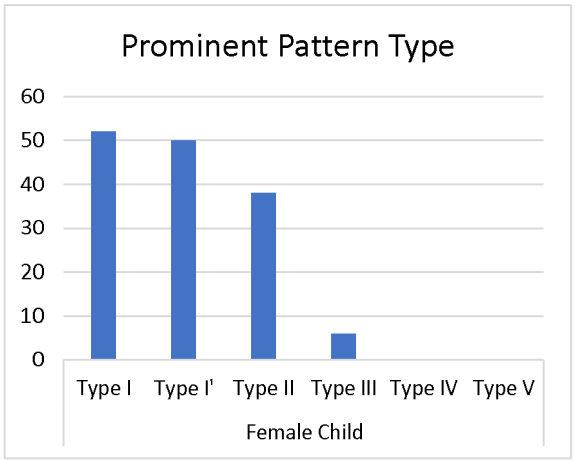


Figure 10: Prominent Pattern Type of Female Child

According to the study Type I is seen to be most prominent in both Mothers and Fathers participants, whereas Type II was seen more in Mothers, and Type I' and Type III was seen slightly

more in Fathers. The male child has Type I most prominent and Type V as the least prominent, whereas the female child has Type I' and Type II as the most prominent and Type III as the least prominent pattern type. The prominent patterns observed in the prints were compared with the mother and father of both male and female child for the association of the hereditary factors.

Family	Male child					
	Type I	Type I'	Type II	Type III	Type IV	Type V
F1	1	1	1			
F2	1	1	1			
F3	1	1				
F4	1	1	1			
F5	1	1				
F8	1	1				
F9	1	1	1			
F12	1		1			
F13	1		1			
F14	1	1	1			
F15	1	1				
F16	1	1				
F17	1	1				
F19	1	1		1		
F21	1	1				
F26	1	1				
F27	1	1				
F33	1	1				
F35	1	1		1		
F37	1	1				
F38	1	1				
F39	1	1		1		
F41	1	1				
F42	1	1	1			
F43	1	1				
F44	1	1				
F45	1	1				
F46	1	1		1		
F47	1	1	1			
F49	1	1				
F51	1	1	1			
F53	1	1				
F55	1		1			
F57	1	1				
F59	1	1				
F65	1	1		1		
F66	1	1				
F68	1	1				
F69	1	1				
F70	1	1				
F74	1	1				
F75	1	1				
F81	1	1				
F84	1	1	1			
F92	1	1				
F93	1	1				
F98	1	1				
F100	1	1				

Figure 11: Most prominent patterns type in Male Child

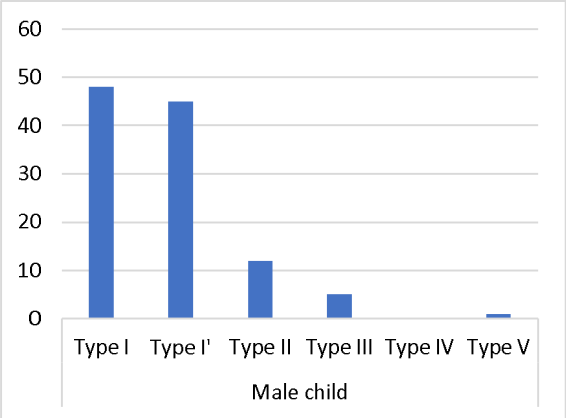


Figure 12: Most prominent pattern type in Male Child

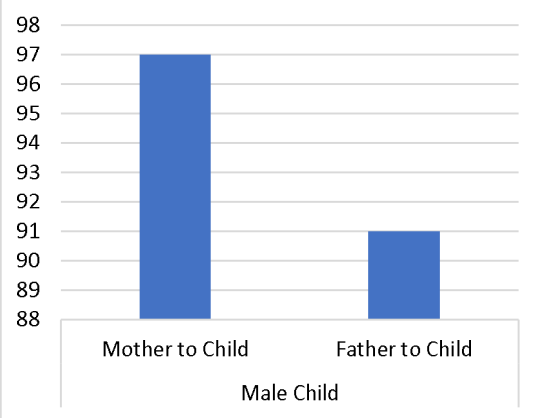


Figure 13: Most prominent pattern type in Female Child

Sr. no.	Family	Female child					
		Type I	Type I'	Type II	Type III	Type IV	Type V
1	F6	1	1				
2	F7	1	1				
3	F10	1	1				
4	F11	1	1	1			
5	F18	1	1	1			
6	F20	1	1	1			
7	F22	1	1	1			
8	F23	1	1	1			
9	F24	1	1	1			
10	F25	1	1	1			
11	F28	1	1	1			
12	F29	1	1		1		
13	F30	1	1	1			
14	F31	1	1		1		
15	F32	1	1	1			
16	F34	1	1				
17	F36	1	1	1			
18	F40	1	1	1			
19	F48	1	1	1			
20	F50	1	1				
21	F52	1	1	1			
22	F54	1	1	1			
23	F56	1	1	1			
24	F58	1	1	1			
25	F60	1	1				
26	F61	1	1	1	1		
27	F62	1	1	1			
28	F63	1	1		1		
29	F64	1	1	1			
30	F67	1	1	1			
31	F71	1	1	1			
32	F72	1	1	1			
33	F73	1	1	1			
34	F76	1	1	1			
35	F77	1	1	1			
36	F78	1	1	1	1		
37	F79	1	1	1			
38	F80	1	1	1			
39	F82	1	1		1		
40	F83	1	1	1			
41	F85	1	1				
42	F86	1	1	1			
43	F87	1	1	1			
44	F88	1	1				
45	F89	1	1	1			
46	F90	1	1	1			
47	F91	1	1	1			
48	F94	1		1			
49	F95	1	1	1			
50	F96	1	1				
51	F97	1		1			
52	F99	1	1	1			

Figure 14: Most prominent patterns type in Female child

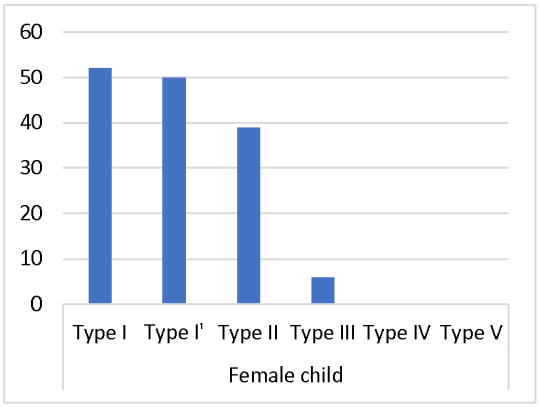


Figure 16: Most prominent patterns type in Female child with the

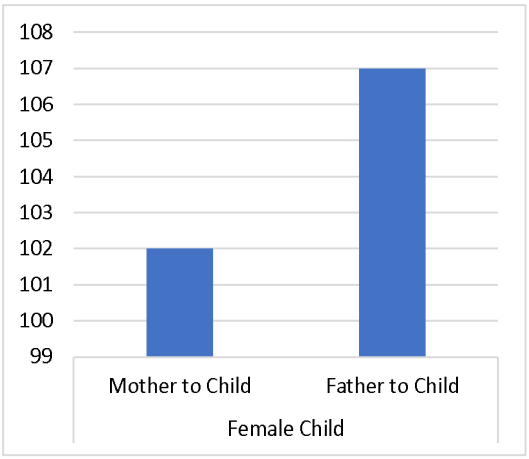


Figure 17: Most prominent patterns type in Female child with the association of father and mother

According to the observation of the print of the participants, it is observed that the Type I is most prominent in both Male and Female child, Type II is more prominent in Female child.

The mother and father association observed in the Male and Female child showed that the most prominent pattern types of the male child associates more with the Mother than that of to their Father, whereas female child associates more with Father than that of to their Mother.

Table 17: Percentage associations of the parents with male and female child.

Percentage of Associations	Parents	Percentage of Associations of lip print patterns in Female child	Percentage of Associations of lip print patterns in Male child
	Father	35.66	30.33
	Mother	40.66	32.33

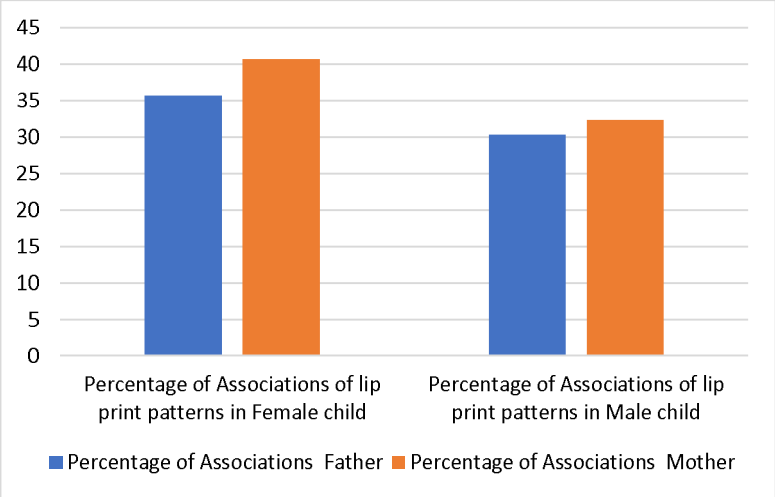


Figure 17: Percentage association of lip print patterns in both Male and Female child

On comparison of association of lip print patterns in both Male and Female child showed that among the both Male and Female child, Mothers pattern types associates more with the children as compared to the Fathers pattern types.

Discussion

Conclusion

The study of lip prints is known as Cheiloscopy. The lip prints have distinct patterns and grooves. The lips' mucosal surface, known as the Klein's zone, is covered in wrinkles and grooves that

create the distinctive lip prints and patterns [1]. The present study was conducted on the Maratha Population to know the hereditary resemblance between mother, father, and child. The result found from the study was Type I was found to be more prominent in both Mothers and Fathers, as well as it was more prominent in their Children. Whereas Type II was seen more in Mothers, and Type III was seen mostly in Fathers and Type IV and V was the least in both Mothers and Fathers. The male and female child has Type I most prominent and Type V as the least prominent in male child. The prominent patterns observed in the prints were compared with the mother and father of the child for the association of the hereditary factors. it is observed that the Type I is most prominent in both Male and Female child, Type II and III is more prominent in Female child. On comparison of association of lip print patterns in both Male and Female child showed that among the both Male and Female child, Mothers pattern types associate more with the children as compared to the Fathers pattern types. To resemble more about the hereditary factors a greater number of sampling is required for the resemblance of the hereditary factors between Mothers, Fathers, and Child.

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Tables

Table 1: Prominent types of patterns in all population

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV	Type V
Male	148	148	129	50	17	-	1

Female	152	152	136	104	17	-	-

Table 2: Prominent types of patterns in Mother and Father

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV
Mother	100	100	86	66	11	-
Father	100	100	84	37	12	-

Table 3: Prominent types of patterns in Male and Female child

Lip print pattern / population	Total population	Type I	Type I'	Type II	Type III	Type IV
Male child	48	48	45	13	5	-
Female child	52	52	50	38	6	-

Table 4: Percentage of Associations of lip print patterns in Female child and male child.

Percentage of Associations	Parents	Percentage of Associations of lip print patterns in Female child	Percentage of Associations of lip print patterns in Male child

	Father	35.66	30.33
	Mother	40.66	32.33

Appendixes

CONSENT FORM

TITLE OF STUDY: Tracing Identity: Cheiloscopy Examination in Maratha's Population

PLACE OF STUDY: Govt. Institute of Forensic Science, Chhatrapati Sambhajinagar

ABOUT THE STUDY:

Lip print examination, also known as cheiloscopy, is a forensic technique used to analyze the patterns, grooves, and characteristics of lip prints left on surfaces such as glass, paper, or other objects. Lip prints are unique to individuals, similar to fingerprints, and can be used for identification purposes in forensic investigations. Lip print examination involves studying the shape, size, and arrangement of lines and grooves on the lips to identify individuals.

In simple terms, lip prints refer to the unique patterns and lines that are naturally present on a person's lips. Just like fingerprints, lip prints are specific to each individual and can be used for identification purposes. Lip print examination involves studying these patterns and characteristics to help identify a person, especially in forensic investigations.

Note: Your identity will be completely confidential and it will not be revealed at any point in this research.

CONSENT: I hereby give you my consent for collection of my handwriting samples. The project work was explained to me and I have understood all the information. I agree that my participation is voluntary for the study.

NAME OF PARTICIPANT: _____

SIGNATURE:

DATE AND TIME:

SUBJECT INFORMATION FORM

NAME: _____

First name Middle name Last name

DATE OF BIRTH: | | | |

Day Month Year

AGE:_____ **GENDER:** **MALE** ☐ **FEMALE** ☐

ADDRESS: _____

QUALIFICATION: _____

OCCUPATION: _____

HABIT:

SMOKING ☐ **CHEWING TOBACCO** ☐

LIP BITING ☐ **LIP LICKING** ☐

LIP CARE HABITS:

LIP BALM ☐ **LIPSTICKS** ☐

LIP GLOSS ☐ **HYDRATION (MOISTURISER)** ☐

ANY OTHER _____

ARE YOU SUFFERING FROM ANY DISEASE REALTED TO LIPS?

YES ☐ **NO** ☐

IF YES, THEN WHAT DESEASE YOU ARE SUFFERING?

_____

DO YOU HAVE ANY OTHER HEALTH ISSUE?

_____

FAMILY HISTORY IF ANY (related to lips):

_____

"Samruddhi Mahamarg Accident Data Analysis using K-Mean clustering"

Avinash Khambayat[1], Ashwini Sampatrao Salunkhe[2]

1- School Of science, Sandip University, Nashik, Maharashtra, India,
2- Research Scholar Sandip University, Nashik, Maharashtra, India,

Abstract:

Road accidents is more frequent today due to variety of reasons. This paper contains the study of road accidents on Samruddhi Expressway (Mahamarg) in Maharashtra. We are identifying distinct patterns, high-risk locations contributing factors and trends in accident data. We are using data analysis by K-Mean clustering for this case study.

Keywords: Big Data Analysis, clustering, K-means, Traffic data Analysis

Introduction:

The Samrudhhi Mahamarg or Nagpur-Mumbai Expressway (Officially known as Hinduhrudaysmrat Balasaheb Thakare Maharashtra Samruddhi Mahamarg) and ME-2 is partially opened 6 lanes wide (Expanded to 8) 701 Km long access-controlled expressway in Maharashtra India. It is almost the country's longest greenfield road project, which will connect the two capital cities of the states, its capital Mumbai, and its third largest and alternate capital city Nagpur.

The Mumbai-Nagpur Expressway will travel through 10 key districts directly and 14 districts indirectly, via feeder roads, 24 talukas, and 392 villages. The total project cost is around 55,000 crore and the Government of Maharashtra believes that the expressway will become a prosperity corridor for the overall socio-economic growth of the state. But still this road has been mired in controversy since the beginning due to frequent accidents on it. A total 1282 accidents have taken place on the expressway since December 2022.[1]

What is Big Data:

Big Data refers to extremely larger and diverse collection of structured, unstructured, and semi-structured data that continues to grow exponentially over time. These datasets are so huge and complex in volume, velocity, and variety that traditional data management system cannot store, process, and analyse them. Big data describes large and diverse datasets that are huge in volume and rapidly grow over time. Big data is used in machine learning, predictive modelling, and other advanced analytics to solve business problems and make informed decisions. Cost reduction, product

development, strategic business decisions, risk management are some benefits of big data analysis. [2][5]

K Mean:

Clustering is the process of partitioning or grouping a given set of patterns into disjoint cluster. Clustering has been a widely studied problem in a variety of application domain. K Means is one of the simplest and popular unsupervised machine learning algorithms. [4]

Materials and Methods:

Karl Pearson's Coefficient of Correlation:

Correlation is a measure of a monotonic association between two variables. In corelated data, the change in the magnitude of one variable is associated with a change in the magnitude of another variable, either in the same or in the opposite direction. "Correlation" is used in the context of such a linear relationship between two continuous, random variables, known as Pearson's correlation, which is commonly abbreviated as "r."

Regression of Line:

Regression allows the researchers to predict or explain the variation in one variable based on another variable. And a regression line is a straight line that describes how a response variable y changes as an explanatory variable x change.

Karl Pearson's Coefficient of Correlation Formula

$$r = \frac{Cov(x,y)}{\sigma_x \sigma_y} = \frac{\sum xy}{\sqrt{(\sum x^2)(\sum y^2)}}$$

Where r = Pearson's correlation coefficient

$\sum x^2$= Sum of the squares of the x values

$\sum y^2$= Sum of the squares of the y values

$\sum xy$ = Sum of the product of x and y

Regression Line Co-variation:

A regression line is a statistical tool that depicts the correlation between two variables specially, it is used when variation in one (dependant variable) depends on the value of other (independent variable).

Regression equation of x on y

$$(x - \bar{x}) = b_{xy}(y - \bar{y})$$

Where $b_{xy} = \frac{\sum xy - n\bar{x}\bar{y}}{\sum x^2 - n(\bar{x})^2}$

N = Number of pairs of values

$\bar{x}$ = Mean of x and $\bar{y}$ = Mean of y

$\bar{x}\bar{y}$= Product of $\bar{x}$ *and* $\bar{y}$

$\sum xy$ = Sum of the product of x and y and

$\sum x^2$ = Sum of the squares of x values

Table1: The following data shows the number of accidents will be happens during the period of coming 1000 days

X(Days)	**100**	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**
Y(Accidents)	135.56	162.56	189.54	216.54	243.53	270.52	297.91	324.50	351.49	378.48

X = Number of Days

Y = Number of Accidents

Table2: Now we form a cluster of above data into 3 groups (By Assuming)

Cluster A **Days 325**	**Cluster B** **Days 325**	**Cluster C** **Days 325**
1282	1714	2556

Table3: Days and number of accidents data of Samrudhhi Mahamarg from December 2022

No. Of Days (x_i))))	**21**	**31**	**28**	**31**	**30**	**31**	**30**	**31**	**31**	**30**	**31**
No. Of Accidents (y_i)	84	124	112	124	120	124	120	124	124	120	124

Table4:

Sr. No	x_i	y_i	xy	x_i^2	y_i^2
1	21	84	1764	441	7056
2	31	124	3844	961	15376
3	28	112	3136	784	12544
4	31	124	3844	961	15376
5	30	120	3600	900	14400
6	31	124	3844	961	15376
7	30	120	3600	900	14400
8	31	124	3844	961	15376
9	31	124	3600	961	15376
10	30	120	3844	900	14400
11	31	124	3600	961	15376
Total	$\sum x = 325$	$\sum y = 1282$	$\sum xy = 38206$	$\sum x^2$ =9691	$\sum y^2 = 150916$

$\sum x$ = Number of days
$\sum y$ = Number of Accidents
$\sum x^2$= Sum of the squares of the x values
$\sum y^2$= Sum of the squares of the y values
$\sum xy$ = Sum of the product of x and y

Results:

1.Karls Pearson's Correlation Coefficient

$$r = \frac{Cov(x,y)}{\sigma_x \sigma_y} = \frac{\sum xy}{\sqrt{(\sum x^2)(\sum y^2)}} = \frac{38206}{\sqrt{(9691)(150916)}} = \frac{38206}{38242.9988} = 0.999\ (< 1)$$

A positive correlation coefficient of 0.999 (< 1) implies that x and y are positively corelated. In other words, as x increases y also increase and vice versa.

2.Regression Line x on y

$$\bar{x} = \frac{\sum x}{n} = \frac{325}{11} = 29.55 \qquad \bar{y} = \frac{\sum y}{n} = \frac{1282}{11} = 116.54545$$

$$b_{xy} = \frac{\sum xy - n\bar{x}\bar{y}}{\sum x^2 - n(\bar{x})^2} = \frac{38206 - 11(29.55)(116.54545)}{(9691) - 11(872.9339)} = 3.705$$

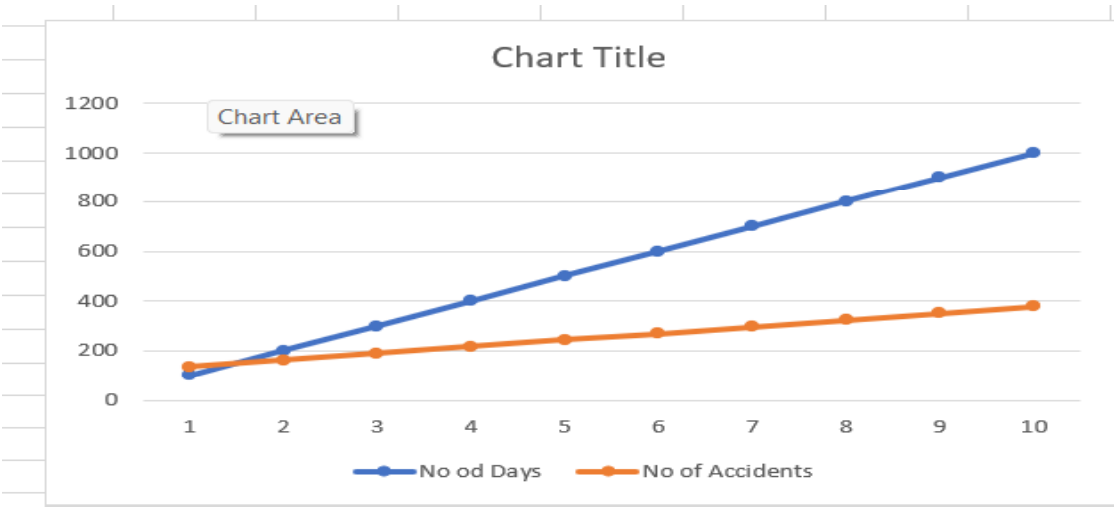
$$(x - \bar{x}) = b_{xy}(y - \bar{y})$$

When $x = 1000$

$$(1000 - 29.55) = 3.705(y - 116.55)$$
$$y = 378.48$$

Table5:

Sr. No.	No. Of Days	No Of Accidents
1	100	135.5648
2	200	162.5554
3	300	189.546
4	400	216.5365
5	500	243.5271
6	600	270.5176
7	700	297.5082
8	800	324.4987
9	900	351.4893
10	1000	378.4798
		2571



Therefore 2571 Approx. accidents will happen within 1000 days.

Conclusion

In this study, we make the clusters of present data obtain by the MSRDC. As we focus on first cluster it is observed that as the number of days increased, no of accidents also increase. The main thing is that the coefficient of correlation is lies in good range (less than 1) no of accidents are frequently increased. To control these increment ratios, we must take preventions using safety measures. The government can lower the number of fatalities and injuries caused by traffic accidents by taking the required action and can create a safer environment for all road users.

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"A Review on Recent Advancement in Chemosensors Ligands for Metal Ions"

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ABSTRACT:

The development of chemosensors for metal ions is a vital area of research due to the significant biological and environmental roles of these ions. This review focuses on the synthesis and characterization of Schiff base ligands and naphthoquinone derivatives, exploring their potential as selective and sensitive chemosensors for metal ions in aqueous solutions. Schiff base ligand 3-((2-hydroxyphenylimino)methyl)-4H-chromen-4-one (SL) was synthesized and demonstrated exceptional selectivity and sensitivity towards Cu^{2+} , Fe^{3+} , and V^{5+} ions. The complexation between SL and these metal ions resulted in distinct color changes, with detection limits of 7.03 µM for Cu^{2+} , 5.16 µM for Fe^{3+} , and 5.94 µM for V^{5+} , and binding constants of 1.37×10^4 M^{-1}, 2.01×10^4 M^{-1}, and 1.82×10^4 M^{-1}, respectively. Additionally, naphthoquinone-based ligands showed significant promise as chemosensors, exhibiting remarkable molecular

recognition abilities and providing easily detectable signals for various metal ions such as Cu^{2+} , Ni^{2+} , and Co^{2+} . These findings highlight the potential of Schiff base ligands and naphthoquinone derivatives in the development of advanced chemosensors for practical applications in environmental monitoring and biological systems. Future research directions include expanding the scope of metal ion detection and enhancing the selectivity and sensitivity of these chemosensors for real-world sample analysis.

Keywords: Chemo sensors, selectivity, Schiff Base, Naphthoquinone Derivatives, Ligands

Introduction:

Recent years have seen significant advancements in the development of chemosensors for the detection of biologically and environmentally active ionic species. There is a continuous demand for chemosensors that are highly selective for metal ions due to their ability to offer accurate and low-cost detection of both anions and metal ions with high selectivity and sensitivity. [1]

Numerous chemosensors have been developed for the selective qualitative analysis of various target molecules, utilizing unique host-guest interactions such as hydrogen bonding, electrostatic forces, metal-ligand coordination, hydrophobic interactions, and van der Waals forces. Most of these chemosensors are utilized in solution, leveraging spectroscopic analysis for its simplicity, convenience, and cost-effectiveness. [2]

The design of metal ion chemosensors involves coupling a metal-binding unit with a signaling unit, resulting in sensors with high binding affinity and selectivity towards specific metal ions. Over the years, many luminescent-based chemosensors have been developed and studied for their applications in physiology and medical diagnostics. [3]

Recently, substantial efforts have been directed towards designing chemosensors for anions due to the critical importance of anion detection in biological and environmental systems. This heightened interest underscores the vital role of anion detection in these fields. [4]

A sensor is a device capable of detecting analytes either chemically or electrochemically. The chemical sensing of an analyte is referred to as a chemosensor. Chemosensors are molecular probes designed to identify various chemical species, including metal ions, anions, biological contaminants, and explosives, through photophysical changes. They play a critical role in identifying contaminated areas and implementing remediation strategies to protect human health and the environment. [5]

Chemosensors can detect and quantify the presence of specific chemical species, such as metal ions, within a sample. They are engineered to target specific analytes in biological or environmental samples and typically consist of three main components: a recognition group that selectively binds to the target analyte, a spacer that connects the recognition group to a signaling unit, and a signaling unit that produces a measurable response upon binding to the analyte. [6]

Various types of chemosensors have been developed, including colorimetric, fluorescent, absorption-based, and electrochemical sensors. Among these, colorimetric and fluorescence chemosensors are the most advanced. These sensors operate through mechanisms such as fluorescence emission, colorimetric changes, electrochemical signals, and other spectroscopic methods. Advanced mechanisms in chemosensors include chelation-enhanced fluorescence (CHEF), intramolecular charge transfer (ICT), photoinduced electron transfer (PET), charge transfer (CT) from chelator to fluorophore, and deprotonating mechanisms. Chemosensors offer several advantages, such as simple and rapid detection, high sensitivity and selectivity, and the capability to detect analytes in complex samples. [7]

The continuous development of new and improved chemosensors is essential to address the challenges in sensing applications, particularly in biological and environmental analysis, due to rapid industrialization and the emergence of new pollutants. Numerous chemical species, including metal cations, anions, biological pollutants, explosives, and antidotes, can exhibit varying levels of toxicity depending on their concentration. Some pollutants can contaminate environmental sources like food and water due to industrial activities.

Chemosensors play a crucial role in monitoring metal ions, anions, and other chemical species involved in biological and environmental processes. Recently, chemosensors and optical sensors have garnered significant attention due to their unique features, such as simplicity, high sensitivity, rapid detection, low cost, ease of operation, and real-time detection capabilities. These advantages make chemosensors highly desirable for various applications compared to conventional detection methods. [5,7].

Materials and Methods:

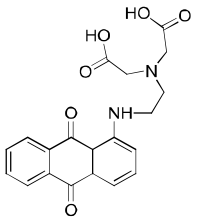
1. **Chemosensor Ligands for Metal Ions**

   1.1 Cu^{2+} (Copper Ions)

A ligand is an ion or molecule that donates a pair of electrons to a central metal atom or ion, forming a coordination complex. A wide variety of sustainable chemosensors have been developed to detect metal ions, with copper being a crucial transition metal ion. Copper plays a significant role

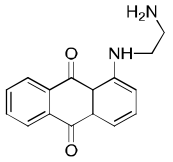
in biological functions such as hemoglobin synthesis, oxygen binding, nerve function, and electron transfer processes. Low levels of Cu^{2+} in cells can affect enzyme activity, while excessive Cu^{2+} levels can cause serious diseases such as Wilson's disease and Alzheimer's disease. Wilson's disease is a rare genetic disorder characterized by excess copper stored in various body tissues, particularly the liver and brain. However, Cu^{2+} is an essential micronutrient for both plants and humans.[8,9]

The synthesis of chemosensors for Cu^{2+} is an active area of research due to the high demand for selective and sensitive chemosensors for in vitro and in vivo purposes. Das et al. reported a 1,8-diaminonaphthalene-based ratiometric and colorimetric off–on fluorescent receptor (E), which is a representative tool for the quantitative detection of target ligands. This receptor detects Cu^{2+} ions selectively among other transition and heavy metal ions. Kumar et al. reported diamide-based chemosensors (A, B, C, D) possessing anthracene 9, 10-dione as a chromogenic moiety, enabling color-based detection. These receptors are highly selective and sensitive for colorimetric recognition of Cu^{2+} ions. The selective deprotonation of an aryl amine NH by Cu^{2+} is responsible for significant color changes, with the stability of the Cu^{2+} complex in the pH range of 7–12. [10, 11]



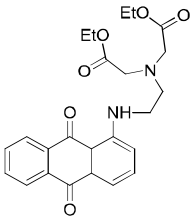
2,2'-((2-((9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl)amino)ethyl)azanediyl)diacetic acid

[A]



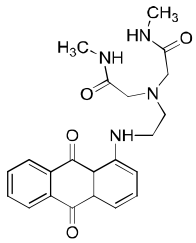
1-((2-aminoethyl)amino)anthracene-9,10(4a*H*,9a*H*)-dione

[B]



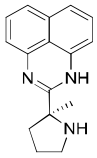
diethyl 2,2'-((2-((9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl)amino)ethyl)azanediyl)diacetate

[C]



2,2'-((2-((9,10-dioxo-4a,9,9a,10-tetrahydroanthracen-1-yl)amino)ethyl)azanediyl)bis(*N*-methylacetamide)

[D]



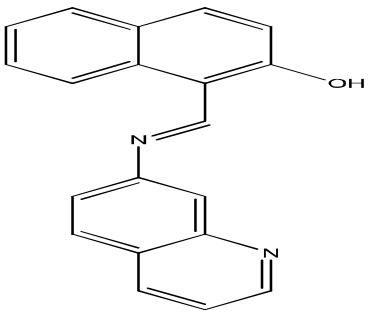
(*S*)-2-(2-methylpyrrolidin-2-yl)-1*H*-perimidine

[E]

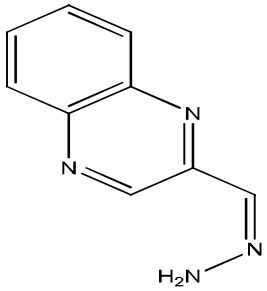
1.2 Ni^{2+} (Nickel Ions)

Ni^{2+} is an essential metal ion in biological systems, playing an important role in respiration, metabolism, and biosynthesis. However, excess Ni^{2+} can result in various diseases such as pneumonitis, asthma, respiratory disorders, and lung cancer. Nickel has widespread industrial applications, including electroplating, welding, painting, and pigment production, making the detection of nickel crucial from both industrial and biological perspectives. [12]

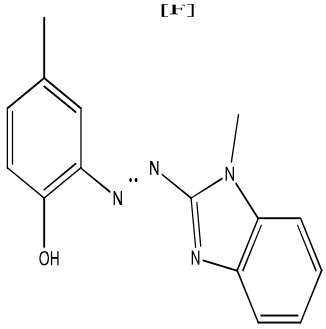
Zhang et al. reported a highly selective chemosensor that can detect Ni^{2+} ions in aqueous solutions, showing high selectivity and sensitivity towards these ions. It is understood that Ni^{2+} forms coordination complexes with the chemosensor. Sarkar et al. designed and synthesized a monohydrazone quinoxaline aldehyde (HOQA) chemosensor, which shows a remarkable colour change from colourless to yellow upon specific and selective binding to nickel, detectable even by the naked eye. HOQA is a highly selective ratiometric and colorimetric probe for Ni^{2+} ions, with cation detection ability monitored by UV-Visible and ^1H NMR titration, as well as DFT (Density Functional Theory) studies. HOQA can recognize nickel cations at a limit of 5.59 μM over other interfering cations in CH_3 CN solution. Mondal et al. reported a benzimidazole-based ratiometric and colorimetric chemosensor for Ni^{2+} ions, which is highly efficient in detecting Ni^{2+} over other metal ions commonly coexisting with Ni^{2+} in physiological and environmental samples. The cation sensing ability of this receptor has been determined by UV-Visible spectroscopy.[13,14]



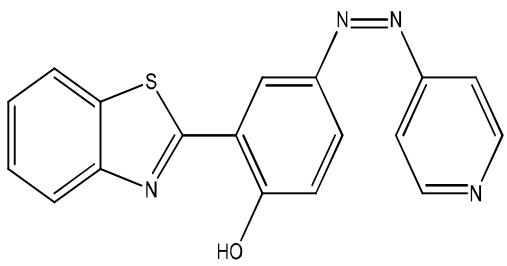
(*E*)-1-((quinolin-7-ylimino)methyl)naphthalen-2-ol
[F]



(*Z*)-2-(hydrazonomethyl)quinoxaline
[G]



[H]



(*Z*)-2-(benzo[*d*]thiazol-2-yl)-4-(pyridin-4-yldiazenyl)phenol

1.3 Naphthoquinone based Chemosensors

The α,β-unsaturated carbonyl derivatives of 1,4-naphthoquinones are attracting increasing interest due to their diverse biological activities, including anticancer, antimicrobial, antifungal, antitumor, and antiviral properties. These compounds' molecular features, such as conjugation and electrophilicity, are crucial in determining their metabolic pathways and redox cycling processes essential in chemotherapy. The redox switching of these naphthoquinones, resulting in the formation of radical anions or dianions upon electron acceptance, is particularly intriguing. Additionally, the intra-molecular charge transfer (ICT) transition is highly influenced by the amine substituent, especially the charge density on the nitrogen center. [21]

Prajkta et. al. in their research paper named Naphthoquinone based chemosensors for transition metal ions: experiment and theory they worked on These ligands act as chemosensors, demonstrating exceptional molecular recognition and providing easily detectable, sensitive signals.

Naphthoquinone-based chemosensors, such as 2-((pyridin-yl)methylamino)naphthalene-1,4-dione (H-1), 2-((thiophen-2-yl)methylamino)naphthalene-1,4-dione (H-2), and 2-((pyridine/thiophen-2-yl)ethylamino)naphthalene-1,4-dione (H-3 and H-4), have been synthesized and characterized using FT-IR, 1H and 13C NMR, and single-crystal X-ray diffraction studies.

The chemosensing capabilities of these ligands with various metal ions, including La^{3+} , Hg^{2+} , Cd^{2+} , Zn^{2+} , Cu^{2+} , Cu^{+} , Ni^{2+} , Co^{2+} , Mn^{2+} , Cr^{3+} , and Ca^{2+} in methanol, methanol-water mixtures, and in the presence of a mild base like triethylamine, have been evaluated. Notably, the chemosensors H-1 and H-3 have proven effective for detecting different metal ions such as Cu^{2+} , Ni^{2+} , and Co^{2+} , with observable colour changes detectable by the naked eye and further analyzable through UV-visible and fluorescence experiments. The synthesis and characterization of 2-((pyridine-2-yl)methylamino)naphthalene-1,4-dione (H-1), 2-((thiophen-2-yl)methylamino)naphthalene-1,4-dione (H-2), and 2-((pyridine/thiophen-2-yl)ethylamino)naphthalene-1,4-dione (H-3 and H-4) have been completed. The molecular recognition abilities of these ligands toward transition metal ions in various solvents, including methanol, methanol-water, methanol-triethylamine, and methanol-water-triethylamine mixtures, have been evaluated. Stoichiometries and association constants for H-1 and H-3 were determined, showing that both coordinate to metal ions via two nitrogen atoms and one oxygen atom.

Notably, H-1 and H-3 exhibit remarkable selectivity for Cu^{2+} ions in methanol or methanol-water mixtures, with the complexation resulting in a color change from orange to intense blue. The limits of detection (LOD) for Cu^{2+} with H-1 and H-3 are 1.48×10^{8} mol/L and 1.59×10^{8}

mol/L, respectively. Additionally, vibrational spectra, ^{1}H NMR chemical shifts, and optical properties of H-1 to H-4, derived from density functional theory, are presented.[21,22]

1.4 Schiff base Ligands for Chemoselective metal ions

Ali Q. Alorabi et. al. in their research paper named Schiff Base Ligand 3-(-(2-Hydroxyphenylimino) Methyl)-4H-Chromen-4-One as Colorimetric Sensor for Detection of Cu2+ , Fe3+ , and V5+ in Aqueous Solution. In their study they have synthesised Schiff bases derived from 3-formyl chromone have been extensively studied and have garnered significant interest among chemists and researchers due to their ability to easily bind with metal ions, forming complexes with various applications. Chromone-based azomethine colorimetric chemosensors, as reported by Rezaeian et al., have been successfully used to detect and recognize Cu^{2+} , Zn^{2+} , and CN^{-} ions. Continuing our research, we present the design and synthesis of a multidentate Schiff base ligand, 3-((2hydroxyphenylimino) methyl)-4H-chromen-4-one. This ligand was prepared by condensing 3-formyl chromone with 2-aminophenol and has demonstrated the ability to bind to metal ions, forming colored complexes. We further tested its efficacy as a colorimetric chemosensor for detecting metal ions in aqueous solutions.synthesized the Schiff base ligand 3-((2-hydroxyphenylimino) methyl)-4H-chromen-4-one (SL) and investigated its use as a chemosensor for detecting metal ions in aqueous solutions. The compound SL demonstrated remarkable selectivity and sensitivity towards three metal cations: Cu^{2+} , Fe^{3+} , and V^{5+} . Experimental results indicated a 2:1 (SL) binding stoichiometry for the SL-Cu^{2+} and SL-Fe^{3+} complexes, while a 1:1 stoichiometry was observed for the SL-V^{5+} complex. The limits of detection (LOD) and binding constants were determined to be 7.03 µM and 1.37×10^{4} M^{-1} for Cu^{2+} , 5.16 µM and 2.01×10^{4} M^{-1} for Fe^{3+} , and 5.94 µM and 1.82×10^{4} M^{-1} for V^{5+} .[24,25]

Conclusion:

In this review, we have explored the synthesis, characterization, and application of Schiff base ligands and naphthoquinone derivatives as chemosensors for metal ion detection. These compounds demonstrate significant potential due to their high selectivity and sensitivity, making them promising candidates for practical applications in environmental monitoring and biological systems.Schiff Base Ligands: The Schiff base ligand 3-((2-hydroxyphenylimino)methyl)-4H-chromen-4-one (SL) exhibited excellent selectivity and sensitivity towards Cu^{2+} , Fe^{3+} , and V^{5+} ions. The distinct color changes observed upon complexation with these metal ions facilitate easy visual detection. The ligand demonstrated binding stoichiometries of 2:1 for Cu^{2+} and Fe^{3+} complexes and 1:1 for the V^{5+} complex, with detection limits and binding constants indicating

strong interaction and stability.Naphthoquinone Derivatives: Naphthoquinone-based ligands showed remarkable molecular recognition abilities, making them effective chemosensors for various metal ions including Cu^{2+} , Ni^{2+} , and Co^{2+} . These ligands provided clear, detectable signals through observable color changes and were further analyzable using UV-visible and fluorescence spectroscopy.

Future Directions: The reviewed chemosensors offer significant promise for real-world applications. Future research should focus on expanding the range of detectable metal ions, enhancing the selectivity and sensitivity of these chemosensors, and validating their effectiveness in complex real-world samples. Additionally, exploring the potential of these sensors in diverse environmental and biological contexts will be crucial for advancing their practical utility.

Overall, the development of Schiff base ligands and naphthoquinone derivatives as chemosensors represents a valuable advancement in the field of metal ion detection, with significant implications for environmental and health monitoring

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Personal Identification Based on Morphological Features of Ears among the Uttarakhand Region of India

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Abstract

The external ear, particularly the pinna, is a crucial physical trait that may aid in personal identification in criminal investigations. Oil on the ear can deposit its impression on surfaces mobile phone screens, glass windows. The ear morphology can be affected by geographical regions. The current study aims to look into variations in ear morphology and analyse ear impressions to have better understanding of their significance in personal identification. The study involved 100 participants, aged between 15 - 30 years from Ramnagar area, Nainital district, Uttarakhand state, northern India. The morphological features such as shape of the ear, size, earlobe shape, concha size, attachment of the earlobe was studied. The ear prints were recorded using ink method and Photographs. Further, the ear impressions were analysed using Image J software to measure data such as ear length, ear breadth, earlobe length, earlobe breadth, concha length, concha breadth. The findings showed that oval shaped ears are common among males and round shaped ears in females with other shapes like rectangle and triangle also present. Similarly, the most common ear lobe shape was square in males and tongue shaped in females. Females had slightly larger averages of ear length and width than males. Significant differences were found between males and females right ear length (p-value =0.05) and right concha length (p-value=0.01). The study contributed to existing research by highlighting the variability in ear morphology among Uttarakhand Region of India. It further emphasizes the need of understanding and analysing these differences in personal identification.

Key words: Ear morphology, ear prints, personal identification, forensic science, North Indian population, ear variations.

Introduction

The external ear is an important instrument in personal identification since it has numerous morphological traits that aid in forensic investigations. Personal identification is the process of determining the identities of people involved in criminal cases based on physiological traits[1]. The ear prints appear as a useful tool, providing a 2-D replica of the human external ear upon contact with surfaces [2]. The distinct morphological characteristics of ear prints serve as key indicators, tying suspects to criminal crimes [3]. This involves examining ear prints found at crime scenes, such as on doors and windows, which can be taken and developed as a tool for identification [4]. The external ear consists of the outer and inner ears. The pinna is a skin-covered flap in the outer ear region, typically positioned on the side of the head. Oil on the external ear aids in accurately detecting the ear. If the suspect is unknown in such circumstances, the latent prints can be matched to a database including prints retrieved from the crime scene [2]. The study of ear structure, known as otoscopy or earology, has drawn interest not just from forensic scientists but also from anthropologists, plastic surgeons, and physicians due to its importance in a wide range of professions[5]. Hirschi was the first to demonstrate the significance of ear prints for personal identification in the field of forensics [2]. Some pioneering studies by researchers, such as Iannarelli's first study, selected ten thousand human ears at random in California and measured twelve distances from certain locations on the ears, demonstrating the uniqueness of the anatomical features of the human external ear [6]. If we trace the history of ears and ear prints, we will come across Darwin’s findings, which captured the scientific world's attention, leading to the ear [7]. Darwin studied primates, focusing on the ear. He noticed a shrinking primitive ear corner, referred to as the 'tubercle of Darwin' [1].

The morphological characteristics of the external ear play a crucial role in Disaster Victim Identification (DVI). Veerappan, a notorious sandalwood smuggler was identified by the Special Task Force in India in 2004, through an examination of his ear morphology [3]. The external ear develops significantly, with evident features appearing as early as the 38th day of fetal development. On the 56th day, the ear takes on its defined position, and on the 70th day, the external ear shape is recognized [8]. The predominance of human pinna size after development emphasizes its usefulness in forensic scenarios [9]. Piercings and other changes

to the earlobe, however feasible, have no major impact on the diagnostic traits required for identification [7]. The anatomical features of the human external ear vary among different individuals [10]. The external ear has significant variations in form, size, contour, and depression, making it a distinguishing trait for identification [11]. The human ear is well-known for its peculiarity and is among the most noticeable facial features [3]. The key morphological features to be researched are size, shape, moles, earlobe, tragus, anti-tragus, helix, and antihelix [12]. Taking photographs helps in making exact measurements of ear dimensions possible, increasing their utility in forensic investigations [12]. Despite potential variations caused by deliberate modifications or disease, the physical features of the human external ear are typically stable, facilitating in personal identification [4]. However, some traits can be changed purposefully through piercings or alterations, while others may be caused by disease, but these changes have little effect on the diagnostic features of the human external ear [2]. Climate can have an impact on the physical properties of human ears. However, environmental factors such as migration and the period since the print was left to when it is lifted, may have an indirect effect on ear identification. Human ears form throughout fetal development and are relatively stable throughout life unless subjected to severe trauma or pathological circumstances [12]. Variations in the genetic histories of different populations can impact the ear's structure, size, and characteristics. Geographically unique ear characteristics may originate from genetic variability among populations. The climate, which includes factors like humidity and temperature influence ear shape, with colder climates having ears that are larger and more shaped like those in warmer climates. Environmental adaptations and cultural customs also impact ear shape over time. [10]. The current research aims to look into variations in ear morphology and analyse ear impressions to have a better understanding of their significance in personal identification.

MATERIALS AND METHOD:

100 participants, aged 15 - 30 years from the Ramnagar area in Nainital district, Uttarakhand state, northern India were selected. Individuals with physical abnormalities of the ear were excluded from the study. Random sampling was used to ensure a representative distribution among the stated age and gender groups.

Local community centres, educational institutions, and public areas were approached to recruit individuals. Individuals were provided with information about the research study's aims and methods. Before participation, each volunteer was provided written informed consent. Instruments that were used for data collection included a ruler, vernier calliper, fingerprint ink (ivory black), glass slab, and roller. Two techniques were used to record ear prints: (1) Ink and (2) Photographs. Ensured that the ear and the surrounding area were cleaned and dried to facilitate the transfer of ink and obtain accurate prints. Fingerprint ink was applied evenly onto a clean glass slab using a roller. This ensures uniform coverage of ink on the slab's surface. The ear was placed against the inked surface of the glass slab. The pressure was applied evenly to ensure that the ink transfers uniformly onto the ear's surface. As the pressure was applied ink was transferred from the glass slab onto the ear, creating a clear impression of the ear's morphology. For getting the print, the inked ear was carefully lifted from the slab and placed onto an A4 sheet or another suitable surface. Ensured that the inked ear made full contact with the paper to transfer the print accurately. Once the print was obtained, the characteristics presented in the ear print were studied as mentioned in Fig.2 For taking photographs the subject was positioned in a shadow -free area to obtain clear image of the ear. The mobile camera was set up at an appropriate distance, approximately 1 meter away from the individual, to get a detailed image of the ear. Ensured that the subject's ear was visible and positioned properly within the mobile camera's frame. Once the photographs were captured, they were transferred to a computer or any digital device for further analysis using Image J software. This information was stored digitally for further analysis as given in Fig.3 Digital images can be of great importance when the physical prints cannot be recorded. The ear impressions were analysed using ImageJ™ software for measurements of data. This software is used for image processing and analysis, which makes it easier to examine the morphological features of the ear for personal identification and investigating variations in these features. This software measures area, min and max of selection or entire image. Measures lengths and angles.

The following morphological characteristics were examined in the Ear impressions:

(1) Ear length: The distance between the base of the earlobe to the top of the helix.

(2) Ear breadth: This is the width of the ear, commonly measured from the outside edge of the helix on one side to the outer edge of the helix on the opposite side, at the widest point of the pinna.

(3) Earlobe length: The earlobe's length, measured from the attachment site to the ear's lowest point.

(4) Earlobe breadth: The width of the earlobe, usually measured at its broadest point.

(5) Concha length: The distance measured along the concha, the ear's bowl-shaped cavity that is adjacent to the ear canal.

(6) Concha breadth: The concha's width, usually measured at its broadest point.

(7) Ear shape: The overall structure of the ear, which might include round, oval, triangular, or rectangular features.

(8) Earlobe form: The earlobe's shape, which might include square, triangular, arched, or tongue-shaped features.

(9) Earlobe attachment: This refers to whether the earlobe is fixed to the side of the head or, in the case of a free-hanging earlobe, if it is detached from the head [3][10]. Fig. 4, 5, and 6 demonstrate the morphological characteristics of the ear.

RESULTS:

The results show an average ear length of 5.27 cm while the average width of the ear was 2.813 cm. A previous study Verma [3] shows the average ear length was approximately 6.42 cm and average ear width was approximately 3.53 cm. Similarly, the average ear lobe length was 0.646 cm and average ear lobe width was approximately 1.464 cm. And the average concha length was approximately 2.139 cm while the average concha width was approximately 1.296 cm.

The distribution of ear shapes for both the ears among the male individuals was observed and result shows total of 65 oval shapes, followed by 20 round, 11 rectangle, 4 triangle Fig. 7. Oval-shape of the ear was observed to be most common with 65%, Round shape of the ear was 20%, Rectangle was 11%, Triangle shape of the ear was least common with 4%. Previous study Krishan [10] shows the oval shape more commonly seen with 40 % in left ear and 40 % in right ear so the average of both the ear shapes was approximately 40% of both the ears with oval shape.

The distribution of ear lobe shapes for both the ears among the male individuals was observed and result shows total of 25 arched shapes ears were observed, followed by 38 squares, 35 tongue, 2 triangles Fig. 9. Arched shapes of the ear were observed to be 25%, Square shape of the ear was more common with 38%, Tongue shape was 35%, Triangle shape of the ear was least common with 2%. Previous study Krishan [10] shows the arched shapes was commonly seen with 67.8% in left and 74.4% in right ear so the average of the both the ear lobe shapes was approximately 71.1%.

The distribution of ear lobe attachment for both the ears among male individuals was observed and result shows total of 37 free ear lobe attachment, 41 partially attached and 22 attached ears Fig. 11. Free ear lobe attachment was observed to be 37%, partially attached was 41%, attached was least common with 22%. Previous study Krishan [10] shows the attached ear lobe attachment to be commonly seen with 50% in left ear and 53.3% in right ear so the average of both the ear lobe attachment was approximately 51.65%.

The average ear length was 5.612 cm and average width was 2.982 cm in female samples and the average ear lobe length was approximately 0.662 cm while the average ear lobe width was approximately 1.556 cm. And the concha length was approximately 2.256 cm while the average concha width was approximately 1.439 cm.

The distribution of ear shapes for both the ears of females was observed and result shows total of 34 oval shapes ears were observed, followed by 47 round, 16 rectangle, 3 triangle Fig. 13. Oval-shape of the ear was observed to be 34%, Round shape of the ear was most common with 47%, Rectangle was 16%, Triangle shape of the ear was least common with 3%. Previous study Krishan [10] shows the oval shape more commonly seen with 44.8 % in left ear and 46 % in right ear so the average of both the ear shapes was approximately 45.4% of both the ears with oval shape.

The distribution of ear lobe shapes for both the ears of females was observed and result shows total of 26 arched shapes ears were observed, followed by 35 squares, 39 tongue, no triangle shape was observed in females Fig. 14. Arched shapes of the ear were 26%, Square shape of the ear was 35%, Tongue shape was 39%. Previous study Krishan [10] shows the arched shapes was commonly seen with 67.8% in left and 72.4% in right ear so the average of the both the ear lobe shapes was approximately 70.1%.

The distribution of ear lobe attachment for both the ears of females was observed and result shows total of 48 free ear lobe attachment, 20 partially attached and 32 attached ears were observed Fig. 15.

Free ear lobe attachment was observed to be more common with 48%, partially attached was least common with 20%, attached was 32%. Previous study Krishan [10] shows the attached ear lobe attachment to be commonly seen with 56.3% in left ear and 56.8% in right ear so the average of both the ear lobe attachment was approximately 51.55%.

Findings also indicated the significant differences between males and females ear characteristics, such as right ear length with significant difference showing p- value of 0.05 and right concha length with significant difference showing p- value of 0.01 Table-1.

DISCUSSION:

The results of the present study highlight the uniqueness of ear morphology among individuals, by studying the variations in shape, size, and other morphological features. Each ear exhibits unique characteristics that distinguish it from others. This uniqueness can be attributed to the diverse shapes observed in the ear across different individuals, emphasizing the importance of understanding and analysing these variations for forensic and identification purposes.

CONCLUSION

This study investigated the morphological features of ears among the Uttarakhand Region of India. The ear feature including length, width, earlobe characteristics, concha measurements, and overall ear shape were examined from ear prints and photographs. The results were studied using descriptive statistical analysis. Males had an average ear length and width of 5.27 cm and 2.813 cm, with an earlobe length of 0.646 cm and a width of 1.464 cm. The concha, or inner ear, measured 2.139 cm in length and 1.296 cm in width. Oval-shaped ears were the most common, followed by round, rectangle, and triangle shapes. Females had slightly larger averages of 5.612 cm and 2.982 cm, with an earlobe attachment of free attachment, arched and square-shaped earlobes, and partial attachment. The results showed the significant differences between males and females ear characteristics, such as right ear length with p- value of 0.05 and right concha length with p-

value of 0.01. The findings highlight the diversity and similarity of ear morphology in geographical region. The distinct features of ear morphology may offer significant clues for identifying people in criminal cases. In the future studies, other geographical regions may be explored to study the variations in morphology of ear.

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CONFLICT OF INTEREST: nil

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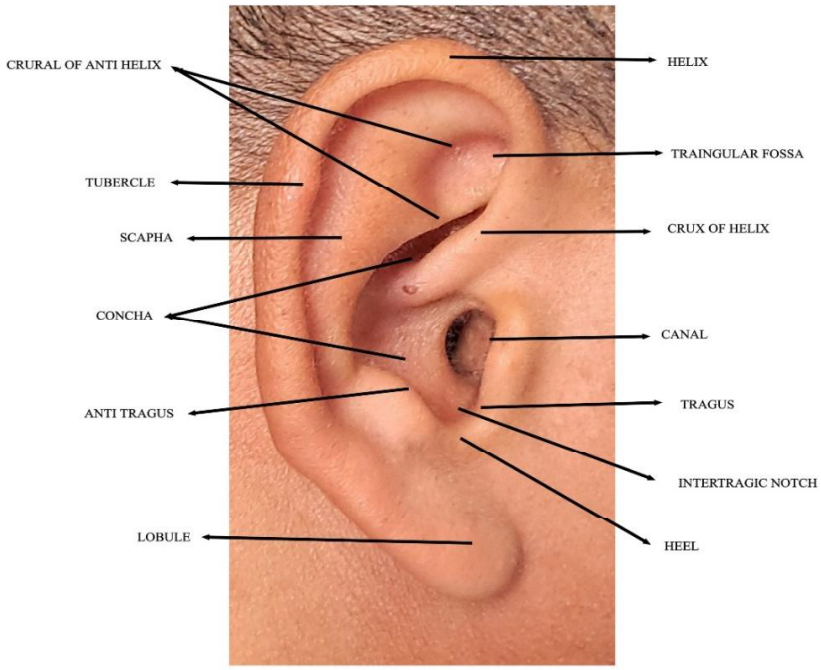


Fig.1 Shows the morphology and different parts of the ear.

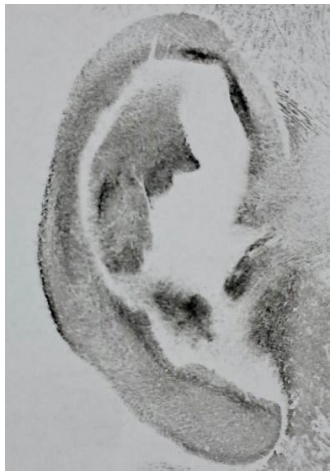


Fig. 2 Shows the ear impression taken by ink method.

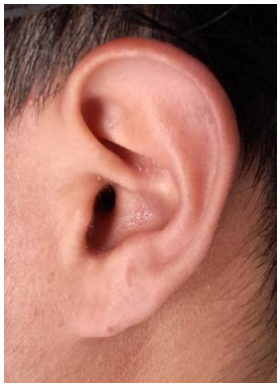


Fig.3 Shows the ear sample taken by photographic method.

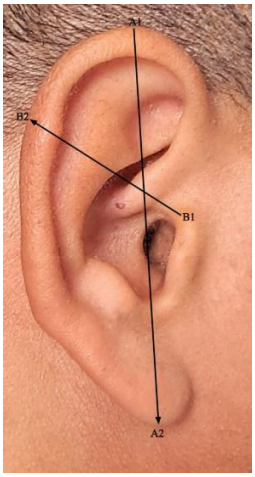


Fig.4 Ear length and breadth.

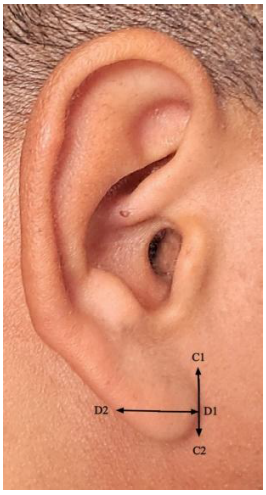


Fig. 5 Earlobe length and breadth.

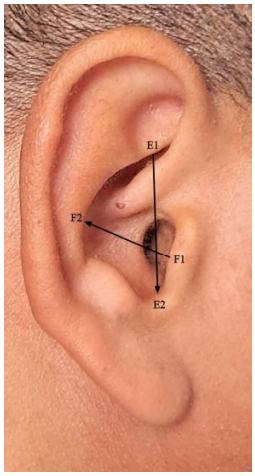


Fig.6 Concha length and breadth.

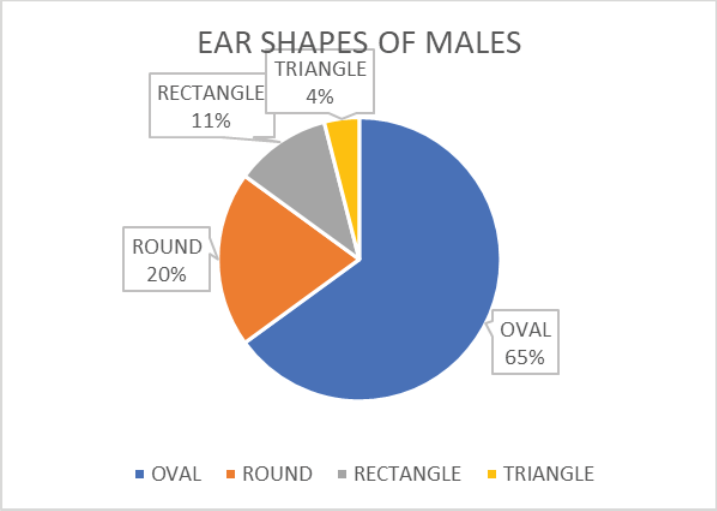


Fig. 7. The pie chart shows the distribution of ear shapes in male samples.

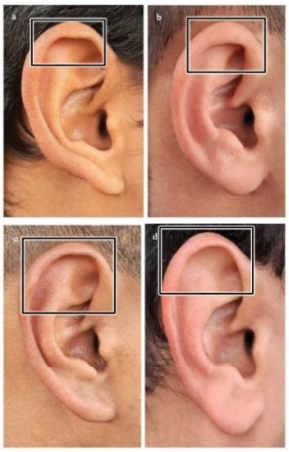


Fig. 8. Photograph showing different ear shapes: (a) rectangle (b) triangle (c) round (d) oval.

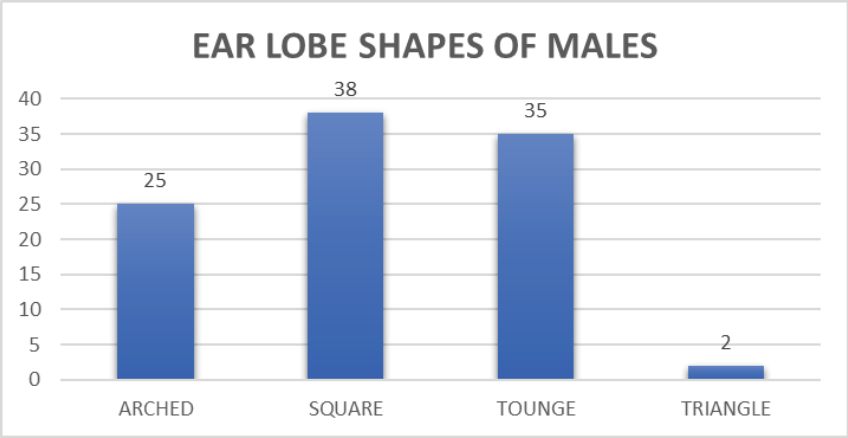


Fig. 9. Shows the distribution of earlobe shapes in male samples.

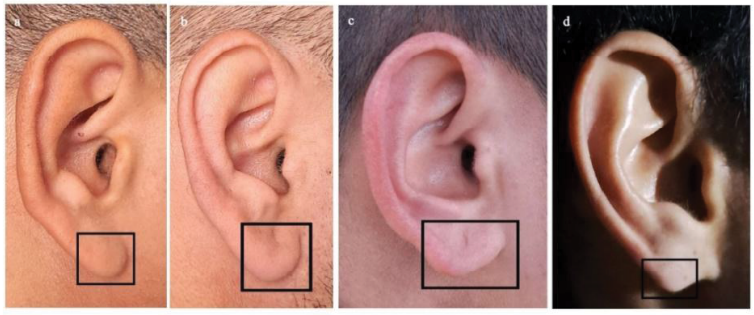


Fig. 10. Photograph showing different earlobe shapes: (a) arched (b) tongue (c) square (d) triangle.

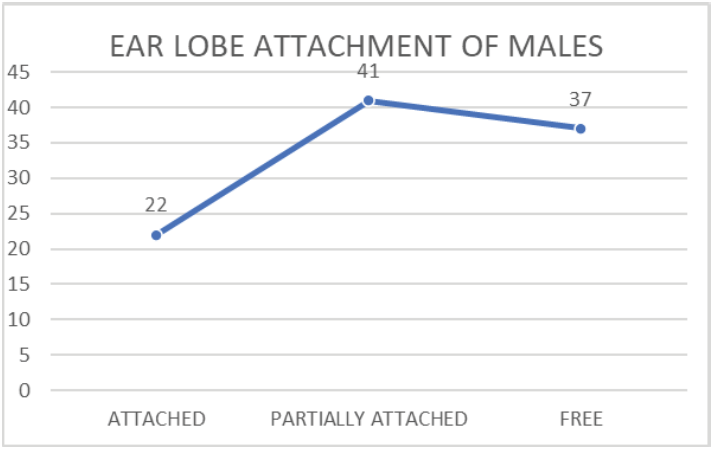
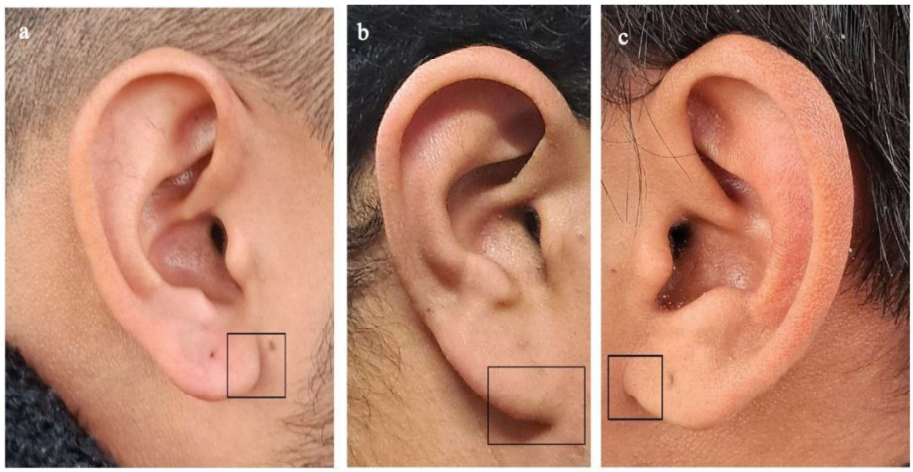


Fig. 11. Shows the distribution of earlobe attachment in male samples.



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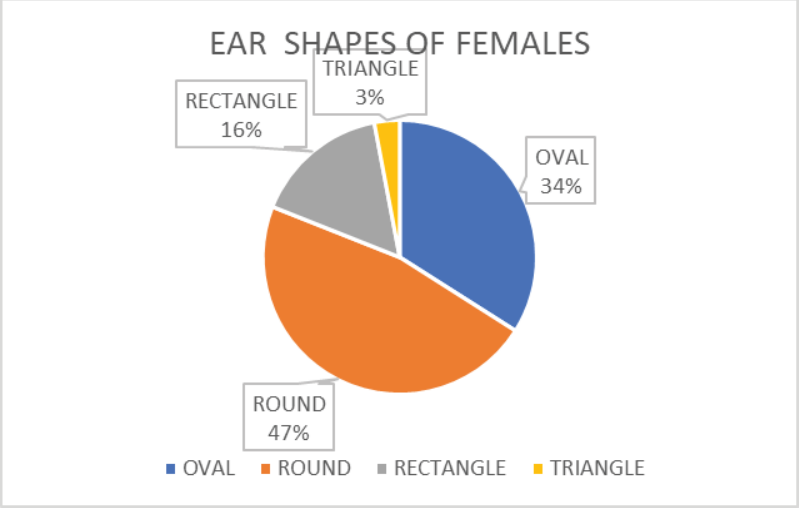

Fig. 13. Shows the distribution of ear shapes in female samples.

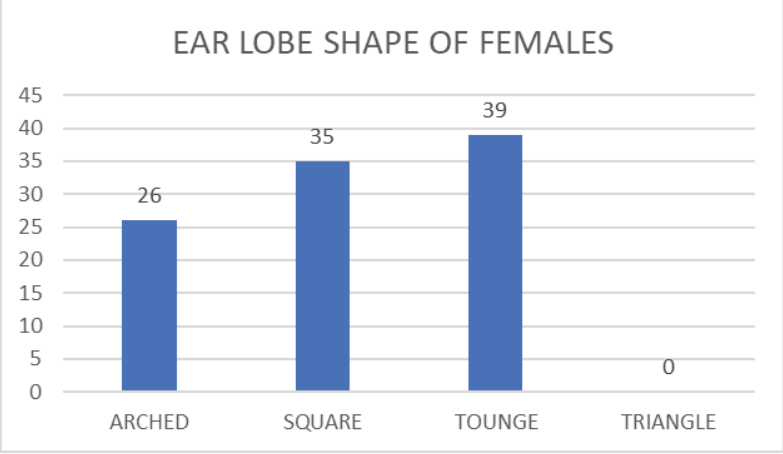

Fig. 14. Shows the distribution of earlobe shapes in female samples.

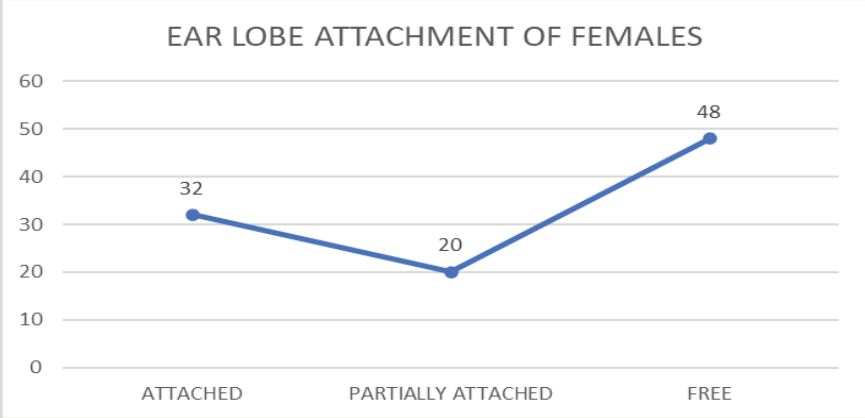

Fig. 15. Shows the distribution of earlobe attachment in female samples.

Table-1 Findings from the previous studies.

SL NO	Author & year	No of samples	Ear characteristics	Selected population	Findings	Reference
1	Verma, et al. 2013	100 samples	Ear length, Ear length above tragus, ear length below tragus, Tragus length, Ear breadth, concha length, concha breadth, lobule height, lobule width.	Greater Noida-Uttar Pradesh.	Significant differences in lobule height, width, length, and width were seen especially in males. The location of the ears on the head also differed significantly, with 39.78% of ears placed in the posterior region and 60.21% in the centre. Subjects' ear types differed.	[1]
2	Angelakopoulos, et al. 2023	Females-633, Males - 778.	Helix, Antihelix, Concha, Lobe.	Brazil, India, Japan, Russia, South Africa, Turkey.	Cameriere's ear identification approach is highly distinctive, according to a multi-ethnic study, with a low possibility of matching numerical codes across six countries	[2]
3	Rani, et al.2022	Females-264, Males-233.	33 Morphological Characteristics.	Solan-Himachal Pradesh	Studied 1056 morphological traits, and found significant correlation in 5 factors, Substantial correlation with 15 factors in external ear shape.	[6]
4	Krishan, et al. 2019.	Females-87, Males-90.	Overall shape, Size, Shape of tragus, Ear lobe, Shape of helix, Darwin	Upper regions of Himachal Pradesh.	The study found that 40% of males and 44.8% of females have an oval-shaped ear, while other types like oblique, rectangular, round, and triangular are also	[13]

			tubercle.		present.	
5	Rani, et al. 2021	Females-69, Males-71.	Ear length, Ear breadth, Ear length from tragus, Distance from tragus to helix, Distance from tragus to anti helix, Lobule height, Lobule breadth.	Sirmaur district-Himachal Pradesh	Study found six differences in males, and developed discriminant function model for sex identification.	[3]
6	Chattopadhyay, et al. 2009.	79 samples	Ear length, Ear breadth, Position of ear, Ear lobe types.	Lucknow	Study found that difference in the indices is within ten percent, Ear is mostly found at the posterior 1/3 of the head, Oblique ear and free lobe is most frequent in samples.	[14]
7	Fakorede, et al. 2021.	Females-176, Males-131.	Shape of ear, Form of helix, Shape and attachment of ear lobe, Shape of ear tragus, Darwin's tubercle.	Hausa, Igbo, Yoruba.	Study found that ear shapes varied with round and triangle shapes more common in Hausa males in oval shapes in Igbo females.	[15]
8	Nedunuri, et al. 2018.	Females-16, Males-20	Ear length, Ear breadth, Ear length above tragus, Ear length below tragus, Tragus length, Concha breadth, Lobule height, Lobule width.	Asian and African population	Study found that the parameters Ear length below tragus, Lobule height, Lobule width of both the sides show significant difference ($p < 0.05$) between both the population groups.	[16]

Table- 2 Shows the descriptive statistics for different ear morphological features studied.

(Measurements in cm)

ATURES	MEAN		STANDARD DEVIATION		MINIMUM		MAXIMUM		*P-VAL*
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	
RIGHT EAR ENGTH	5.203	5.546	0.721	1.021	3.834	3.183	7.285	7.6	0.05
FT EAR ENGTH	5.387	5.560	0.860	0.956	3.919	3.154	8.078	7.755	0.3

RIGHT EAR WIDTH	2.854	3.015	0.399	0.567	2.186	1.436	3.621	4.258	0.1
EFT EAR WIDTH	2.853	2.961	0.497	0.590	1.858	0.658	3.939	4.147	0.3
RIGHT AR LOBE ENGTH	0.714	0.648	0.222	0.201	0.295	0.187	1.468	1.087	0.1
EFT EAR LOBE ENGTH	0.719	0.654	0.225	0.190	0.306	0.142	1.536	1.137	0.1
RIGHT AR LOBE WIDTH	1.548	1.687	0.306	0.531	0.853	0.195	2.106	3.302	0.1
EFT EAR LOBE WIDTH	1.522	1.626	0.401	0.501	0.398	0.4	2.349	2.827	0.2
RIGHT ONCHA ENGTH	2.068	2.304	0.403	0.566	0.022	0.656	2.859	3.26	0.01
LEFT ONCHA ENGTH	2.172	2.329	0.314	0.595	1.613	0.533	3.185	3.544	0.1
RIGHT ONCHA WIDTH	1.347	1.360	0.239	0.359	0.946	0.455	1.869	2.403	0.8
LEFT ONCHA WIDTH	1.348	1.379	0.327	0.374	0.68	0.317	2.444	2.267	0.6

***Significant Differences**

A Review on systematic approach for the process development of Active pharmaceutical ingredient and impurity profiling

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Abstract: -.The pharmaceutical industry is one of the most essential industries in the world since it significantly improves people's health and well-being. Research and innovation are the key components to drive the sector's growth by creating new treatments and medicines to treat diseases and to improve people's quality of life. The developmental process for APIs (Active Pharmaceutical Ingredients, also called drug substances) is a most challenging task. The many process factors in the article can help today's chemists understand and apply a systematic, progressive approach in research and development to build documented, regulated synthetic processes. This may assist to consistently meet the goals for product quality, as well as serve as a strong foundation for achieving the goals. Managing impurities in pharmaceuticals is a critical aspect of drug development and production, and it plays a significant role in ensuring patient safety and product efficacy and it is essential for meeting quality standards and regulatory requirements

Keywords: - KSM, Intermediate, Impurities, Genotoxic, regulatory guidelines, drug substance etc.

Introduction:-

Active pharmaceutical ingredient (API) and API Process development:-The pharmaceutical industry is one of the most essential industries in the world since it significantly improves people's health and well-being. Research and innovation are the key components to drive the sector's growth by creating new treatments and medicines to treat diseases and to improve people's quality of life. The pharmaceutical sector presents unique problems that necessitate focusing on research, innovation, and strategic methods to maintain long-term success. With poor success rates and stringent clinical trial standards, obtaining regulatory clearance for new drugs and pharmaceuticals takes time. Government involvement in pricing and reimbursement further complicates the sector's profitability. Pharmaceutical companies have shifted towards becoming more research- and science-driven to meet these problems. They have also outsourced their research efforts and used mergers, acquisitions, and joint-development agreements to benefit from foreign breakthroughs. But integrating acquired resources is difficult because of cultural disparities and knowledge transfer restrictions. Innovation is fuelled by recent scientific

information, cutting-edge technology, and tactics for enhancing innovation capabilities. Although their relevance in the clinical development phase is restricted, models emphasising customer demands and feedback loops are the foundation of the shift towards science-driven organisations. The pharmaceutical business continues to be an understudied field of research regarding the correlation between innovation and sustainability [1].

Impurity Profiling:-Impurity is defined as the presence of any foreign matter or substance in product, which may be organic, inorganic, and volatile or non-volatile residual having different chemical, physical, pharmacological and toxicological effects. A description for both identified as well as unidentified substances might be included in the impurity profile. According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) guideline on impurities in new drug substances, an impurity is defined as 'any component of the new drug substance that is not the chemical entity defined as the new drug it is or get generated out of synthesis or undesirable chemicals that remains present with APIs. The characteristic of drug product is exceptionally altered by impurity present in API or Finished drug. Pharmaceutical product would be accomplished to serve their predetermined therapeutic activity only when they are free from impurity hence , an impurity present in an active pharmaceutical ingredient to be identified, and quantify by the of modern analytical approaches[2-3]

Active pharmaceutical ingredient (API): The active elements in a pharmaceutical medicine that have the desired impact on the body to treat a condition are known as Active Pharmaceutical Ingredients (APIs). In the API Process Development, chemical substances are processed to create APIs. Paracetamol, which is found in Anti fewer tablets, is an illustration of an API. Bulk process intermediate refers to the biological drug's active component (BPI). The insulin included in an insulin pen cartridge used by diabetics is an illustration of a BPI In general, the API Process Development and Production involve several processing processes, including reaction, crystallization, separation and purification, filter cake washing, solvent swapping, and solvent exchange.

Two major categories can be used to classify APIs: natural and synthetic. Based on the synthesis method, synthetic APIs are further divided into novel and generic synthetic APIs. Small molecules, or synthetic chemical APIs, make up a significant portion of pharmaceutical medicines. Biologics, which are increasingly common medications on the market, are made using natural APIs. Despite the rising demand, there are very few numbers of biologics available than the small molecule drugs.[1]

Active Ingredients in Common OTC Medications	
Brand Name	Active Pharmaceutical Ingredient
Advil	ibuprofen
Benadryl	diphenhydramine
Claritin	loratadine
Mucinex	guaifenesin
Neosporin	bacitracin
Pepcid AC	famotidine
Prilosec	omeprazole
Tylenol	acetaminophen

Examples of Active Pharmaceutical Ingredients

Some common OTC and prescription medications and their active pharmaceutical ingredients include [3-4]**.** The developmental process for APIs (Active Pharmaceutical Ingredients, also called drug substances) is a most challenging task. Contradicting goals must be balanced, and these include the consideration of quality, safety, robustness, costs, and time constraints (Fig.1).

The workflow outlined here defines the methodology used at Siegfried Ltd, Zofingen. The company manufactures APIs (and key intermediates) on an exclusive basis for several pharmaceutical companies, as well as for the generic pharmaceutical industry. [5]

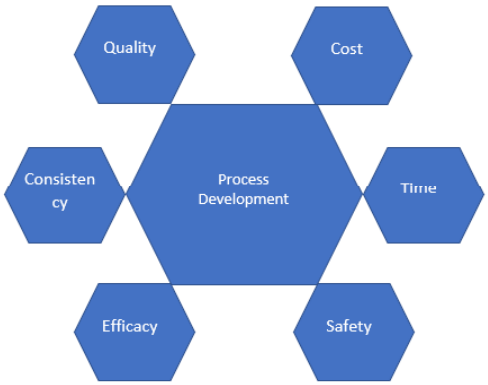


Fig.1 Parameter of process optimization

All aspects of the API that influence its identity, strength, purity, and quality are awarded top attention. Because the drugs are prescribed to patients, the health authorities have established guidelines for manufacturers to follow that address the quality of the production process. The safety of the process is a target dictated by a sense of responsibility: At no time may a process pose a significant risk to operators, equipment, or the environment. To ensure smooth production, a process must be as sturdy and robust as possible. The ranges set for specific parameters (*e.g.,* temperature, reaction time, concentration), and within which the process can be operated without failure, determine the robustness of the process. Naturally, costs must be kept under control, both in the developmental stage of a drug and during the commercial manufacturing process once the drug is on the market. In the early stages of drug development, resource allocation for individual projects is limited; most of these candidates will never reach the market having failed in clinical trials. Later, however, during the commercialization phase, the drug must be competitively priced, and its manufacturing process optimized. Time spent during the clinical development determines the entry date of a drug to market and, eventually, the lifespan of its patent protection. It is thus important that the development time of the API synthesis does not restrict the drug development process [6]

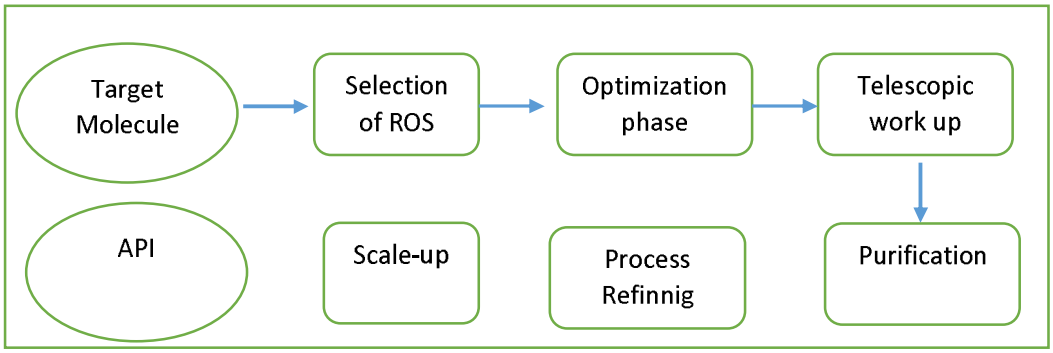


Fig. 2. Development cascade from the target molecule to the API

Fig. 2 outlines the developmental cascade. At Siegfried, this has proven to be a versatile scaffold for the design of chemical processes. A sequential transition down the cascade ensures good results and enables the company to align the process requirements to the corresponding clinical development status.

• First, based on route finding or 'ground-breaking' chemistry, the chemical route to the target molecule is chosen.

• Second, in an initial optimization phase, a process draft is submitted for reagent screening; additionally, optimization with respect to maximum conversion is performed.

• Third, further optimization focuses on

Work-up and purification as well as the telescoping of steps.

• Fourth, the unit operations are refined and, during the scale-up, adaptations to the equipment are carried out.

• Fifth, for commercialization purposes, the process must be validated. A change control system guarantees that, prior to implementation, any process changes and improvements are carefully assessed with respect to product quality. Performed at scale-up and, eventually, the final commercial process is executed and validated on a plant-scale. [7]

Process research and development:- Process research and development in pharmaceutical companies aim to produce a process for the manufacture of a chemical intermediate or an active pharmaceutical ingredient (API) at minimal cost with high quality. However, the cost of the "product" is dependent on the time of its inception in the market and decreases gradually as it becomes generic. Hence, the process chemistry plays an important role to gain the competitive advantage in pharmaceutical companies. In comparison, in the generic API industry, the issues are to be non-infringing and cost-effective all under stringent timeline. Exploring new routes or exploiting the existing chemical schemes to enhance the yield and quality are the potential solutions for the chemist engaged in the process development of generic APIs. In this report, we discuss our attempts to meet the process development aspects of Apixaban , Lurasidone hydrochloride and a well-known anticoagulant and antipsychotic drugs[8].

The eventually target of API process development research is to replicate the laboratory process of making milligram amounts to a commercial production process on a higher scale while maintaining high quality and reproducibility at the lowest cost. The term 'process development' in the pharmaceutical industry is broad and can apply to the process improvement work that prompts the productive, reproducible, economical, safe and environmentally friendly synthesis of the active pharmaceutical ingredient (API) in a regulated environment

Given the continuous stringent regulatory requirements and global nature of the pharmaceutical business, the pharmaceutical industry is continuously being challenged, resulting in increased competition and the need to produce high-quality APIs. The process development of the API, whether it is new or generic, has subsequently gained more attention because of the possibility to establish early control over the process at the R&D stage by identifying and addressing the related issues a priori. Thus, a systematic and prospective approach in R&D is key to achieving successful prospective API validation and scale-up. These activities are important and are frequently under the

scrutiny of FDA investigators. This article highlights the important process considerations for API development in the R&D laboratory, which can help chemists to understand and adopt a systematic and prospective approach in R&D, and to have documented, controlled synthetic processes. This can encourage consistent product quality and provide a good basis for achieving the goals of prospective validation and scale-up activities in the plant[9].

Process research and development in pharmaceutical companies aim to produce a process for the manufacture of a chemical intermediate or an active pharmaceutical ingredient (API) at minimal cost with high quality. However, the cost of the "product" is dependent on the time of its inception in the market and decreases gradually as it becomes generic. Hence, the process chemistry plays an important role to gain the competitive advantage in pharmaceutical companies. In comparison, in the generic API industry, the issues are to be non-infringing and cost-effective all under stringent timeline. Exploring new routes or exploiting the existing chemical schemes to enhance the yield and quality are the potential solutions for the chemist engaged in the process development of generic APIs. In this report, we discuss our attempts to meet the process development aspects of Apixaban , Lurasidone hydrochloride and a well-known anticoagulant and antipsychotic drugs.

API development: It is important to gather all the current literature and possible future developments of the API in question and keep these in the one place. The most important challenges to overcome at the API development stage include.

- Patent infringement issues
- Inconsistency in raw material quality and supply
- Hazardous/nonregulated raw materials
- Costly raw materials
- Unsafe/environmentally hazardous reactions
- Lower yields
- Difficult-to-achieve level of purity (For example, enantiomers)
- scale-up issues
- Difficult-to-handle processes
- Polymorphism issues
- Stability issues regarding intermediates/products R&D chemists should devise a route that overcomes as many of these challenges as possible.
- Genotoxic impurity
- Nitrosamine impurity.

The various process parameter mentioned in the above paragraph can help the modern-day chemist to understand and adopt a systematic and prospective approach in R&D to achieve documented,

controlled synthetic processes. This can help in meeting the product quality objectives consistently and can build a good basis for achieving the goals of prospective validation and scale-up activities in the plant, which are considered to be important and are frequently under the scrutiny of the regulatory authorities such as FDA investigators.

Quality management system for Documentation Standard operating procedures (SOPs) must be created and followed for important tasks, such as the qualification and calibration of equipment like weighing balances, standard weights, temperature indicators, and reference standards. The data produced in the R&D laboratory must be precise, repeatable, and dependable. Maintaining accurate and comprehensive documentation of these qualification and calibration procedures, along with other laboratory tests, observations, and associated analytical data, is also essential.

API development: - It is essential to collect and retain in a single place all the available research on the API in issue, as well as any potential future improvements. These are the main obstacles to be addressed during the API development stage: • concerns about patent infringement. The challenges associated with scaling up include: lowered yields; unsafe or ecologically harmful reactions; expensive raw materials; inconsistent raw material quality and supply; difficult-to-achieve levels of purity (such as enantiomers); and scale-up difficulties. • Complex procedures; • Problems with polymorphism; • Stability concerns with intermediates and products R&D chemists ought to come up with a plan that gets around as many of these obstacles as they can.

The cost:-The primary cost-factors are manual labour, operations, raw materials, and packaging materials. R&D chemists may decrease process costs by: • recommending less expensive substitute reagents or synthetic pathways • cutting down on the number of raw materials used (via process optimization studies); • shortening the cycles of the processes; and • reusing the resources whenever feasible

Greener Approach: Today's R&D scientists are supposed to practice "green," or environmentally friendly, chemistry. High yielding processes should ideally be created such that by-products may either be "treated" to remove pollution or are not pollutants in the first place. The unreacted components, by-products, and solvents should be recovered by trying additional processing of the mother liquor and solvent washes. For instance, a recovered solvent may undergo treatment to enable it to meet the required quality standards once more, allowing for recycling inside the same process step. A thorough scrub down of the gaseous products is necessary. After going through the scrubber and other processes, the final used materials should be evaluated for their impact on the environment and managed in a way that doesn't harm it.

Selection of Solvent: The International Conference on Harmonization (ICH) guidelines have classified the solvents based on the risk to human health.1 Class 1solvents should not be employed

in the manufacture of APIs. These include solvents such as • benzene (carcinogenic) • carbon tetrachloride (toxic and environmental hazard) • 1, 2-dichloroethane (toxic) • 1, 1-dichloroethane (toxic) • 1,1,1-trichloroethane (environmental hazard). The Solvents in Class 2 should be limited because of their inherent toxicity, for example: • toluene • dichloromethane • chloroform • ethyleneglycol. Solvents in Class 3 may be regarded as less toxic and of lower risk to human health. These include • acetone • ethanol • 1-butanol • formic acid [10].

Process adaptability: R&D chemists should adapt a process to the plant environment. For example, to isolate a product, R&D chemists should avoid evaporating the solvent(s) to 'dryness' because this is not feasible in a plant. Instead, a suitable technique such as crystallization or precipitation should be developed because, in such cases, the product can be isolated by centrifugation or filtration process in the plant. Similarly, the purification of product should be achieved by means of crystallization or selective precipitation, instead of column chromatography as this is not feasible in the plant. Methods of handling viscous materials in the plant must also be considered because the large surface area of plant equipment and piping can pose problems during material transfer. Solutions include performing a one-pot reaction using a suitable solvent to transfer such materials. Reactions involving low temperatures or high pressures are difficult to handle in the plant and an alternative route should be considered

Safety precautions Material safety. At the time of route finalization, R&D chemists should collect all raw material safety information (normally Material Safety Data Sheets [MSDS]). The storing and handling risks of such materials should be assessed, and appropriate measures taken to minimize them.

Process safety. During process development, significant consideration should be given to the safety of the chemistry being developed. The majority of industrially useful reactions are exothermic, suggesting the need for risk assessment. Figure 1 shows the enthalpies of two common reactions to indicate the high degree of process hazard associated with them.2 The magnitude of overall heat release can be influenced by the type of solvents used, concentration, other simultaneous processes taking place, and so on. Together, this can have an enormous destructive power, if not controlled properly. Therefore, all such chemical processes that have the potential to be performed in the pilot plant should be subjected to safety evaluation. Initially, the thermal stability of the compounds (raw materials, intermediates, etc.) should be screened by differential scanning calorimetry (DSC) to detect endothermic or exothermic behaviour. These results can then be used to decide if more careful measurements are required. In some scale-up cases, an exothermic reaction can lead to thermal runaway, which begins when the heat produced by the reaction exceeds the heat removed. The rate of heat produced may increase exponentially. Once the control of reaction is lost, the

reaction vessel may be at risk from over-pressurization because of violent boiling or rapid gas generation. The elevated temperatures may initiate secondary, more hazardous runaway or decomposition. The possibility of such a reaction hazard should be assessed in the laboratory by employing methods such as thermal gravimetric analysis (measuring the thermal stability of the reactants or products) and the adiabatic calorimetry (measuring the decomposition and release of gases). A process should not be performed in the pilot plant before such safety assessment. Other process hazards such as 'dust explosion' during milling or storage may also be assessed at an early stage, depending on the potential of such risks [11].

Materials and vendors: To ensure consistent quality and supply of raw materials, packaging materials and other process components (such as the filtration media; gaskets;'O' rings that may come into contact with the raw materials, process fluids, intermediates or the API) vendor audit/approval has gained importance. Vendors should be selected using criteria such as their market recognition, past record, ability to supply consistent quality materials in time and customer orientation. The Certificate of Analysis (CoA) and MSDS of the materials should be obtained from the vendors. Such information is useful while designing the specifications of raw materials and packaging materials, and for recommending storage conditions [12].

Developing the specifications: - The in-house specifications can be developed based on 'user trials' results and the CoA of the vendor's samples

Scale up challenges: It is important for R&D chemists to identify prospectively the potential plant challenges and try to address them suitably at the R&D stage itself. Laboratory studies like the ones below can help to address many issues a priori to avoid 'surprises' occurring in the plant scale-up batches

Operation correlation between of the R&D plant environment: Once the route is finalized, the plant environment in R&D should be simulated as far as possible by • Using similar material of construction [MOC]; shape of vessel; type of stirrer; number of baffles; D: L ratio (diameter: length) of vessel and so on. • Using the same charging sequence of the raw materials. • Using similar mixing patterns/stirring parameters that are achievable in the plant vessels (similar tip speed, power requirement per unit volume of the reaction mass, etc. can be maintained in R&D). • Developing suitable in-process sampling procedures that are feasible in the 'controlled' environment in the good manufacturing practice (GMP) plant. • Using similar filtration cloth/filtration medium. • Using a similar type of dryer and drying parameters. Such simulation experiments can help achieve better reproducibility atplant scale because the possible deviations can be minimized [13].

Determining the scale-up factor: Many scale-up operations require more time than laboratory-scale experiments because of the larger volumes of materials. R&D chemists should take into

account the scale to which the process can be operated in the plant and the required time cycles for such process steps. They should then increase the process time cycles to match the plant conditions in a laboratory experiment. The process steps that may be considered for such studies could include the following: • addition of reactant • mixing • filtration • centrifugation • drying • maintaining temperature. The effect of such 'increased' cycle time on the product quality and yield should be assessed. Thus, a scale-up factor, which the process can be operated without affecting the quality and yield, can be determined prospectively.

Critical process parameters: While performing a laboratory experiment, R&D chemists can test the limits of some operating conditions such as time, temperature and pH, or the quality parameter of a key raw material (For example, water content/impurity level). The effect of such 'challenge' on the product quality and/or yield should be assessed. If a parameter adversely affects either of these, it should be identified as a critical process parameter (CPP) and be documented during the development stage. When scaling up, it is necessary to strictly control such parameters to ensure consistent product quality and yield [14].

Critical observations During the R&D experiments:-, it is important to record observations, such as any signs of exothermic or endothermic activity during reaction, frothing, fuming, sublimation, pressure development, change in colour and change in phase. Similarly, observations regarding the reaction rate (vigorous/mild), filtration rate, flow characteristics of fluids, nature of product (sticky/fluffy/amorphous/crystalline/ semi solid, etc.) should be noted because the appropriate measures can be taken in the plant during scale-up

Chemical compatibility studies: Certain 'process chemicals', such as process fluids and intermediates, may react chemically with 'plant items' such as process equipment, piping, flexible hoses and filters while in direct contact with them, which can lead to serious quality issues including contamination and impurity formation. R&D chemists should consider all the process chemicals involved in the synthesis of the API and obtain the data on their compatibility with various MOC of all the plant items that may be involved during the operations (see Table 1 for the illustration of some chemical compatibility data3). In the absence of such data, in-house data should be generated by simulating the exact contact conditions in a laboratory experiment and the observations recorded. For example, to determine the compatibility of a filter cloth with a process fluid, the sample piece of a filter cloth can be kept in contact with the process fluid for the specified time/temperature/pressure in a laboratory and the effect of the process fluid on the weight/size/shape/colour, and so on, of the sample cloth can be recorded. Such observations can help in deciding the suitability of various plant items involved in the process. It is recommended that during the purchase of such plant items, an MOC certificate and chemical compatibility information should be obtained from the vendors [15].

Equipment cleaning procedures: To develop a prospective cleaning method for plant equipment, a suitable cleaning procedure for similar laboratory apparatus should be established. In a typical cleaning procedure, a similar 'flow pattern' of the cleaning solvent(s) as that in the plant equipment should be followed. The solvent(s) for cleaning, such as acetone, methanol, and water, should be selected based on the solubility of the concerned product which is to be 'washed' from the empty apparatus. Cleaning observations and the results of the rinse samples may help to develop a prospective cleaning method for plant equipment during the scale up.

Stability data: The short-term stability data of the critical raw materials and intermediates should be generated in R&D under conditions similar to the plant environment. Based on these data, the packaging and storage conditions for the critical raw materials and intermediates can be established.

'**Finalisation of specifications:-** As the processes are fully developed and optimized, the specifications of the in-process controls, intermediates, API and the packaging materials can be 'frozen'. No change in the process should be allowed without a 'change control' assessment and approval.

Working standards: A 'working standard' is a sample of highest purity that can be synthesized in R&D and purified to the maximum extent by repetitive crystallization/column **chromatography**. It can then be 'qualified' by comparison with a suitable reference standard, for example, a pharmacopoeia reference standard.

Stability For stability investigations, a representative sample of the R&D batch obtained using a "Finalized" process ought to be retained. As a result, if the same procedure is used, "early indications" on the stability profile of upcoming API scale-up batches can be acquired.

Other important challenges. In recent years, API process development has become more challenging because of the need for making APIs with the desired 'enantiomeric purity' and 'polymorphic form'. The following examples justify the need for the stringent regulatory requirements in these areas [16]

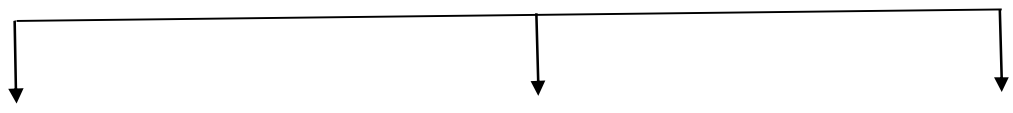
Polymorph synthesis:-. 'Polymorphism' is the ability of a substance to exist in two or more crystalline phases that have a different arrangement and/or conformation of molecules in crystal lattice. Many APIs exist in various polymorphic forms with different properties such as crystallinity, bulk density, solubility and bioavailability. Some interesting cases of product recall as a result of the issue of drug polymorphism are given below.6 Abbot Laboratories had to withdraw its HIV protease inhibitory drug Norvir (Ritonavir) from the market because an unwanted polymorph of the drug had been produced (Form II) during shelf life. This form has a different dissolution rate to the known polymorph (Form I), so the bioavailability of the drug was affected. Chloramphenicol-3-palmitate has 'Form B' as a metastable form having 8-fold higher bioavailability than the other polymorph

'Form A'. This cancreate a danger of fatal dosages when the unwanted polymorph is unwittingly administered as a result of alterations in process and/or storage conditions. These examples suggest the need to identify all the polymorphs of an API at R&D stage. One can establish the polymorphs by determining physicochemical properties, thermodynamic stabilities and by studying conditions of inter-conversions. Some useful tools for such determinations are Fourier Transform Infrared Spectroscopy (FTIR), X-ray Powder Diffraction (XRPD) and Differential Scanning Calorimetry (DSC). The formation of a specific polymorph may depend upon the type/composition of the solvent(s), temperature, synthetic route, storage conditions etc. An interesting example of solvent composition giving different polymorphs is cholamide7, which gives needle-like crystals (Form I) by recrystallization from a solution of 1:1 acetonitrile: water and platelet-like crystals (Form II) by recrystallization from a solution of 25:1 acetonitrile: water. (Figure 2). Once the desired polymorph has been identified, the process of the API must be validated to obtain the desired polymorph consistently. In addition, the drug's stability protocol needs to incorporate appropriate tests to ensure that the polymorphic form remains unchanged in these scenarios [14]

Impurity Profiling/Impurity Management:-Managing impurities in pharmaceuticals is a critical aspect of drug development and production, and it plays a significant role in ensuring patient safety and product efficacy. Compliance with ICH guidelines and industry best practices is essential for meeting quality standards and regulatory requirements

It's absolutely important that impurities in pharmaceutical products are a major threat for drug manufacturers. Even a single unknown impurity, when introduced in the final product, can lead to batch rejection due to quality concerns. Managing impurity profiles is crucial to ensure the safety and efficacy of pharmaceuticals. The International Conference on Harmonization (ICH) provides guidelines and standards to help the pharmaceutical industry in identifying and characterizing impurities, which is essential for regulatory compliance and product quality assurance [15].

Classification of impurities



Organic Impurities **Inorganic Impurities** **Residual solvents**

Organic impurities: Organic impurities may arise during the manufacturing process and/or Storage of the new drug substance. They may be identified or unidentified, volatile or non-volatile, and include

1. Starting materials

2. By products

3. Intermediates

4 Degradation products

5. Reagents

6. Ligands

7. Catalysts

b) **Inorganic impurities**: Inorganic impurities may derive from the manufacturing process.

They include

1. Reagents.

2. Ligands.

3. Catalysts.

4. Heavy metals

5. Inorganic salts

6. Other materials (filter aids, charcoal, etc ....)

c) **Residual solvents:** Residual impurities are organic or inorganic liquids used during the manufacturing processes.

Impurity profiling: - The impurity profile is a description of Identified and unidentified impurities. The impurity may be developed either during formulation or in the final product upon ageing. The Various instrumental approaches for isolating and identifying the process related impurities and degradation products are Mass spectroscopy (MS), Nuclear magnetic spectroscopy (NMR),High performance liquid chromatography (HPLC) etc., has been established to review a summary of the problems and the various possibilities offered by modern analytical chemistry. The identification and qualification of impurities in Active Pharmaceutical Ingredients (APIs) and pharmaceutical products, is a very important step performed at many levels of the drug discovery and beyond. Impurity is a substance which exists with original drug that is starting material or intermediates or the substances which are formed during any side reactions, during the manufacturing process of the drug [16].

Regulatory bodies around the world like the United States food and drug administrating and International Conference on Harmonization (ICH) directed those impurities in drug substance present at the threshold levels to be isolated and characterized.[17]

The ever-increasing regulatory authority's requirement on not only emphasizing not only the purity profile but on impurity profiling (identification, isolation and characterization of impurity) for the approval of drug its manufacturing and marketing licensing and regulatory related issues for particular APIs e and drug product. Different approval agencies like British Pharmacopoeia (BP),

European Pharmacopoeia (EP), Indian Pharmacopoeia (IP), Japanese Pharmacopoeia (JP) and United States Pharmacopoeia (USP) are also continuous revising their monographs for the drug substances and drug products every year by adopting modern methodology for the identification and also stringent the acceptance criteria of impurities introducing concept like genotoxic and nitrosamine impurities and other different types of impurities.[18]

The sources of impurities are the major concern for any drug manufacturer. Some time it has been observed that in the final stage of the production is badly affected by the introduction of a single unknown impurity and due to that unknown impurity; whole batch can get rejected as per the quality criteria. This impurity profile can be explained using multidisciplinary approach including the manufacturing or aging of both active pharmaceutical ingredient (API) and formulation. According to International Conference on Harmonization (ICH) guidelines identifying and characterizing all impurities that are present at a level of 0.10% or more are recommended [17-20].

ICH Guidelines for impurity profiling -It is now getting an important critical attention from regulatory authorities. The International Conference on Harmonization has published various guidelines on impurities in drug substances and drug products as well as residual solvents

You are absolutely correct in noting that regulatory authorities, including the International Conference on Harmonization (ICH), have placed significant emphasis on addressing impurities in drug substances and drug products, as well as residual solvents. These guidelines play a vital role in ensuring the quality, safety, and efficacy of pharmaceutical products [21-23]

1. Q1A-"stability testing of new drug substances and products"
2. Q3A (R2) - "Impurities in New Drug Substances"
3. Q3B (R2) - "Impurities in New Drug Products"
4. Q3C (R5) - "Impurities: Guidelines for Residual Solvents"

Regulatory Guidelines on impurity:-International Conference on Harmonization guidance of Technical Requirements for Registration of Pharmaceuticals for Human Use is inscribed by The United States Food and Drug Administration (FDA)

The FDA has the assigned responsibility of ensuring the safety and efficacy of drugs. The various regulatory guidelines regarding impurities are as follows

It's important to note that the FDA's regulatory guidelines are continually updated and refined to reflect advances in scientific understanding and changing industry practices. Compliance with these guidelines is essential for pharmaceutical manufacturers seeking approval for their products and for ensuring that drugs on the market are safe and effective. The FDA plays a critical role in maintaining the high standards of the pharmaceutical industry in the United States. [24-25]

1. ICH guidelines —stability testing of new drug substances and products"- Q1A

2. ICH guidelines —Impurities in New Drug Substances‖- Q3A
3. ICH guidelines —Impurities in New Drug Products‖- Q3B
4. ICH guidelines —Impurities: Guidelines for residual solvents‖- Q3C
5. US-FDA guidelines —NDAs -Impurities in New Drug Substances‖
6. US-FDA guidelines —ANDAs – Impurities in New Drug Substances
7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia

Impurities associated in with APIs –

According to ICH guidelines, impurities associated with APIs are classified into the following categories:

- Organic impurities (Process and Drug-related)
- Inorganic impurities
- Residual solvents

ORGANIC IMPURITIES – Efforts to minimize and control impurities are essential not only to meet regulatory standards but also to ensure the safety and efficacy of pharmaceutical products. Quality assurance and good manufacturing practices are integral components of the pharmaceutical industry to address impurity-related concerns and deliver safe and effective medications to patients. These are common impurities in any API, if proper precautions are not taken during multi-step compilation there is a risk that undissolved residues will remain, unless manufacturers are careful about contamination although the final product is always washed with solvents. These types of impurities form during the manufacturing process or during storage of the drug substance. The sub-types of these impurities are given below [25-27]

Starting Materials or Intermediate Impurities – During multistep synthesis process there are high chances of impurities formed as by products, intermediates are produced. So, special care is needed. It results in unreacted starting material in the final product. The impurities that arise from starting materials or intermediates is found in every API unless proper care is not taken in every step involved in the multi-step synthesis. Although the end product are always washed with solvents, there is always chance that the residual unreached starting material remain, except the manufactures are very careful about the impurities. In Paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for someone manufacturer or be an intermediate for other.

By-products – Formation of by product mainly observed due to variety of side reactions, such as incomplete reaction, dimerization, rearrangement, reaction instability, isomerization, and side rection reactions between key starting material with each other or reagents. For example, Toluene is biproduct in benzyl deprotection reaction in case of no of API and intermediate.

Degradation products – An impurity resulting from a chemical change in the drug substance brought about during manufacture and/or storage of the new drug product by the effect of, for example, light, temperature, pH, water, or by reaction with an excipient and/or the immediate container closure system[ICH Topic Q 3 B (R2) Impurities in New Drug Products]

Impurities can also be formed by degradation of the product during manufacturing of bulk drugs. However, degradation products resulting from storage or formulation to different dosage forms or aging are common impurities in the medicines. The degradation of penicillins and cephalosporins is a well-known example of degradation products

INORGANIC IMPURITIES –

Impurities like reagent , phase transfer catalyst , metal catalyst and inorganic salt used during the work and isolation of product inorganic impurities are also obtained from physical operation like charcolisation , filtration etc . They impurities are well known, and process identified impurities. Inorganic impurities are well described f Inorganic impurities are normally detected and quantified using different pharmacopeia or other appropriate standards [28].

Reagents, ligands, and catalysts -Chemical reagents, ligands, and catalysts used in the synthesis of a drug substance can be carried over to the final products as trace-level impurities. The possibilities of having these impurities are uncommon however, in certain processes, these could real challenge for the process chemist unless the proper care not taken during Manufacturing and design of the process.

Heavy Metals - Water is the main source of heavy metals, like Ar, Cd, Cr, Na, Mg, Mn, etc manufacturing process cannot be run without water it is essential during manufacturing process. Heavy metal contaminant can be avoided using modern techniques like demineralization of water, reverse osmosis technique that generate mineral free water.

Other materials - The medium used routinely at commercial scale for the filtration of product , biproduct such as centrifuge bags many cases, activated carbon is also used are in the bulk drugs manufacturing plants. For getting product free from contaminates like black particle and suspended fibres continuous monitoring is essential.

RESIDUAL SOLVENT –Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. It is very difficult to remove these

solvents completely by the work-up process; however, efforts should be taken to the extent possible to meet the safety data. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into three classes.[25-30]

Class I solvents -These solvents are either avoided or restricted to a limit in the manufacture of excipients and drug substances because of their unacceptable toxicity or their deleterious effects. These are generally carcinogens

Class II solvents - As Class II solvents are inherently toxic, their usage should be limited in pharmaceutical Industry. These are generally Non-genotoxic, animal carcinogens and possible neurotoxicants

Class III Solvents - As they are less toxic and possess lower risk to human health than class I or class II solvents, they do not have any serious health hazard. According to several data‘s, long term toxicity is generally not reported. The amount of these residual solvents of 50 mg or less would be acceptable. E.g. for this class of solvents are Acetic acid, Acetone, Anisole, 1-Butanol.

CONCLUSION:

The many process factors in the article can help today's chemists understand and apply a systematic, progressive approach in research and development to build documented, regulated synthetic processes. This may assist to consistently meet the goals for product quality, as well as serve as a strong foundation for achieving the goals of potential validation and scale-up activities in the plant, which are considered as critical and often come under the scrutiny of regulatory bodies such as FDA investigators. This review provides a perspective on impurity profiling in drug substance and drug product. Impurity profiling in pharmaceutical world is an increasing importance and drug safety receives more and more attention from regulatory agencies . This article provides valuable information regarding the types of impurities and critical factors has to be considered while the preparation of the bulk drugs

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Handwriting Characteristics of Attention Deficit Hyper-Activity (ADHD) Individuals – A Review

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Abstract

The act of handwriting, much like any other behavior, is governed by the brain, operating predominantly on a subconscious level and that is closely related to brain impulses. In recent years, there has been growing research interest in understanding the handwriting features of individuals with ADHD. This review paper aims to synthesize and analyze the existing literature on the various aspects of handwriting in individuals diagnosed with ADHD. By investigating the complex relationship between ADHD and handwriting, this review explores the unique patterns, challenges, and implications of handwriting in this population. Studies reveal notable changes in handwriting features such as letter size, formation, spacing, and alignment among individuals with ADHD as compared to neurotypical individuals. Utilizing both manual and digital handwriting analysis, identified reliable markers of ADHD - related handwriting traits These findings have significant implications for forensic practice, particularly in cases involving documents where the author may have ADHD. The synthesis of this literature highlights the importance of understanding these handwriting characteristics, not only for academic and clinical purposes but also for practical applications in forensic document examination. By identifying the distinctive handwriting patterns associated with ADHD, professionals can improve the accuracy of document analysis and provide better support for individuals with ADHD in various contexts

Keywords – Attention deficit hyperactivity, Handwriting, Forensic, Neuro-muscular disorder

1. Introduction

Handwriting is a skill that is learned and involves a complex combination of perception & motor skills. Sometimes referred to as a neuromuscular task. Handwriting is a vital part of human language and motor skills. It is necessary for academic learning, communication, and cognitive development. It's a multidimensional ability that combines language, physical, and cognitive functions (Yang et al., 2022). Handwriting is the result of the movement of the hand, wrist, and forearm. The hand is a very delicate and sophisticated mechanism made up of about 27 bones governed by over 40 muscles, a complex network of tendons links the majority of the muscles, which are situated in the lower arm, to the digits. Their hand, arm, and finger movements are controlled by a brain system that uses a timing mechanism to synchronize

their abilities to manipulate a writing instrument perfectly. The complexity of the pattern that the writing instrument records depends on the exact placement and timing of the movements (Huber & Headrick, 1999.). Handwriting results from a fixed motor program as mentioned in Figure 1. There is a fixed motor program for every individual. A motor program is a memory structure comprising abstract codes that can be converted into movement patterns. The motor program of a person is developed through the learning process and further leads to form fixed handwriting habits. The manifestation of the habits of a writer is the reason for the individuality of handwriting. The repetition of the motor program several times results in individual features of handwriting (Teulings, 1996). The habit of writing is something you establish on your own, no two writers are exactly alike visually, and No two people write precisely the same way twice under the same circumstances. (Low et al., 2009; Srihari et al., 2005)

According to neuroscience, the brain is organized into distinct modules specialized in tasks. While writing involves a sequence of motor commands to control the muscles in the hand and arm, these commands can be flexibly applied to produce writing patterns of varying sizes or with different limbs. Studies have shown that certain psychomotor features, such as timing speed (Keele & Hawkins, 1982), timing accuracy, and force-amplitude accuracy (Keele, Ivry, & Pokorny, 1987), are correlated between different body parts like the fingers and arms. However, timing accuracy and force-amplitude accuracy do not correlate with each other, suggesting that different brain modules control timing and force. Specifically, neurological evidence indicates that the cerebellum is responsible for timing computations, while the basal ganglia regulate force (Keele, 1990, Teulings, 1996). The neocortex is located on the brain's outer layer responsible for advanced functions such as sensory perception, motor commands, spatial reasoning, conscious thought, and language processing. When there's damage to this area (neocortical lesions), it can lead to specific deficits in various cognitive functions. Neocortical lesions and basal ganglia disruptions can impact letter formation through distinct mechanisms. Neocortical lesions affect cognitive aspects, leading to specific errors like irregular shapes inconsistent spacing, or alignment issues. This results from impaired coordination and control of fine motor movements necessary for writing. On the other hand, basal ganglia disruptions affect neuromuscular control, causing difficulties in executing proper letter sizes due to improper motor activity (Margolin & Wing, 1983, Teulings, 1996). Handwriting is one vital human skill that, if impaired, might result in difficulties in school or failure as well as lower self-esteem (Brossard-Racine, Majnemer, Shevell, et al., 2011). The act of handwriting, being a neuromuscular activity, can be influenced by various internal and external factors. Internal factors pertain to those within the individual, such as health conditions like neurological disorders such as "ADHD", Autism, and Parkinson's, and psychological factors like stress, anxiety, depression, age-related tremors, or lack of literacy the external factors are the writing instrument and writing surfaces(Mahajan, 2019). In recent years, researchers have been increasingly focusing on handwriting difficulties as a significant area within learning disabilities.

These difficulties commonly involve illegible letter formation with increased height and width (Shen et al., 2012) (Frings et al., 2010), difficulty in spacing (Dirlikov et al., 2017) which leads to irregular spacing, narrower or wider spacing, spelling errors (Li-Tsang et al., 2018), and disruptions in syntax and composition, regardless of whether they are linked to slow writing speed. The analysis done by (Farhangnia et al., 2020) reveals that children with ADHD had significantly lower legibility scores compared to their healthy peers ($p = 0.001$). Additionally, children with ADHD performed significantly worse in word formation ($p = 0.000$). Because of the discrepancy in the number of words between the Chinese and English handwriting tasks, it's unsuitable to directly compare writing times. In general, participants exhibited slower writing speeds ($p = .0247$), along with reduced variability in writing speed ($p = .0021$) (Li-Tsang et al., 2018). Using ANOVA it shows he ADHD group also had worse letter-form scores compared to the TD group across conditions (copy: $p = .093$; trace: $p = .017$; fast trace: $p = .02$) (Dirlikov et al., 2017). Handwriting analysis is a crucial forensic examination tool, often used to authenticate documents, identify individuals, and detect potential fraud or forgery. However, the impact of "ADHD" on handwriting features in forensic contexts remains relatively unexplored. "ADHD" is known to affect various cognitive functions, including attention, impulse control, and motor coordination, which can influence handwriting quality and consistency (American Psychiatric Association. (2013). DSM-5). Understanding these effects is crucial for forensic handwriting experts tasked with analyzing and interpreting handwritten documents in legal and investigative contexts, by reviewing the studies that used handwriting for the assessment of "ADHD" individuals can identify potential differences and unique features that may be indicative of the disorder. Those findings from the previous studies may make a significant impact on forensic practice, particularly in cases involving documents where the author may have "ADHD" and this could lead to more studies in the future on the handwriting characteristics of "ADHD" authors in the forensic context.

1.1 Understanding Attention-Deficit/Hyperactivity Disorder (ADHD)

"ADHD" is a neurodevelopmental condition distinguished by a substantial level of inattention, disorganization, and hyperactivity-impulsivity that delay normal functioning or growth (DSM 5; American Psychiatric Association, 2013). Although the etiology of "ADHD" is complex, researchers point to differences in the central nervous system as significant factors in the development of this disorder. A single factor does not cause "ADHD" but arises from a combination of genetic, environmental, and neurobiological influences. That differences in the central nervous system play a significant role in the development of the disorder. The imaging studies show that anatomical changes in the brains of both adults and children with "ADHD" individuals exhibit widespread decreases in the volume of the total brain and reduced size of several brain regions including the caudate nucleus, prefrontal cortex, corpus callosum, and cortical gray matter, particularly in regions responsible for advanced cognitive functions like the

prefrontal cortex(Bush et al., 2005; Durston, 2003; Hynd et al., 1987; Kieling et al., 2008; Swanson et al., 2007; Tripp & Wickens, 2009; Valera et al., 2007; Xavier Castellanos et al., 2007).This could relate to difficulties in areas like impulse control, attention regulation, and decision-making commonly observed in "ADHD". Additionally, there is a decrease in the volume of specific subcortical structures like the basal ganglia, and anterior cingulate cortex, these regions are involved in processes like motor control, reward processing, and emotion regulation, which are often dysregulated in individuals with "ADHD". and cerebellum are also observed in individuals with ADHD(Amico et al., 2011; Batty et al., 2010; Duda et al., 2014; Durston et al., 2004; McAlonan et al., 2007; Narr et al., 2009; Romanos et al., 2010; Seidman et al., 2011; Shaw et al., 2006). When compared with children who do not have "ADHD", those with the disorder exhibit unusual activation patterns during tasks involving attention, inhibition, motor control, and executive function, characterized by decreased activity in the prefrontal cortex, basal ganglia, and cerebellum (Bush et al., 1999; Duda et al., 2014; Durston et al., 2003; Posner et al., 2011; Rubia et al., 1999; Yeo et al., 2003). Attention deficit hyperactivity disorder (ADHD) first manifests in early years and can worsen in school-aged children, and last into adulthood (Farhangnia et al., 2020; Lazzaro et al., 1999; Shin et al., 2023) "ADHD" can significantly influence various facets of a child's life, extending beyond the individual to affect parents and siblings, disrupting family dynamics and marital relationships. These effects evolve as the child transitions from school to adolescence, with various dimensions of the disorder becoming more noticeable at various developmental stages (Harpin, 2005; Shin et al., 2023). Furthermore, "ADHD" can persist into adulthood, impacting both personal and professional life. Additionally, it's linked to heightened healthcare expenses for patients and their families. While a consistent pattern of inattention or hyperactivity/impulsivity responses is a key feature of "ADHD", the disorder is varied on many levels. The main diagnostic indicators of "ADHD" consist of difficulties with focus and attention, frequently accompanied by hyperactivity or impulsiveness (Fenollar-Cortés et al., 2017).

Early onset and normal development are impacted by neurodevelopmental disorders (ND), which cause delays in predicted social, emotional, linguistic, and cognitive functioning. Between 2 and 7% of school-age children globally are estimated to have ADHD, making it one of the most prevalent NDs. High levels of impulsivity, hyperactivity, inattention, and disruptive conduct are symptoms of ADHD. It can be divided into three clinical presentations based on which symptoms predominate: mostly hyperactive-impulsive, primarily inattentive, and a combination if six or more symptoms of each category persist for a minimum of six months(Español-Martín et al., 2023)Along with its primary symptoms, motor impairments are seen in 30–50% of children with ADHD, which may lead to a coexisting developmental coordination disorder (DCD) 30–60% of a child's school day is spent time to fine motor activities, of which 17–37% are paper-pencil tasks, which include handwriting (3–18% of all tasks). Therefore, it is indisputable that deficiencies

in graphomotor movements can affect children's development generally as well as academic achievement in particular (e.g., reduced self-worth, growing frustration over time-consuming writing) because fluency in handwriting letters predicts written expression. (Rothe et al., 2023). Signs of “ADHD” include a deficiency in sustained attention, forgetfulness, disorganization, distractibility, nervousness, and impulsivity. This condition can be diagnosed either by itself or in combination with several other conditions, such as anxiety disorder and oppositional defiant disorder(Brossard-Racine, Majnemer, Shevell, et al., 2011).but the disorder also affects other essential skills for daily functioning, such as time management, problem-solving, emotional and motivational self-regulation, and deficiencies in motor coordination. Most of these issues could negatively impact academic performance directly or indirectly it may persist as a long-term impact on children’s academic performance. Children diagnosed with “ADHD” often exhibit difficulties with their handwriting which is the ability to coordinate hand movements during writing which causes Illegibility, Children with “ADHD” may have handwriting that is difficult to read or decipher. This could be due to inconsistencies in letter formation, inadequate spacing between alphabets or words, or irregular sizing of alphabets. Then Children with “ADHD” are more likely to make mistakes while writing, like spelling errors, letter reversals, or omitted words. These errors can contribute to the overall illegibility of their handwriting. Lack of organization, Handwriting produced by children with “ADHD” tends to be less structured or organized compared to that of their peers without “ADHD”. This may manifest as uneven margins, erratic letter placement, or disorganized overall presentation on the page. These difficulties with handwriting can have negative consequences for academic achievement and in forensic scenarios, these characteristics can give an indicative of “ADHD” handwriting during the examination of “ADHD” affected individual handwritten documents. Illegible, making mistakes, and disorganized handwriting may make it challenging for teachers to assess students' work accurately, leading to potential misunderstandings or underestimations of their knowledge and abilities. Additionally, poor handwriting can affect students' self-esteem and confidence in their academic abilities (Brossard-Racine et al., 2011; Duda et al., 2019; Racine et al., 2008). It has been proposed that fine motor coordination deficiencies may account for up to 30–50% of ADHD cases. Handwriting quality and quantity were lower in “ADHD” children than in control children, as evidenced by unclear writing products and sluggish execution. Improving handwriting skills could make texts simpler to read, but it might not always result in higher academic results. Given that processing readable content is linked to higher ratings of finely handwritten essays, this would benefit children with “ADHD”. In other words, assignments that teachers deem to be "easy" to read are more likely to receive higher grades (Fenollar-Cortés et al., 2017). Since handwriting is a crucial learning skill, one of the best ways to look into how “ADHD’ manifests itself is through handwriting testing. In actuality, handwriting issues are frequently present in “ADHD” students. Research conducted in the United States of America discovered that nearly 70% of students diagnosed

with “ADHD” experienced difficulties in their handwriting (Li-Tsang et al., 2018) .According to the study children with “ADHD” exhibited poorer word formation compared to healthy children, those individuals produced larger letter formation while copying (Ivančević et al., 2020) .The writing challenges experienced by individuals with “ADHD”, which are linked with attention problems are the result of deficits in graphemic buffer functioning, where the cognitive component is involved in temporarily storing and processing the information related to writing such as letter formation, words, and their order, and the impairment in kinematic motor production where the physical execution of writing needed (Ivančević et al., 2020; Rodríguez et al., 2017) .There was no notable variation in the speed of writing between the “ADHD” and Non-ADHD groups (Farhangnia et al., 2020).

1.2 Handwriting characteristics of ADHD individuals

size:

The height width and size of letters are self-explanatory; this analyses the proportion of the handwriting. The size of letters in writing refers to the physical measurements of individual letters along their vertical and horizontal dimensions (Huber & Headrick, 1999). previous studies it show that “ADHD”-affected individuals show larger size in handwriting which increases the character width and height, (Shen et al., 2012) in repeated writing of the same sentence leads to an increase in letter height. (Frings et al., 2010). The sample I collected also shows the bigger letter size among “ADHD” individuals most common (figure 2&3.)

Spacing:

Spacing in writing refers to the habitual patterns of distance and arrangements that individuals develop between letters, words, and lines. The spacing can be narrow, wide, uniform, and irregular (Huber & Headrick, 1999) .“ADHD”-affected individuals show difficulty in letter spacing (Dirlikov et al., 2017) . ADHD individuals often exhibit narrow spacing between letters and words, lacking the typical uniformity seen in the handwriting of neurotypical individuals. Conversely, some individuals may show wide letter spacing. These variations in spacing can offer valuable insights to forensic document examiners when assessing handwriting authenticity. I collected some samples of “ADHD handwriting” and it shows narrow spacing and an absence of spacing between letters and words (Figure 4,5&6)

Alignment:

Alignment is the relation of the letters of a word or signature to an actual or imaginary baseline.

It may be classified as straight, irregular, arched, ascending or descending (Huber & Headrick, 1999) .The alignment of the writing is in an ascending or descending manner or irregular So this feature can help

distinguish between a neurological disorder individual and a typically developing individual. The sample I collected shows descending and ascending alignment in the writing (Figures 7,8,9 &10).

Letter formation:

Formation is the manner of reconstruction of strokes in forming a particular letter and it completely depends on the habit of writing. Regarding letter formation, letter size "ADHD"-affected individuals are exposed to poor letter formation, and the size will increase in repeated writing (Frings et al.,2010) .The sample I collected shows poor letter formation in "ADHD" individuals' handwriting (figures 11&12). In normal individuals, the letter formation is in good manners and also shows uniform letter size (Frings et al., 2010) .The poor letter formation is caused by the impairment in hand-eye coordination and prioritizing speed over the accuracy of word formation and application of suitable pen pressure while finishing the task as soon as possible (Farhangnia et al., 2020; Li-Tsang et al., 2018)

spelling mistakes and missing letters:

The spelling mistakes and missing letters in handwriting done by "ADHD" were seen because of impairment in hand-eye coordination and symptoms of inattention. "ADHD" writers tend to make mistakes while copying longer words causing Spelling mistakes and missing letters are shown in Figure 13 (Li-Tsang et al., 2018). The sample I collected indicates the spelling mistakes or missing letter seen as common among "ADHD" individuals (figures 13 &14)

1.3 Effect of medical treatments on ADHD handwriting

The medication can be used for improving handwriting in "ADHD" individuals. Table 3(b) outlines the previous research regarding how the medication affects the handwriting of individuals with "ADHD". The effects of treatment on handwriting in people with ADHD are complex and varied.. While stimulant medication like methylphenidate has shown improvements in handwriting accuracy and legibility (Brossard-Racine et al., 2015). It can also lead to decreased handwriting fluency. However, the extent of improvement in legibility may vary among individuals, with those experiencing more severe difficulties potentially showing greater benefits. Despite these improvements, handwriting remains challenging for many children with ADHD. The relationship between medication and graphomotor skill is complex, with some studies suggesting improvements in basic movements and visual-motor integration, particularly in tasks with reduced stimuli. The precise mechanisms underlying these enhancements are not fully recognized, but it is believed that medication may enhance attentional focus on handwriting, leading to smoother and more automated movements. In conclusion, while stimulant medication may offer some benefits in improving handwriting legibility in individuals with ADHD, for improving these deficits

among has medications gives better results for them. Such as parental psychoeducation Parental psychoeducation treatment resulted in unexpected improvements in graphomotor skills, particularly in fluency and velocity in the dominant hand. Possible factors contributing to these improvements include the adoption of structured routines, increased parental support through motor activities, increased self-confidence in children, and reduced stress levels due to changes in parenting approaches (Rothe et al., 2023).

2. Handwriting Assessment tools

The assessment of handwriting in individuals with "ADHD" can be conducted using various tools The Table. 3a. shows the tools employed in previous studies for assessing "ADHD" related Handwriting characteristics. The handwriting characteristics like quality and speed of an individual who is affected with neurological disorders can identified using measuring tools, for attention deficit hyperactivity disorder has handwriting measuring tools that can be useful in assessing those features. these tools are useful in manual examination like paper pens and new technology such as such as digital pens and tablets, to capture and analyze handwriting samples more accurately

2.1 Pen Stroke Test (PST)

PST measures fine motor abilities by recording handwriting strokes on a digitizer, the test provides a quick and efficient means of identifying motor skill issues in children diagnosed with ADHD and could help to improve the diagnosis of ADHD, The assessment involves quickly creating 30 straight lines, one after another, from a designated starting point to a target area specified on a instruction sheet. Accuracy and 3exact direction aren't crucial. The only requirement is that the lines exceed 13 centimeters in length. The parameters extracted from the PST test reveal that children identified with ADHD have poorer writing ability so this technique can differentiate between children having ADHD and those absent of ADHD (Laniel et al., 2020). Forensic handwriting examiners may utilize this technique to identify the individual who has the influence of ADHD from the normal individual.

2.2 Persian Handwriting Assessment too (PHAT).

PHAT is a handwriting assessment tool utilized to analyze writing abilities in students of primary school. The tool contains 2 parts, a demographic part, and a handwriting part. Information about class, gender, hand dominance, using spectacles, and wearing hearing aids was included in the demographic section. There were copying and dictation tasks in the handwriting section. Assessing all the legibility dimensions of the written task a 5-point scale, from very bad to excellent, can be used. A five-point scale, spanning from very little to very big, was used to evaluate the size. And for analyzing speed use a clock in seconds. From the study, it was determined that the legibility of written works produced by ADHD children is very

poor (Farhangnia et al., 2020).The PHAT measures in the dictation and copying domains showed acceptable to excellent internal consistency, according to the results. When assessing handwriting components in children in grades 2 and 3, PHAT is a reliable method. Additionally, it can help detect kids in elementary school who struggle with their handwriting (Havaei et al., 2018). The tool is likely to be effective if it can reliably and accurately identify elementary school-aged children who may have specific learning disorders. Its psychometric properties indicate satisfactory reliability and validity, along with acceptable to high diagnostic accuracy. This makes the tool suitable for use in assessment programs aimed at early identification and intervention for such disorders (Meimandi et al., 2022).

2.3 BVSCO-2 tool (Battery for the assessment of writing skills in children)

BVSCO-2 is a tool discovered in Italy for the evaluation of writing abilities in children. This tool can assess both the velocity and proficiency of handwriting samples from children diagnosed with ADHD. It involves a task where children were assigned to write numbers in cursive beginning with one and finishing the assignment as quickly as possible on a sheet of paper. Another task used the performing continuous alternated letter production of cursive “l” and “e”. They gave the result that the writing battery can be utilized for analyzing the handwriting samples regarding both the quality and speed of the writer who is affected by ADHD. Another study utilized BVSCO-2 for assessing the speed, and in addition to assessing the quality of the writing adopted a “Handwriting Legibility Scale” developed by Woodcock-Johnson (Borella et al., 2011; Capodieci et al., 2018, 2019).

2.4 Minnesota Handwriting Assessment Tool (MHA)

The Minnesota Handwriting Assessment (MHA) is a tool that copies a nonsense sentence “The brown jumped lazy fox quick dogs over”. Which is concise and includes all characters of the alphabet. The words are arranged in an irregular order to eliminate the speed and diminish the memory advantage of proficient readers. The test where began and ended in 2.30 minutes and circled the end word. They are given time to complete the entire task to evaluate the quality(Dirlikov et al., 2017; Falk et al., 2011). Assess the handwriting performance of ADHD children in terms of quality and speed by administering the MHA writing tool manually and automated on a digitizing tablet which copies a nonsense sentence Digitalizing tablets employ tracing over the content normally and as quickly as possible (Dirlikov et al., 2017). MHA is used as a handwriting assessment tool Regarding the creation of an objective method for computer-based handwriting evaluation that can measure children's handwriting ability(Falk et al., 2011).

2.5 Basic Reading & Writing Comprehensive Test (BRWCT) Tool & THPC= Tseng handwriting problem checklist.

BRWCT is a standardized assessment tool to evaluate the fundamental Chinese reading and writing abilities of primary school-aged students. It consists of the following tasks: (1) determining a component character linked to a multi-character word that is presented either visually or orally; (2) creating a character based on its phonetic representation or pronunciation; (3) speaking a character; (4) constructing a term with many characters using a component character; and (5) replicating a term or sentence from a textbook (proximal copy) or the blackboard (distal copy). the test yields two composite scores: in reading and writing and the writing composite comprises dictation and far-point copying tasks. The scores obtained can be convertible into percentile ranks based on age allowing comparison of an individual student's performance relative to their peers of a similar age. (Cheng et al., 2011; Shen et al., 2012)THPC is a checklist comprising 24 items aimed at evaluating handwriting difficulties among elementary school students in China. It assesses six aspects of handwriting, including Formation, Arrangement, behavior, precision, motor skills, and alignment. Items are scored on a 4- 4-point scale using this scale can identify whether the writing performance was poor or good, the assessment was conducted by an occupational therapist who was unaware of the group allocation (Shen et al., 2012).

3.Discussion

ADHD is a neurodevelopmental condition characterized by a substantial level of inattention, disorganization, and hyperactivity-impulsivity that delay normal functioning or growth,(DSM 5; American Psychiatric Association, 2013) that appears in childhood and may persist until childhood (Fenollar-Cortés et al., 2017). ADHD can impact various aspects of the individual's life including motor skills like handwriting. In the handwriting examination of ADHD individuals, two important components need to be investigated: legibility and speed. Individuals with ADHD encounter difficulties with the readability of their handwriting. Handwriting legibility involves factors such as letter formation, spacing between letters and words, uniformity in size and slant, and overall neatness.in ADHD writers aim to finish the task quickly, prioritizing speed over the accuracy of the word and application of pen pressure this also affects the legibility of the writing (Li-Tsang et al., 2018) .Legible handwriting is clear and understandable to the reader without significant effort or confusion. In handwriting, crucial elements for legibility are letter production, which includes variation in letter height,(Adi-Japha, E.,et al 2007)spacing between letters and words, and positioning of letters on baseline.(Tucha & Lange, 2001) In ADHD individuals show bigger letter formation while copying the word this could affect the legibility of the writing.(Ivančević et al., 2020; Rodríguez et al., n.d.) The inconsistency in the production of letters lies in variations in the letter

height which tends to letters into larger sizes..) In letter formation when writing the same sentence repeatedly the letter height increases, even at a higher height of the letter the sentence is legible. Improving the size and formation of words can compensate for any issues with spacing, slant and alignment in a written sample, ultimately it leading to increased readability ADHD children make more spelling mistakes in their writing even when it is copying, in the study by *Li-Tsang et al. 2018* shows that copying longer words tends to make more spelling errors (Li-Tsang et al., 2018) .and the study done by Capodieci et al.2018 also shows that ADHD individuals have difficulty in spelling correctly (Capodieci et al., 2018). In ADHD individuals can't pay more attention to where the point they reached the copying letters this makes them more spelling mistakes leads to affects the readability of the written sentence. Additionally, variability in these gets worse when longer passages need to be written, indicating that ADHD children may struggle to maintain stable writing for longer periods (Borella et al., 2011).The poorer writing performance of ADHD children is mainly related to word formation. Visual-motor integration involves aligning visual perception with motor abilities, particularly hand-eye coordination (Farhangnia et al., 2020) .In the context of handwriting, visual-motor integration is essential for accurately translating visual information (such as letters and words) into motor movements required for writing. Individuals with strong visual-motor integration skills are typically able to produce neater and more legible handwriting because they can accurately control the movements of their hands and fingers based on visual cues (Farhangnia et al., 2020; Li-Tsang et al., 2018). Conversely, difficulties in visual-motor integration may result in challenges with letter formation, spacing, and overall handwriting legibility. Research has not shown a significant contrast in writing speed between individuals with ADHD and those without. However, one study did demonstrate a significant distinction, indicating that non-medicated children with well-established ADHD tend to write at a slower speed (Borella et al., 2011). Lack of accuracy in handwriting was found in the study done by Langmaid et al.2016 which pointing those children with ADHD displayed lower accuracy compared to the typically developing group when a cursive “l” under two conditions: 10mm and 40 mm. However, this discrepancy in accuracy, particularly in missing the upper target line more frequently, was evident only in the 40mm condition. For instance, some features observed in ADHD handwriting can also manifest in other neurological disorders like Parkinson's, schizophrenia, and arthritis. Parkinson’s individual show reduced stroke size in width and height (Smits et al 2014, Rosenblum et al 2013) schizophrenic individuals possess larger handwriting and increased repetition in letters (Kömür 2015, Gallucci et al 1997), in Arthritis, also possess deterioration in letter formation which declines the quality of writing and downward alignment and also has narrow spacing between the words that found in ADHD handwriting (Saini et al 2021).

In a forensic investigation involving authors with neurological disorders, identifying critical aspects of the author's style, such as legibility, including variations in letter height, spacing, alignment, letter formation, and spelling errors can provide valuable clues. Handwriting examiners rely on reliable handwriting tools to identify such variations. In discussing the various handwriting assessment tools, it is evident that each has its strengths and limitations. The Persian Handwriting Assessment Tool (PHAT) Demonstrates validity and reliability in measuring writing performance among primary school students, particularly focusing on legibility dimensions. However, its limited scope and age restriction to second and third-grade students may hinder its applicability to older age groups or individuals with diverse educational backgrounds (Farhangnia et al., 2020). Similarly, while the BVSCO-2 task offers a standardized assessment of handwriting speed and accuracy, it may overlook other aspects of writing proficiency and may not be universally applicable due to its emphasis on cursive scripting (Borella et al., 2011; Capodieci et al., 2018, 2019). On the other side, the Minnesota Handwriting Assessment (MHA) integrates digital technology for both manual and automated assessment, providing flexibility but requiring specific resources and potentially introducing variability in results(Dirlikov et al., 2017). Finally, the Tseng Handwriting Problem Checklist (THPC) and Basic Reading and Writing Comprehensive Test (BRWCT) offer comprehensive evaluations but may be limited by subjective ratings and language-specific focus, respectively. (Shen et al., 2012). Considering the need for a versatile tool, the MHA stands out for its integration of manual and automated assessment methods, providing a balance between comprehensive evaluation and practicality. Therefore, researchers and practitioners should carefully consider the strengths and limitations of each tool when selecting the most appropriate assessment method for their specific context and objectives, Utilizing appropriate tools can aid investigators in understanding variations in handwriting, particularly in authors affected by ADHD. By utilizing these tools, Forensic investigators can analyze handwriting patterns and inconsistencies, ultimately aiding document-related investigations and identification of authors, especially in cases involving individuals with ADHD.

4. Conclusion

The handwriting characteristics of individuals with ADHD provide valuable insights for forensic investigation, particularly in identifying unique features such as alignment, spacing, and spelling errors. Recognizing these distinctive ADHD handwriting patterns can aid forensic handwriting examiners in accurately analyzing and identifying authors affected by ADHD. However, selecting the appropriate handwriting assessment tool is crucial, considering the strength and limitations if each option. Future advancements in digital analysis tools can further improve accuracy in handwriting assessment, while ongoing research into neurological disorders could lead to the development of better tools customized to specific populations, such as those who have ADHD. Despite the potential benefits, it is important to note that some features observed in ADHD handwriting can also manifest in other neurological disorders, such

as Parkinson's, schizophrenia, and Arthritis. By effectively utilizing these tools and considering these limitations, forensic investigators can enhance their ability to analyze handwriting patterns and inconsistencies, ultimately aiding document-related investigations and author identification, especially in cases involving individuals with ADHD

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Author's contribution

Muhammed Afeef. R.K – manuscript drafting, investigation, editing, visualization, and result analysis

Priya Sharma – manuscript drafting, conceptualization, editing, reviewing, and supervision

Compliance with ethical standards

Samples utilized in this study taken by proper consent

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Tables:

Table 1 shows the ADHD types as per DSM-V guidelines (Puyjarinet et al., 2023).

ADHD diagnosis	**Description**
Predominantly Inattentive (ADHD/I)	Characterized by significant challenges with sustaining attention and organization tasks.
Predominantly hyperactive-impulsive (ADHD/HI)	Mainly characterized by excessive levels of hyperactivity and impulsivity, with less pronounced attention difficulties.
Combined Presentation (ADHD/C)	Features symptoms of both inattentiveness and hyperactivity-impulsivity, presenting challenges in multiple domains.

Table 2 shows symptoms (Frick & Lahey, 1991)

Main symptoms	
Inattention/Disorganization	**Motor Hyperactivity/impulsivity**
1. Struggles to complete task 2. Frequently appears unresponsive 3. Simply diverted or distracted 4. Hard to maintain focus 5. Difficulty organizing work 6. Requires significant supervision 7. Frequently shifts activities	1. Engages in excessive running and climbing 2. Demonstrate extreme fidgeting 3. Struggles with remaining seated 4. Exhibit restlessness 5. Always on the go 6. Acts impulsively without considering consequences 7. Regularly calls out in class 8. Impatient and struggles with waiting their turn
Associated problems	
1. Academic underachievement Problematic peer relationships 2. low self-esteem 3. Conduct issues 4. Experience negative interactions with parents 5. Negative interactions with teachers	

Table. 3a. shows the tools utilized for the assessment of ADHD Handwriting

Sl.no	Criteria for selection	Handwriting measuring tool	Key findings	reference
1	a formal diagnosis of ADHD,	BVSCO-2 continuous alternated letter production of cursive "l" and "e"	• ADHD has greater intraindividual variability (IIV) than the control group in simple response time (SRT) and handwriting tasks	(Borella et al., 2011)
2	No source	PHAT; Copy task and dictation task	• children with ADHD shows poor handwriting legibility	(Farhangnia et al., 2020)
3	formal diagnosis of ADHD & ad-hoc questionnaire	BVSCO-2; Word production	• Poorer quality and speed of handwriting	(Capodieci et al., 2018)
4	formal diagnosis of ADHD	BVSCO-2; Writing legibility scale; phrases and words creation; Dictation challenges.	• higher spelling mistakes • less legibility of their handwriting. • No variation in the writing speeds.	(Capodieci et al., 2019)

5	formal diagnosis of ADHD	MHA; Copy task	• worse on letter forms in all three circumstances (copy, trace, and quick trace). • There is no dissimilarity in letter-spacing errors. • Showed less fluctuation in speed. • In the copy condition alone, both groups' letter form and WM performance exhibited a strong association.	(Dirlikov et al., 2017)
6	Formal diagnosis of ADHD	Production of cursive letters using a digitizing tablet at 10 and 40 mm	• Inaccurate writing size • Higher pen pressure	(Langmaid et al., 2016)
7	DSM–IV criteria	THPC & BRWCT copy and dictation task	• Low legibility in writing • No difference in writing speed	(Shen et al., 2012)
8	Diagnose by a psychologist and clinical doctor	Copy assignment; handwriting on a tablet computer	• Low Writing quality scores. • Slower writing speed. • Larger size writings. • More variability in motor control. • Worse motor planning. • Worse motor execution	(Laniel et al., 2020)

			on the pen-stroke test.	
9	Diagnosed by pediatric neurologist using DSM-IV criteria	No source	• More letter corrections • Higher deletion of letters • Higher writing duration for complex letters/ words • Faster movement on image-based tasks • Lower accuracy on image-based tasks	((Adi-Japha et al 2007.)

Table 3(b) the studies showing the effect of medication on ADHD Handwriting

Sl.no	Criteria for selection	Handwriting measurement tool	Medication	Key findings	Reference
1	Diagnosed by a psychologist and physician	Writing a word 30 times	On and off state of stimulant medication. Adderall, Concerta, Dexedrine, Ritalin, Vyvanse	• Greater inconsistency in graphomotor fluency. • Motor coordination variation linked to ADHD extends into adulthood • Variation presents regardless of stimulant medication usage	(Duda et al., 2014)

2	Formal diagnosis of ADHD	Writing task in cursive letters.	Methylphenidate	• Visual and cognitive control are crucial for handwriting automation. • Controlled movements show increased NIV (inversion in velocity) • Stimulant-treated ADHD children have heightened NIV during handwriting NIV decreases after stopping medication.	(Tucha & Lange, 2005)
3	DSM-V criteria	Drawing and letter copying task	Methylphenidate	• Methylphenidate usage improves writing kinematics. • Repetition or task increases familiarity and comfort in the writing process lead changes in writing style and technique	(Ivančević et al., 2020)
4	Formal diagnosis of ADHD	ETCH (Evaluation Tool of Children's Handwriting) handwriting tasks	Long-acting stimulant 1) concerta 2) biphentin 3) Adderall XR	• Showed notable enhancements in the legibility of handwriting.	(Brossard-Racine et al., 2015)

5	Formal diagnosis of ADHD	Writing and drawing task	1)Methylphenidate medication treatment(10-40mg) 2)Physiotherapeutic treatment 3)Parental psychoeducation treatment	• inconclusive proof for the superiority of parent psychoeducation over medication or physiotherapy. • parental involvement in Addressing children's challenges may enhance confidence, improve motor skills, and reduce stress levels	(Rothe et al., 2023)
6	Official determination of ADHD.	Copying a sentence	Methylphenidate	• letter height grew when the same sentence was written multiple times.	(Frings et al., 2010)
07	Diagnosed on DSM-IV criteria	Dictation and copy state	Methylphenidate	• Improvement in legibility; spacing • Slower handwriting	(Tucha & Lange, 2001)

Figures:

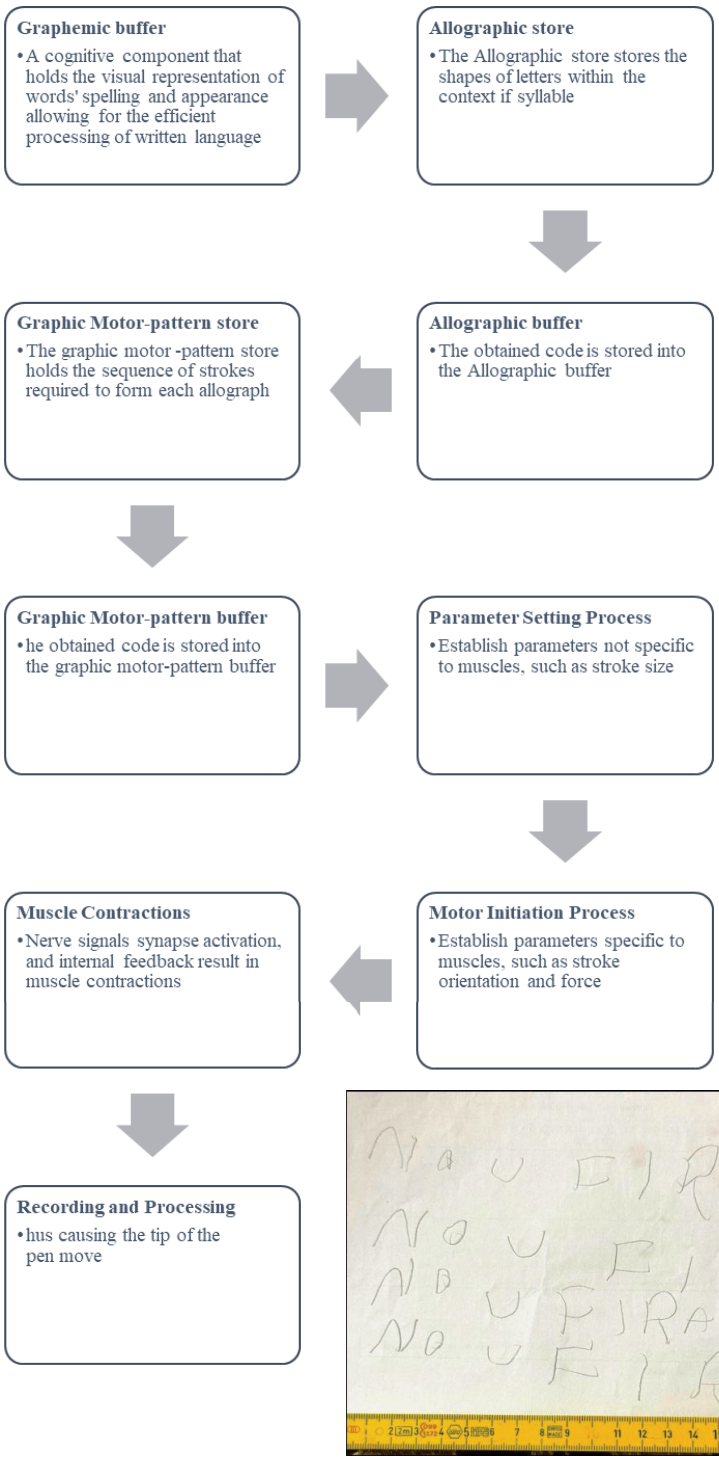

Figure 1. An overview of the handwriting modules, their contents, and the operation taking place between and inside them

Figure 2. shows the bigger size of the letter in handwriting among ADHD individuals

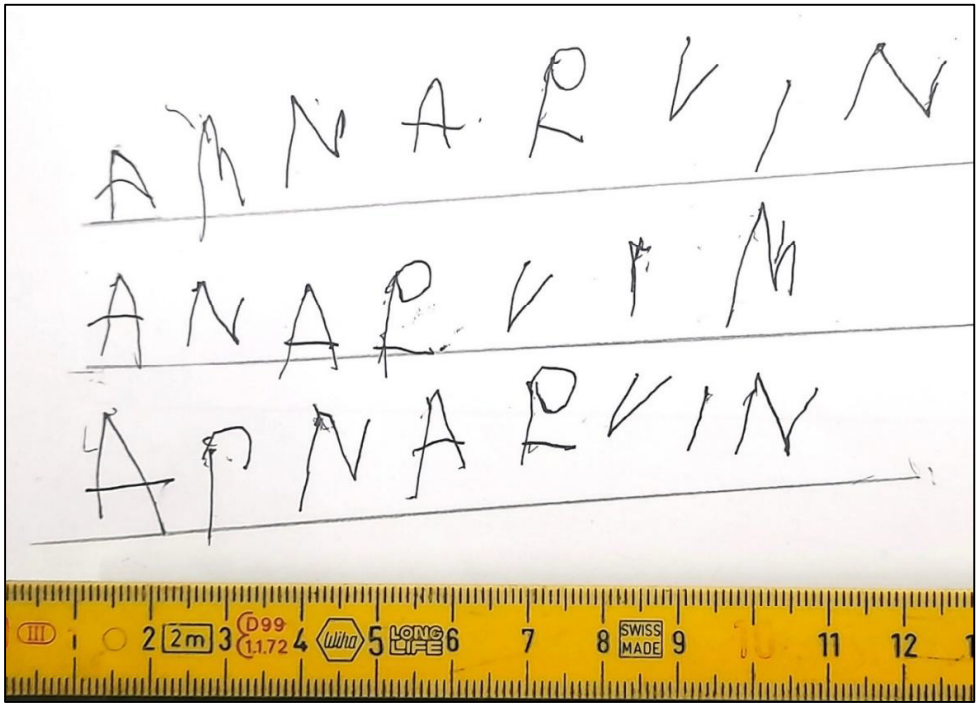

Figure 3. shows the bigger size of the letter in handwriting among ADHD individuals

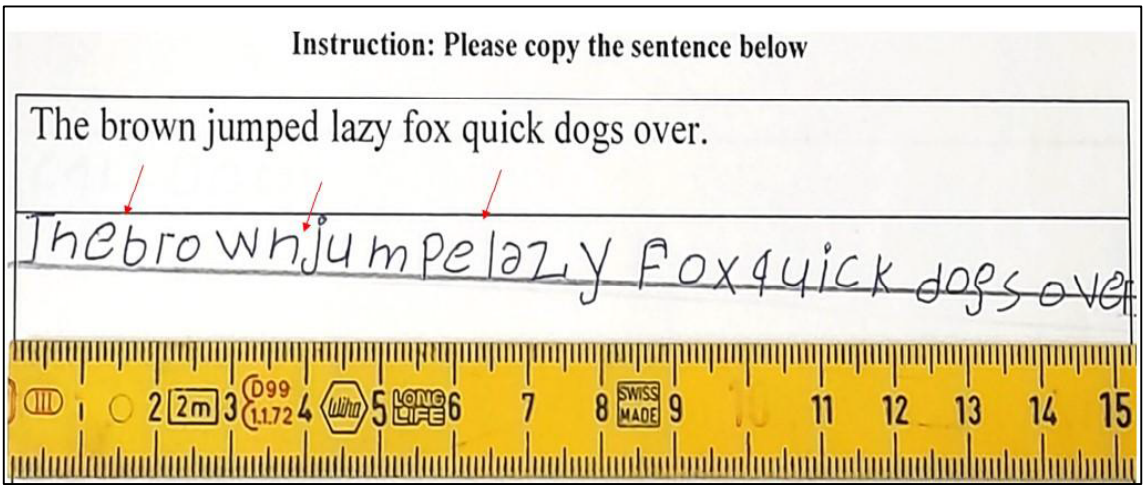

Figure 4. showing narrow spacing between words among ADHD individuals

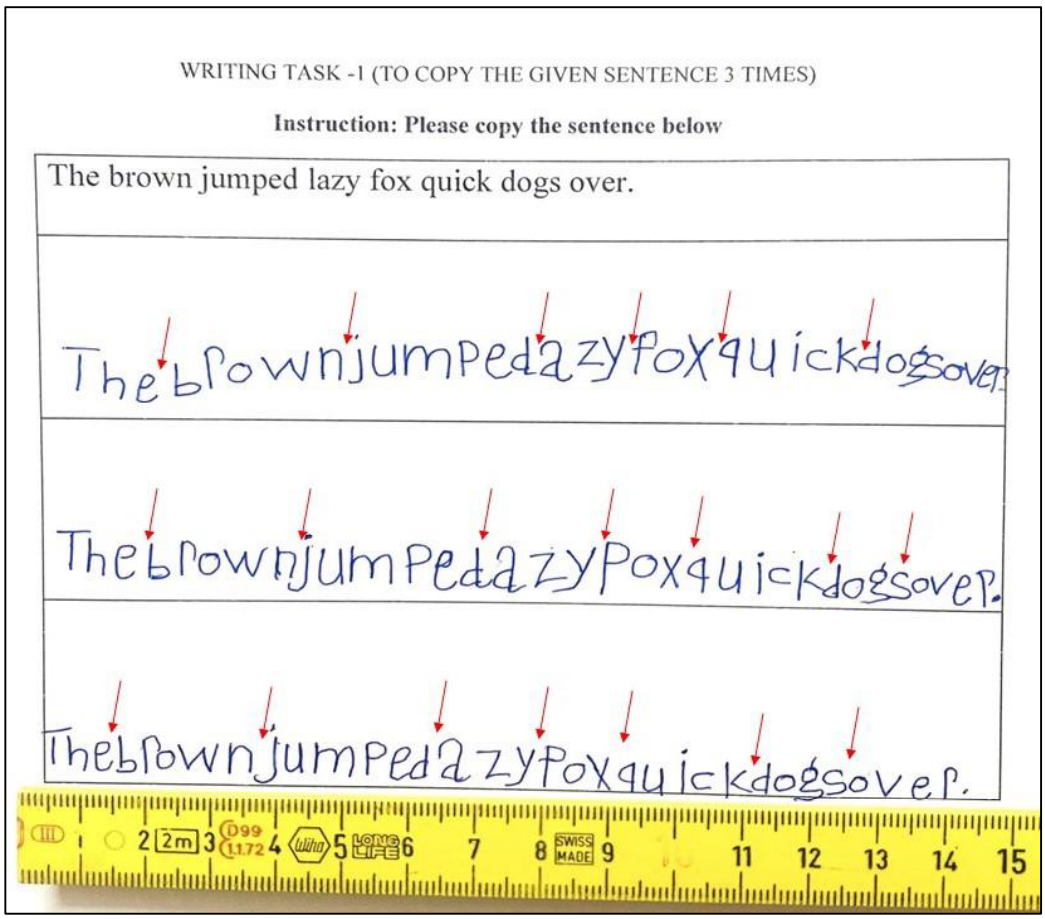

Figure 5. showing absence of spacing between words among ADHD individuals

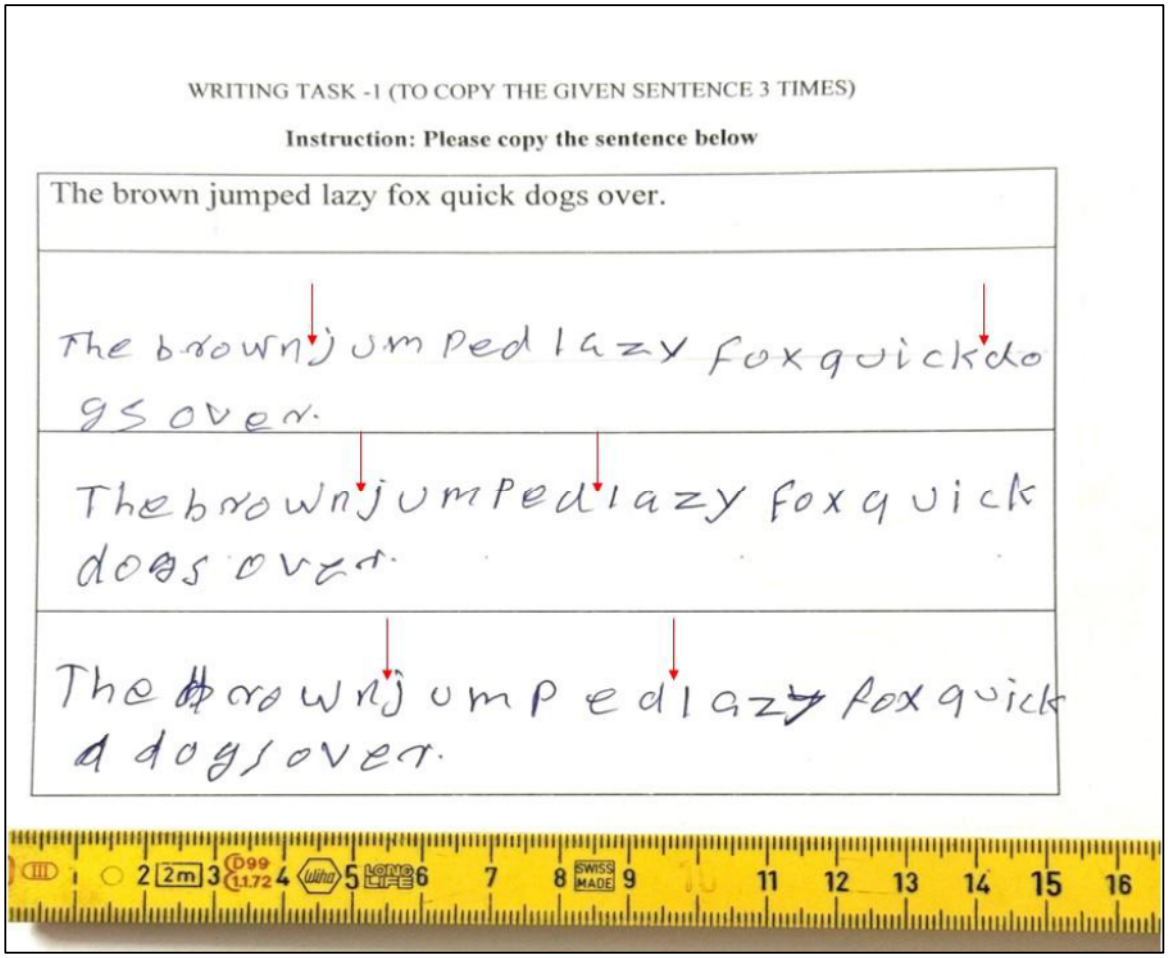

Figure 6. showing narrow spacing between words among ADHD individuals

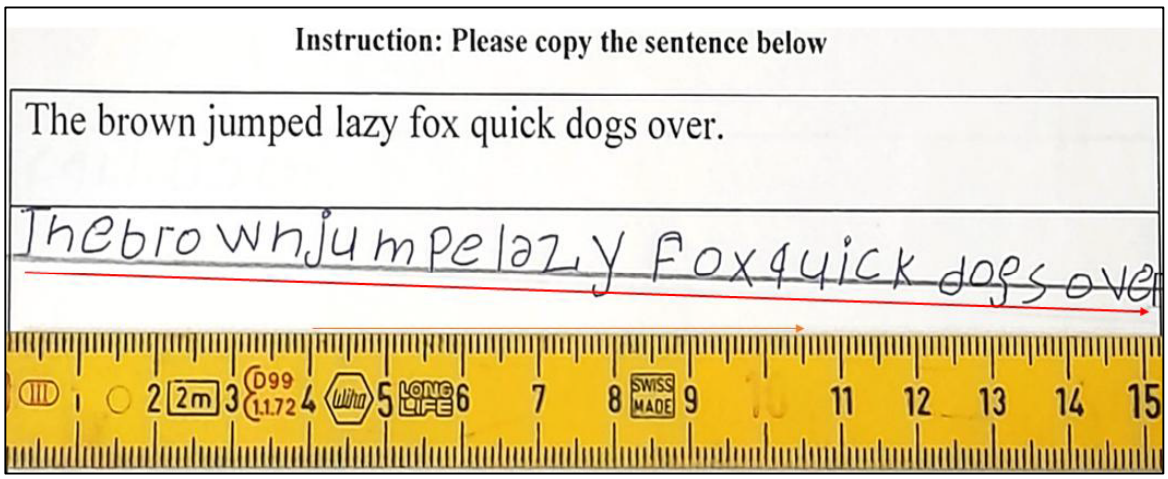

Figure 7. showing descending alignment of handwriting among ADHD individuals

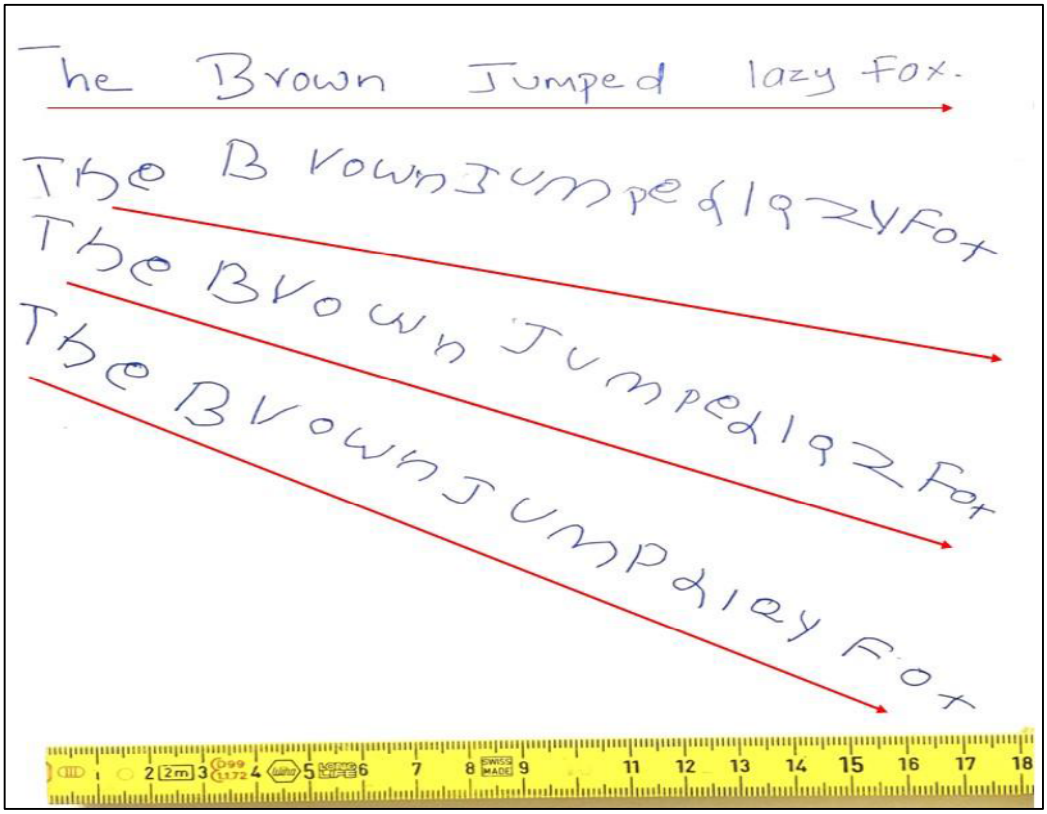

Figure 8. showing the descending alignment of handwriting among ADHD individuals

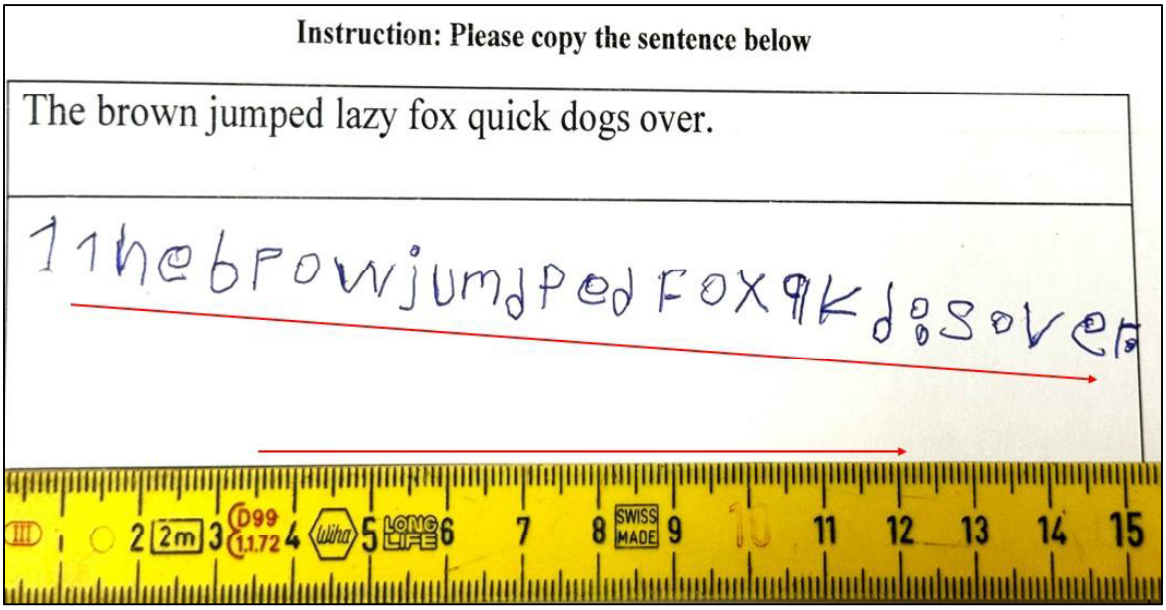

Figure 9. showing the descending alignment of handwriting among ADHD individuals

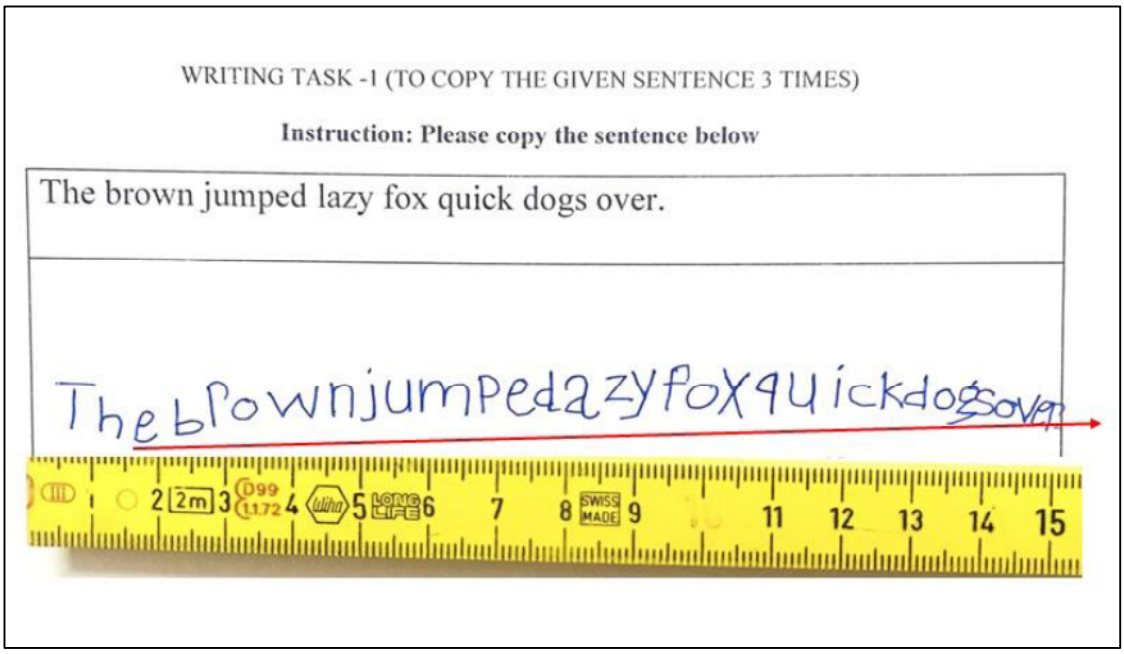

Figure 10. showing the ascending alignment of handwriting among ADHD individuals

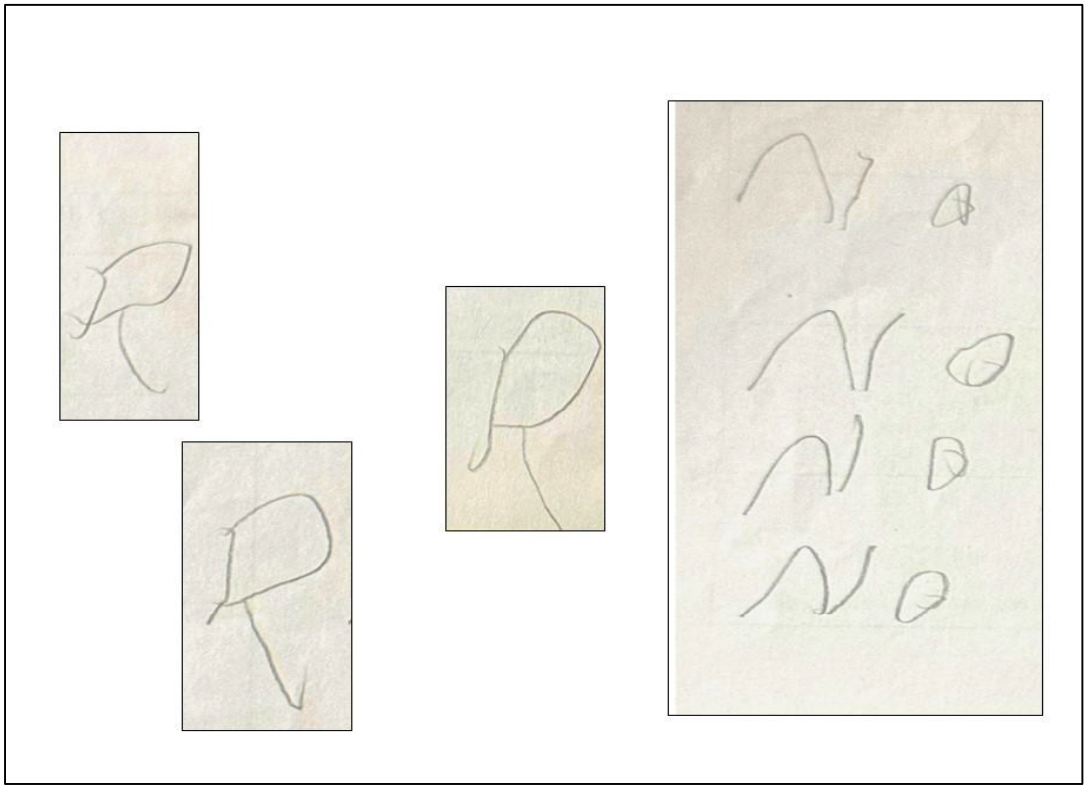

Figure 11. showing letter formation of letters R,N, and O among ADHD individuals

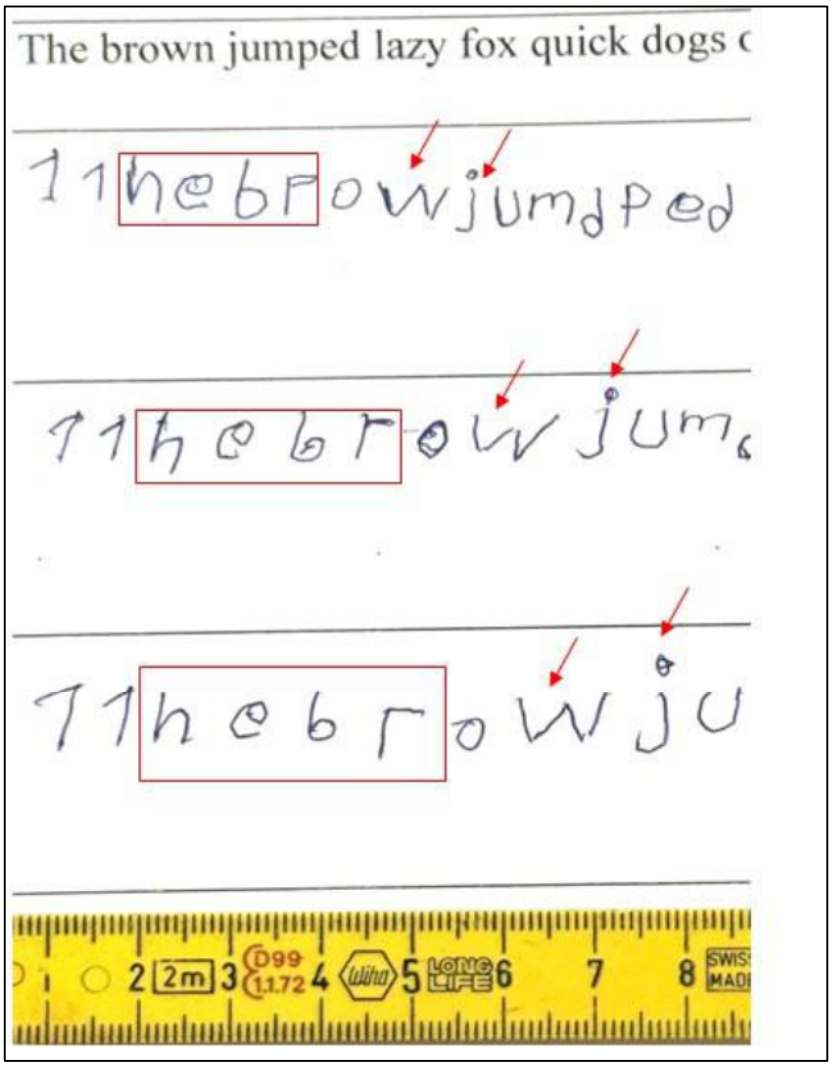

Figure 12. showing the letter formation of letters h, e, b, r, w, and j in handwriting among ADHD individuals

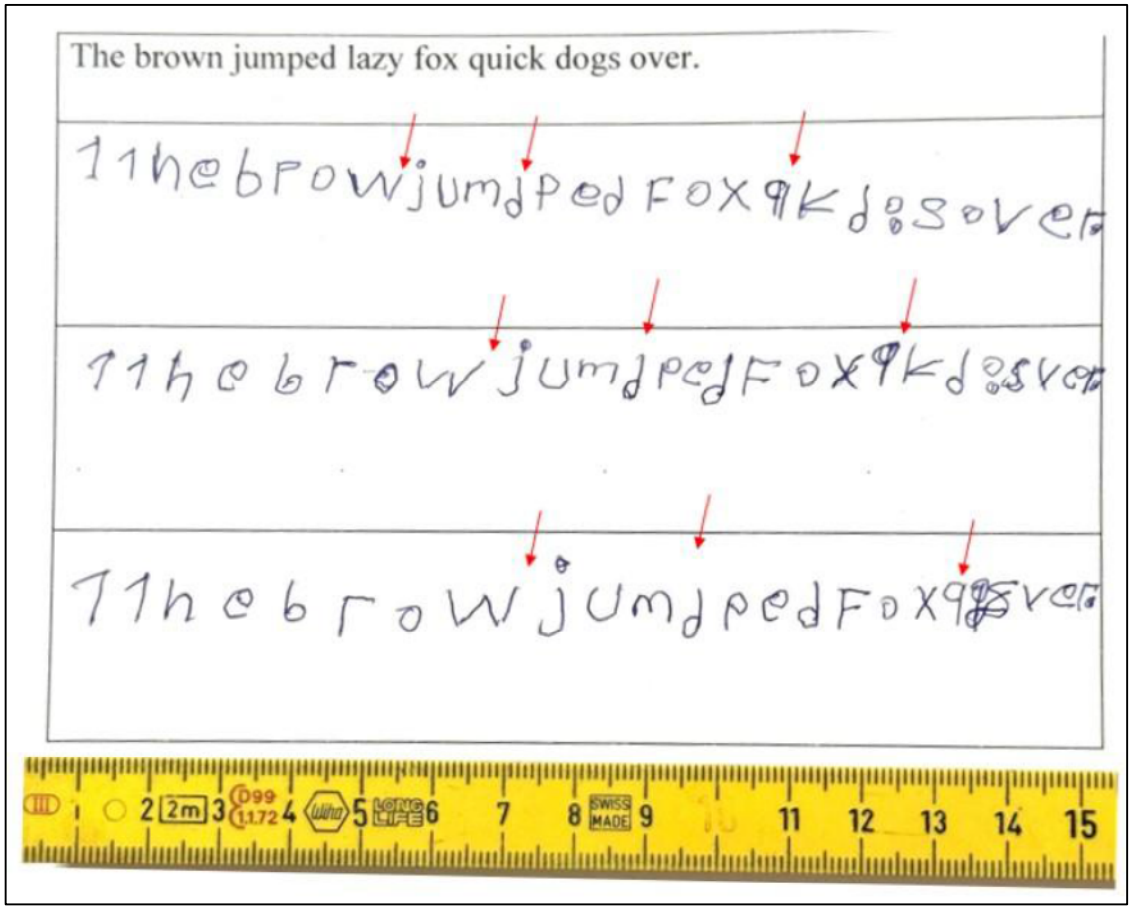

Figure 12. shows the missing letter “n” in the word brown and “uic” in the word quick and the insertion of the letter “d” in the word jumped

WRITING TASK -1 (TO COPY THE GIVEN SENTENCE 3 TIMES)

Instruction: Please copy the sentence below

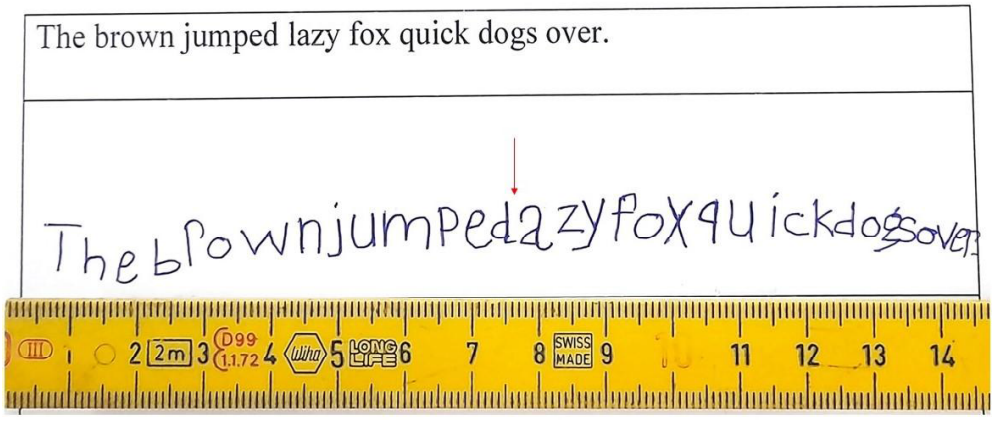

Figure14. showing missing of letter “L” in the word lazy in the handwriting among ADHD individual

Abbreviations:

BRWCT= basic reading &writing comprehensive test

THPC= Tseng handwriting problem checklist.

Role of spectroscopy in forensic examination of alcoholic beverages

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Abstract

Alcoholic beverages are beverages that contain ethanol (alcohol) as a key ingredient. These beverages are produced through the fermentation of sugars by yeast. Alcoholic beverages come in various forms, including beer, wine, spirits (such as vodka, whiskey, rum, gin), and liqueurs. Spectroscopy analysis plays a crucial role in the quality control, authentication, traceability, and characterization of alcoholic beverages. Various spectroscopic techniques such as UV-Vis spectroscopy, infrared spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy have been employed to analyze the composition of alcoholic beverages. UV-Vis spectroscopy is commonly used to determine the alcohol content in beverages by measuring the absorbance of specific wavelengths of light. Infrared spectroscopy helps in identifying functional groups present in the molecules of the beverage, aiding in the detection of impurities and additives. NMR spectroscopy provides detailed information about the molecular structure of compounds in alcoholic beverages, allowing for the identification of flavors compounds and quality control assessment. Spectroscopy analysis not only ensures compliance with regulatory standards but also helps in maintaining consistency in production processes and meeting consumer expectations for high-quality beverages. In conclusion, spectroscopy analysis of alcoholic beverages is a powerful tool that contributes to the understanding and optimization of beverage production processes, ultimately enhancing the overall quality and consumer satisfaction of alcoholic beverages.

Key words – Alcoholic Beverages, UV Vis spectroscopy, IR, NMR, Raman spectroscopy, Authentication, and Traceability

1. Introduction

Food forensics is a term that refers to the ability to determine the authenticity of a food product. By employing forensic techniques, food forensics investigates the probability of authenticating and tracing food products, ensuring their traceability and quality. The correlations between this field and various subjects, such as biology, nanotechnology, geology, mathematics, and many more, are significant [1, 2]. Food forensics is essential for various reasons. It helps ensure the safety and quality of food products by detecting food fraud, identifying contaminants, and tracing the origin of food items. For example, food forensics can uncover if a product has been adulterated, contaminated, or mislabeled, which is crucial for protecting public health and maintaining consumer trust. By using techniques like DNA analysis, spectroscopy, and chromatography, experts in food forensics can investigate food-related crimes, such as food poisoning outbreaks, counterfeit products, or illegal additives. Ultimately, food forensics plays a vital role in upholding food safety standards, preventing fraud, and safeguarding the integrity of the food supply chain. Food-related crimes occur worldwide and can take various forms. Some common food-related crimes include:

1. Food Fraud: This involves intentionally misrepresenting food products for economic gain. Examples include selling counterfeit or adulterated food products, mislabeling the origin of food items, or substituting cheaper ingredients for more expensive ones.
2. Food Contamination: Deliberate contamination of food with harmful substances, such as chemicals, pathogens, or foreign objects, can lead to foodborne illnesses or even intentional food poisoning outbreaks.
3. Illegal Additives: Adding unauthorized substances to food products, such as prohibited chemicals or drugs, can pose serious health risks to consumers and violate food safety regulations.
4. Counterfeit Products: Producing and selling fake or imitation food products under the guise of well-known brands can deceive consumers and compromise their health and safety.

5. Smuggling and Trafficking: Illegal activities related to the import or export of food products, such as smuggling contraband items, can circumvent regulations and endanger public health.

These food-related crimes not only jeopardize consumer safety but also damage the reputation of food businesses and the integrity of the food industry as a whole. Regulatory agencies and food forensic experts play a crucial role in investigating and preventing such crimes to protect public health and ensure food safety standards are upheld globally.

Alcoholic beverages, which include a broad selection of drinks, can have varying concentrations of alcohol (ethanol). Examples of alcoholic beverages that are produced on an industrial scale include beer, wine, China rice wine, as well as various distilled spirits like brandy, whisky, rum, gin, cognac, vodka, tequila, pisco, and China distilled spirit. The complexity of alcoholic beverages is remarkable, as scientists have managed to identify more than 1300 compounds in various types of these beverages. The meticulous production of alcoholic beverages involves the fermentation of grains, sugars, or fruits, resulting in the presence of ethanol as their defining characteristic. Beverages that are referred to as "licit liquors" are those that strictly follow all the legal regulations and licensing requirements during their production, distribution, and sale processes. The role they play is crucial as they contribute to economic growth, protect cultural heritage, and advocate for responsible drinking practices. They contribute significantly to government revenue and support local industries. 'Illicit liquors,' which are also referred to as illegal or bootleg liquors, are produced, distributed, or sold with no regard for legal regulations and licensing requirements. These types of liquors, in both their production and consumption, present formidable risks, and complexities that require careful consideration [3]. Illicit, undocumented, and counterfeit spirits have a detrimental impact on both global and local scales, posing a threat to human health, damaging the economy, and undermining consumer confidence in otherwise reputable brands. Broadly speaking, piracy and counterfeiting involve the act of falsely rebranding and producing consumer goods without permission, which is a serious issue.

Counterfeit consumer goods pose a worldwide problem, encompassing illicit and counterfeit spirits as one of its components. In 2016, it was estimated that the cumulative value of these crimes was close to half a trillion USD. These illicit spirits are significant problems both from an economic and a health stand-point. Each year thousands of deaths and injuries are reported from ingesting toxic and poisonous compounds that were presented as spirits. [4].

Each alcoholic beverage contains constituents that can be classified as major, minor, or trace components. Ethanol and water are commonly the primary constituents that make up most of the composition. Fusel alcohols, organic acids, carbonyl compounds, esters, aldehydes, lactones, sulfur compounds, sugars, preservatives, and colorants are all examples of the minor or trace constituents that can be detected in this mixture. The analysis of the components of alcoholic beverages can be approached in two ways.

2. Forensic significance of alcoholic beverage

2.1.Authentication

Authentication is verifying the features stated on a product tag, this process also ensures whether or not a foodstuff is adulterated. In the authentication procedures, some chemical parameters are used to differentiate authentic food from adulterated ones. Several variables such as geographic origin, variety, and production techniques have to be evaluated while authenticating of the products [5][6]Authentication of alcoholic beverages involves verifying the origin, composition, quality, and authenticity of the drink. Several methods are employed in the forensic examination of alcoholic beverages to ensure their integrity.

1. Label Examination: The first step in authenticating alcoholic beverages is to examine the label for information such as brand name, alcohol content, country of origin, and any certifications or seals of authenticity. Discrepancies in labeling can raise suspicions about the product's authenticity.

2. Physical Examination: Visual inspection of the packaging, bottle, cap, and label can reveal signs of tampering, counterfeiting, or resealing. Any irregularities in the physical appearance of the product can indicate potential authenticity issues.
3. Chemical Analysis: Chemical analysis techniques like spectroscopy (IR, NMR, mass spectrometry), chromatography (gas or liquid), and isotopic analysis are used to identify and quantify the components of alcoholic beverages. This helps in detecting adulterants, additives, or contaminants that may compromise the drink's authenticity.
4. Isotope Analysis: Isotope ratio analysis can determine the geographical origin of alcoholic beverages by analyzing the isotopic composition of elements like carbon, hydrogen, and oxygen. Variations in isotopic ratios can distinguish between products from different regions, providing valuable information for authentication.
5. DNA Analysis: DNA analysis may verify the ingredients used in alcoholic beverages, especially with fruit-based drinks like wines or ciders. DNA profiling can confirm specific fruit varieties or detect any unauthorized ingredients.
6. Forensic Testing: Forensic techniques are employed to investigate cases of suspected adulteration, contamination, or fraud involving alcoholic beverages. These tests can identify toxic substances, illegal additives, or unusual compounds that pose health risks to consumers.

By combining these authentication methods, forensic experts can accurately determine the authenticity and quality of alcoholic beverages, ensuring consumer safety and regulatory compliance in the beverage industry. Authentication processes are essential for maintaining trust in the market and protecting consumers from the risks associated with counterfeit or adulterated products.

2.2.Traceability

Traceability is the process for verifying the relation between a foodstuff and its source of the material. These procedures study the chemical parameters to figure out the paths of the various phases within a production chain. If a food sample could be trace-linked to its raw materials; it could be authentic. Authentication and traceability are original concepts, but follow the share goals which is assuring the quality of food products for consumers' advantage [7]. Traceability refers to the ability to track and trace the entire supply chain of a drink from production to consumption.

1. Supply Chain Monitoring: Traceability involves monitoring the entire supply chain of alcoholic beverages, starting from the raw materials used in production to the distribution and sale of the final product. Each step in the supply chain is documented and recorded to ensure transparency and accountability.
2. Batch Tracking: Alcoholic beverage manufacturers assign unique identifiers or batch numbers to each production batch. These identifiers are used to track the movement of specific batches throughout the supply chain, enabling quick identification and recall of products if issues arise.
3. Barcoding and RFID: Radio Frequency Identification (RFID) and Barcoding technologies are commonly used in the alcoholic beverage industry to track products at various stages of production and distribution. Barcodes and RFID tags provide real-time data on product location, movement, and storage conditions.
4. Digital Platforms: Many companies use digital traceability platforms to record and manage data related to the production, distribution, and sale of alcoholic beverages. These platforms enable real-time tracking, data sharing, and collaboration among supply chain partners.

5. Regulatory Compliance: Traceability systems in the alcoholic beverage industry help companies comply with regulations and standards related to product labeling, quality control, and safety. By maintaining accurate records and traceability data, manufacturers can show compliance with legal requirements.

6. Quality Control: Traceability systems play a crucial role in quality control by allowing manufacturers to identify and address issues such as contamination, adulteration, or product recalls promptly. Traceability helps in maintaining product quality and ensuring consumer safety.

7. Consumer Confidence: Transparent traceability practices in the alcoholic beverage industry build consumer trust and confidence in the products they purchase. Consumers are increasingly interested in knowing the origin, production methods, and quality of the beverages they consume, making traceability a valuable asset for brands.

By implementing robust traceability systems, the alcoholic beverage industry can enhance product safety, quality, and transparency throughout the supply chain, benefiting both businesses and consumers. Traceability is essential for maintaining accountability, preventing fraud, and ensuring the integrity of alcoholic beverages in the market.

2.3.Food frauds/ counterfeiting

The term food fraud simply means selling an inexpensive product at the price of an expensive one. There are different fraud such as slight or complete replacement of a food product with its low-priced counterparts, addition, misrepresentation of food contents, or food packaging, false labeling for economic gains. Many types of fraud are easy to identify because adulterants might contain materials acting as original markers. For instance, lemon juice is replaced with carrot juice. Therefore, despite, highly negative effects on consumers' health, food fraud is often regarded as an economic crime [8]. Counterfeiting of alcoholic beverages is a significant issue that poses risks to consumer health and

safety. Counterfeit alcohol refers to fake or illegally produced beverages that are sold as genuine products.

1. Types of Counterfeiting: Counterfeiting of alcoholic beverages can take various forms, including substitution (genuine products are replaced with fake or inferior quality alcohol); tampering (labels, packaging, or contents are altered to deceive consumers); illegal production (unauthorized manufacturers produce beverages without adhering to quality standards or regulations);

2. Health Risks: Counterfeit alcoholic beverages can pose serious health risks to consumers. These products may contain harmful substances, such as methanol or other toxic chemicals, which can lead to poisoning, illness, or even death.

3. Economic Impact: Counterfeiting affects the revenue of legitimate producers and distributors in the alcoholic beverage industry. It leads to lost sales, damages brand reputation, and undermines consumer trust in authentic products.

4. Detection Challenges: Detecting counterfeit alcohol can be challenging because of sophisticated counterfeiters who replicate packaging, labels, and even the taste of genuine products. Consumers and authorities may struggle to differentiate between real and fake beverages.

5. Combatting Counterfeiting: To address counterfeiting in the alcoholic beverage industry, stakeholders can implement various measures, including traceability where establishing robust traceability systems to track products throughout the supply chain can help identify and prevent counterfeit products from entering the market. It also includes regulatory enforcement where strengthening regulations, conducting inspections, and imposing penalties on counterfeiters can deter illegal production and distribution of fake alcohol are included.

By addressing counterfeiting through a combination of regulatory measures, industry collaboration, and consumer education, the alcoholic beverage sector can mitigate the risks associated with

counterfeit products and protect consumer health and safety. It's essential for both businesses and consumers to remain vigilant and take proactive steps to combat counterfeit alcohol.

2.4.Adulteration

Adulteration is the action in which a food product is changed by adding to it inferior or false products, or taking some of its valuable contents. Adding a false product might be done to increase the weight or appearance of a product to enhance its overall look, or even offer a fake strength to it [9] Adulteration of alcoholic beverages is a significant issue that involves the addition of unauthorized substances to drinks. This adulteration can take different forms, such as dilution with water or other liquids to reduce alcohol content, adding harmful substances like methanol, or using artificial flavorings to mimic authentic products. These adulterants can pose serious health risks to consumers, leading to poisoning or other health complications.

Detecting adulterated alcoholic beverages can be challenging because of the complexity of some adulterants. Advanced testing methods like chromatography or spectroscopy are often needed to identify these substances accurately. To prevent adulteration, the industry can implement quality control measures, comply with regulations, and verify suppliers and ingredients to ensure the authenticity and safety of products. By prioritizing quality control, regulatory compliance, and transparency in production and distribution, the industry can mitigate the risks associated with adulteration. Consumers play a crucial role by inspecting labels, buying from reputable sources, and being aware of the signs of adulterated beverages to protect themselves from potential harm.

3. Role of spectroscopic techniques in forensic examination of alcoholic beverages

3.1.UV spectroscopy

UV-visible and fluorescence spectroscopic techniques are among the most basic and widely used analytical tools for sample characterization. The UV-visible technique is based on electronic transitions and the Beer-Lambert law while the fluorescence technique is based on the emission of fluorescence

when an excited electron falls back to its ground state. Over the years, they have been used in different industries such as pharmaceuticals, textiles, and dyeing industries. In forensic science, they are used for the analysis of body fluid, counterfeit beverages, drugs, fibres, fingerprints, paint, petroleum products, and questionable documents (Hussain et al.). The versatility of these techniques has led to their adoption in various industries, such as pharmaceuticals, textiles, and dyeing (Hussain et al., 2021). The chapter by Hussain et al. (2021) provides a comprehensive overview of the principles, theory, and instrumentation of UV-visible and fluorescence spectroscopy, as well as their specific applications in forensic sample analysis, highlighting their importance in modern forensic investigations

The method is non-destructive so that the sample can be reused. The technique is fairly simple and can be used easily. No prior training is necessary. Measurement can be done in a short span of time, helping easy integration into experiments. Data analysis is simple and requires less processing. Instrumentation is relatively inexpensive and can be procured easily by laboratories

Instruments are not always perfect; hence stray light may interfere with the measurements. Scattering of light because of bubbles or undissolved solid particles in the sample solution causes measurement error. Beer-Lambert Law is only obeyed when a single absorbing species is present in the solution. A sample containing multiple absorbing species cannot be used to determine concentration using absorbance. Improper orientation of the sample holder or misalignment can imbibe errors in the measurement.

3.2.IR spectroscopy

Infrared spectroscopy techniques, including near-infrared (NIR) and mid-infrared (mid-IR), offer rapid and cost-effective methods for analysing alcoholic beverages and their raw materials (Gishen et al., 2010). These vibrational spectroscopic approaches can apply to grapes, juices, wines, and other fermented products like beer and sake. The techniques enable compositional analysis, particularly for alcohol content, as well as fermentation monitoring and authenticity assessment. Gishen et al. (2010)

discuss the challenges and requirements for sample presentation and instrument configurations to optimize performance when analysing these complex matrices. The authors highlight the potential for non-destructive and on-line testing, including the ability to measure the composition of bottled products in situ. These spectroscopic methods offer versatile applications in the alcoholic beverage industry, providing opportunities for quality control, process monitoring, and product authentication throughout the production chain. Infrared spectroscopy and spectroscopic imaging are powerful, non-destructive techniques with high chemical specificity and sensitivity, widely used in forensic science (Ewing & Kazarian). These methods, particularly Fourier transform infrared (FT-IR) spectroscopic imaging in various modes, have shown great potential in analyzing forensic samples. They can provide detailed chemical composition of fingermarks and identify contaminants at crime scenes (Ewing & Kazarian). The review highlights the applications of these techniques in examining fingerprint residues, explosive materials, and counterfeit drugs. The authors emphasize the robustness and label-free nature of these methodologies, making them particularly suitable for forensic applications. The paper also discusses recent developments in the field and considers the implications of this research for analyzing different materials in forensic contexts. Finally, it suggests potential future research directions for applying vibrational spectroscopic methods to forensic sample analysis (Ewing & Kazarian).

The advantages include rapid qualitative and quantitative analysis; minimal or no sample preparation required; high sensitivity and rapid analysis; and versatility in analysis; easy interpretation. Disadvantages include sometimes difficult handling procedures and maintenance of the sample cells. There are no infrared spectra in atoms or monatomic ions, hence it cannot analyze. To use infrared spectroscopy is that it requires very sensitive and properly tuned devices. The sample having aqueous solutions and complex mixtures are complicated to analyze by infrared spectroscopy.

3.3. Raman spectroscopy

Raman spectroscopy has emerged as a valuable tool in forensic analysis for the identification and quantification of cocaine and other illegal drugs. This non-destructive technique offers rapid and cost-effective analysis, preserving evidence for potential re-examination (Penido et al., 2016). The method has proven effective in detecting drugs in various seized samples, including those concealed in legal materials like beverages and clothing. Raman spectroscopy can determine drug concentrations in street cocaine and crack rocks, as well as identify adulterants, providing crucial information for forensic toxicology and criminalistics. Recent advancements in Raman spectrometers, such as portable instruments and new excitation wavelengths, have expanded its applications, enabling on-site investigations at crime scenes. The development of improved data analysis techniques further enhances the potential of Raman spectroscopy in forensic science, making it a promising tool for law enforcement agencies in their efforts to combat drug trafficking and abuse (Penido et al., 2016).

It has following advantages. Many organic and inorganic materials are suitable for Raman analysis. These can be solids, liquids, polymers, or vapors. No sample preparation is needed. Water does not interfere with the final spectrum. Other advantages include non-destructive nature; rapidity, simple sampling, and easy recording and interpretation. Disadvantages include its incapability to be used for metals or alloys, weak Raman effect, and fluorescence effect because of impurities.

3.4. NMR spectroscopy

NMR spectroscopy has emerged as a powerful tool in the forensic examination of alcoholic beverages, offering insights into their composition, authenticity, and quality. This technique enables the detection of sophisticated adulterations that may elude conventional methods like chromatography (Guillou et al., 1992). NMR-based metabolomics has shown promise in profiling the complex chemistry of alcoholic beverages, influenced by factors such as alcoholic content, origin, and fermentation processes (Tabago et al., 2020). The approach can identify specific metabolic signatures and biomarkers related to various forensic concerns, including the estimation of time since death and exposure to drugs of

abuse (Locci et al., 2020). NMR spectroscopy's ability to determine site-specific isotope ratios in low molecular weight molecules like ethanol and flavor compounds makes it particularly valuable for authenticating expensive commodities and detecting economic fraud in the beverage industry (Guillou et al., 1992; Osborne et al., 1993). Nuclear Magnetic Resonance (NMR) spectroscopy has emerged as a powerful tool for metabolomics research, particularly in the analysis of alcoholic beverages and wine authentication (Tabago et al., 2020; Solovyev et al., 2021). NMR offers several advantages, including minimal sample preparation, quantitative metabolite analysis, high reproducibility, and non-destructive nature (Emwas et al., 2019). In wine authentication, 1H NMR spectroscopy enables both targeted quantification of ingredients and non-targeted metabolomic fingerprinting (Solovyev et al., 2021). Despite its lower sensitivity compared to mass spectrometry techniques, NMR excels in isotope detection, mixture deconvolution via 2D spectroscopy, and automation (Emwas et al., 2019). Recent studies have focused on applying NMR-based metabolomics to evaluate the authenticity, variety, age, and fermentation processes of alcoholic beverages, with particular interest in Asian markets (Tabago et al., 2020). As NMR technology continues to advance, it is becoming an increasingly valuable tool in metabolomics research and food authentication.

3.5. Mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is a valuable technique for the forensic analysis of alcoholic beverages, as highlighted in a comprehensive review by Yadav and Sharma (2017). The authors emphasize the importance of forensic characterization of liquor samples in cases involving alcohol-related deaths and crimes. GC-MS analysis offers several advantages, including increased sensitivity, accuracy, and reduced analysis time compared to other methods. The review discusses various sample preparation techniques for GC-MS analysis of alcoholic beverages and their applications in forensic science. The authors note that determining the geographic origin of alcohol samples can be crucial in certain investigations. Overall, the paper underscores the significance of

developing more sensitive analytical methods for alcoholic beverage analysis in forensic contexts (Yadav & Sharma, 2017).

Mass spectrometry is used for both qualitative and quantitative study of chemical substances. These can classify a sample's elements and isotopes, to determine molecular masses, and as a tool for helping to classify chemical structures. This can calculate the purity of the samples and the molar mass.A big advantage of mass spec is that it is incredibly sensitive (parts per million) over many other techniques. It is an excellent tool for identifying or confirming unknown components in a sample. The disadvantages of mass spec are that identifying hydrocarbons that produce similar ions is not very good and it cannot separate optical and geometric isomers. The disadvantages are offset by combining MS with other methods, for example gas chromatography. One disadvantage of mass spectrometry is over hydrocarbons. The method cannot distinguish between hydrocarbons producing similarly fragmented ions.

4. **Conclusion:**

Spectroscopy plays a crucial role in the forensic examination of alcoholic beverages by providing valuable insights into the composition and authenticity of the products. Spectroscopic techniques, such as infrared spectroscopy, UV-Visible spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy, can analyze the chemical composition of alcoholic beverages, detect adulterants, and differentiate between authentic and counterfeit products. These spectroscopic methods allow forensic experts to identify specific compounds present in alcoholic beverages, such as ethanol, methanol, and other volatile substances. By comparing the spectral fingerprints of known authentic samples with questioned samples, spectroscopy can help determine the origin, quality, and potential adulteration of alcoholic beverages. In conclusion, spectroscopy serves as a powerful tool in forensic examinations of alcoholic beverages, enabling accurate identification of substances, detection of counterfeit products, and ensuring consumer safety. By leveraging spectroscopic techniques in forensic analysis, authorities can effectively combat adulteration, counterfeiting, and other illicit

practices in the alcoholic beverage industry, promoting transparency, authenticity, and quality assurance for consumers.

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Trends in craniofacial reconstruction techniques

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Abstract:

Craniofacial reconstruction helps in creating a face from the skull of a decomposed body. It involves estimation of bone and tissue markers and nerve density, along with the graphic/ visual improvements. Positive facial reconstruction can lead to positive personal identification. Over the last decades, newer and better methods have emerged for facial reconstruction, among which one method is 3D reconstruction methods. The present review discusses the current techniques, advancements, challenges, and applications of computerized facial reconstruction. This paper represents the survey on the application and uses of computerised methods or software used for facial reconstruction with forensic human identification and recognition purposes. Information was gathered by searching databases (Google Scholar) studies, articles, books and evaluating existing literature related to computer aided craniofacial reconstruction. Then, by considering computerised techniques, studies containing manual methods were excluded. The present study enabled us to highlight the most important findings or trends in computerized facial reconstruction. This includes breakthrough technologies, limitations of current methods, or emerging trends in the field. As there is a rapid development of digitalization, computer based facial reconstruction is a less time consuming, more effective and a long-term useful method for facial reconstruction or recognition.

Keywords – Facial reconstruction, 3D reconstruction, Personal identification, Anthropology, Forensic Science

Introduction

Craniofacial reconstruction (CFR) is used to reconstruct the facial appearance based on the analysis of skull morphology. Scientists or practitioners can use this technique to recreate the faces of individuals whose remains are discovered at archaeological sites. Facial reconstruction, an interdisciplinary field that combines art, science, and advanced technology, has seen remarkable advancements over recent years. This paper reviews the latest trends in facial reconstruction, including digital techniques, forensic applications. Facial reconstruction is a specialized area that involves recreating the appearance of a person's face from available evidence. Historically rooted in forensic anthropology and archaeology, the field has evolved to encompass medical and

esthetic applications. Recent technological advancements and methodological improvements have transformed ow facial reconstruction is approached, offering new opportunities for accuracy and efficacy.

he goal of Craniofacial Reconstruction (CFR) is to estimate the facial outlook of an individual according to he skull [1]. Traditionally, facial reconstruction relied primarily on manual techniques, such as the application f clay over a skull based on morphological guidelines derived from anatomical studies. As the need for ccuracy and detail increased, the field saw the introduction of photographic and artistic methods that enhanced he visual representation of reconstructed faces. In spite of these developments, traditional methods often faced imitations in terms of reconstructive accuracy and scalability.

omputerized facial reconstruction in forensic science represents a revolutionary advancement in identifying nknown victims and solving criminal cases. This technique employs advanced software and algorithms to enerate a three-dimensional model of a person's face based on skeletal remains. By analysing the shape and tructure of the skull, forensic experts can reconstruct facial features such as the nose, cheeks, and jawline. This rocess not only aids in identification but also serves as a crucial tool in providing closure to families of nissing persons. There has also been additional work for the operation of immersive technology like VR eadsets to enable an immersive crime scene restoration [2], [3], adding 3D scanners to generate 3D models for rime scenes [4], [5] and the capturing of crime scene video sequences to extract 3D models [6]. The ntegration of computerized methods has significantly enhanced the accuracy and efficiency of facial econstructions compared to traditional manual techniques. Using a combination of anatomical data, statistical nodels, and artificial intelligence, modern software can produce highly detailed and lifelike representations.

s technology continues to change, computerized facial reconstruction is becoming more available and easier, mpowering forensic professionals to conduct reconstructions more promptly than ever before. Additionally, he results can be organised in various backgrounds, from digital forensics to cold case investigations, xpanding the scope of its application. The fusion of artistry and technology in this field not only enables the eek for justice but also highlights the significance of forensic science in the wider landscape of criminal nvestigation and victim advocacy.

ata Acquisition

ata acquisition or data collection refers to the process of gathering applicable information and dimensions elated to the facial structure. Data acquisition in facial reconstruction frequently involves exploiting techniques ike CT scans, MRI's, X-rays, 3D scanners, and photographs to capture detailed information about the facial eatures. Researchers collect data on the shape, size, proportions, and asymmetries of the face to create a videspread understanding of the individual's facial morphology. The collected data works as the foundation for

acial reconstruction studies, allowing researchers to analyse the facial structure, identify unique characteristics, nd develop accurate representations of the face.

). Costantino [2016], [7] This study estimates the pertinency of photogrammetry and laser scanner methods in orensic ballistic articles and crime scene reconstructions and their potential in forensic applications. It provides seful and valuable insights into the use of these technologies in real-world forensic scenarios. The paper is estricted to two technologies and does not explore any other crime scene reconstruction methodologies.

echniques recently followed for Data Acquisition:

D Scanners: [8,9,10]

hese devices capture the geometry of a face by scanning it from multiple angles. These scanners utilize arious technologies such as structured light, laser, or photogrammetry to create detailed three-dimensional epresentations of the face. Researchers rely on 3D scanners to obtain accurate measurements of the facial eatures, including the contours, proportions, and asymmetries. By generating high-resolution 3D models of the ace, these scanners enable researchers to analyse facial structures with exceptional detail, aiding in the dentification of unique characteristics crucial for facial reconstruction. The data obtained from 3D scanners is ivotal in creating customized facial prosthetics, planning surgical interventions, and conducting comparative nalyses for forensic purposes. They play a significant role in documenting facial changes over time, acilitating longitudinal studies in facial reconstruction research. 3D scanners are indispensable tools in facial econstruction research, providing researchers with precise and comprehensive data essential for understanding acial morphology, planning reconstructive procedures, and advancing the field of facial reconstruction.

hotogrammetry: [11,12,13,14]

hotogrammetry involves the process of obtaining precise measurements and creating three-dimensional nodels of the face through the analysis of photographs taken from multiple angles. In the perspective of facial econstruction, photogrammetry uses specialized software to analyse photographs of the face, identifying vital eference points and features to reconstruct the 3D structure accurately. By analysing the spatial relationships etween the reference points in the images, photogrammetry can generate comprehensive 3D models of the ace, capturing shades, traces or tones in facial contours and proportions. This method enables the acquisition f detailed facial data without direct contact with the subject, making it suitable for various applications in acial reconstruction research, such as forensic facial reconstruction and facial prosthetics development. The hotogrammetry abilities are famous also in medicine because of its radiation-free image acquisition.

omputer Tomography (CT) and X-rays: [15,16,17]

hese imaging methods are particularly useful for capturing detailed images of the facial internal structures. In acial reconstruction, CT scans are desired for their capability to generate cross-sectional images of the face.

These scans use X-rays to generate detailed and three- dimensional images of the soft tissues, bones, and other tructures and dimensions in the face accurately. X-rays are particularly useful for imaging the skeletal tructure, such as the skull and facial bones. They provide valuable information on the size, shape and lignment of the bones, which is crucial in facial reconstruction research. Both CT scans and X-rays play a vital ole in facial reconstruction research by providing detailed images that help researchers in understanding the acial anatomy, identifying abnormalities, and planning reconstructive procedures.

Depth Sensors: [18,19]

Devices like depth cameras can capture 3D point clouds. This method is effective even in low-light conditions. Depth sensors, such as Time-of-Flight cameras or structured light scanners, use IR light to measure the distance between the sensor and different points on the face, creating a depth map that represents the spatial dimensions of the facial features. These sensors deliver accurate depth data, allowing to capture the outlines, structures, and proportions of the face with extreme accuracy. Through collecting data on the complexity of different points on he face, depth sensors enable the creation of thorough 3D models that are crucial for facial reconstruction tudies. In reconstruction, depth sensors are essential for their capability to improve the data acquisition process by offering detailed spatial information that aids to the accuracy and practicality of facial reconstructions. Researchers influence the abilities of depth sensors to capture complex facial details, allowing a complete nalysis of the facial structure for various applications in anthropology, forensic science and other medical ields.

Magnetic Resonance Imaging (MRI): [20,21,22]

MRI provides high-resolution images of soft tissues, which can be essential for reconstructing facial features ccurately. MRI works by using a radio waves and strong magnetic fields to create detailed images of the nternal structures of the face, comprising soft tissues, bones, and cartilage. In facial reconstruction, MRI provides valued data about the basic anatomical features that guide facial morphology. Researchers influence MRI technology to obtain high-resolution images of the face, granting for the imaging of internal structures hat are vital for accurate facial reconstruction. In facial reconstruction, MRI is often highlighted for its ability iding in the understanding of facial fractions, irregularities, and distinctive features. The information obtained rom MRI scans assists as an important means for researchers in developing accurate, defined and genuine acial reconstructions for numerous applications in forensic science, anthropology, and medical fields.

Facial Soft Tissue Thickness OR Facial Soft Tissue Depths: [23-29]

FSTT / FSTD comprise an important cornerstone of craniofacial identification methods helping to set a systematic basis. These were investigated by Welcker in 1883 and then allowed their advanced inclusion into both craniofacial identification methods and superimposition of facial approximation. Facial soft tissue depths are determined by measuring at various points on the face, the distance between the skin and superficial surface of underlying hard tissue is FSTT/FSTD. These measurements, designate the face fitting over the skull in a general way due to a variety of organs are included in any single measurement and mean values are calculated.

Softwares, Methods and Techniques Studied

1. **Conditional Generative Adversarial Networks (CGAN) [30]**

The method uses CGANs to map skull images to facial images, leveraging depth maps to signify 3D craniofacial shapes. Depth maps are used to capture craniofacial features, making it easier to apply neural networks and recollect high-frequency details. Body Mass Index Classes (BMIC) are presented to increase the accuracy of facial reconstruction by considering the individual's body mass index. The paper intends a new approach for 3D craniofacial reconstruction using Conditional Generative Adversarial Networks (CGAN) based on depth maps. The authors represent 3D craniofacial shapes with depth maps and use a CGAN to map skull images to face images. They introduce Body Mass Index Classes (BMIC) to increase reconstruction accuracy. The method shows accuracy and practicality in craniofacial reconstruction, overtaking existing methods in comparative experiments.

Advantages:

The method shows accuracy and verisimilitude in craniofacial reconstruction results. Using depth maps preserves high-frequency details and is easy to generate and apply to neural networks. This model effectively learns the complex nonlinear relationship between skull and face, improving reconstruction accuracy.

Disadvantages:

Adding geometric information like curvature and elevation can introduce noise, making reconstructions visually obscure. The method relies on a limited dataset, which may affect the robustness and generalizability of the model.

2. **Missing Mandible Reconstruction [31]**

The study used a mathematical method based on linear cranial measurements to plan a virtual reconstruction of Dante's missing mandible. A three-dimensional standard mandible was created and modelled on the size of Dante's skull, which was scanned and introduced into 3D graphic modelling software. The reconstructed

nandible and skull were used to develop a new 3D virtual facial reconstruction of Dante, following methods ommonly used in forensic backgrounds. The study also considered historical descriptions and photographs of Dante to improve the accuracy of the facial reconstruction.

Advantages:

The method uses mathematical equations and 3D scanning to confirm precise reconstruction of the mandible nd facial features. Combines forensic methods and 3D computer graphics, providing a detailed and accurate econstruction.

Disadvantages:

The absence of the original mandible and teeth leads uncertainties in the reconstruction. Regardless of scientific nethods, some artistic elements are necessary, which can affect the accuracy of the final result.

3. CBCT (Cone Beam Computed Tomography) [32]

The study evaluates whether CBCT (Cone Beam Computed Tomography) is a better diagnostic tool for orensic facial reconstruction. The study found that CBCT-based facial reconstructions are reliable and create ccurate facial features. 3D computerized modelling using CBCT data is effective for facial reconstruction, losely mimicking the original appearance The studies included in the review indicate that facial econstructions using CBCT data are reliable and produce accurate results. The facial features reconstructed sing CBCT data demonstrated good levels of accuracy, closely mimicking the original appearance. The najority of the included studies used 3D computerized craniofacial forensic reconstructions, with some using ombination methods or region fusion strategies. The sample sizes in the included studies ranged from 1 to 200, vith reconstructions performed on live patients, existing CT data, and cadaver .

Advantages:

CBCT produces 3D craniofacial reconstructions with minimal radiation exposure. It provides excellent images f the skull and landmarks used in cephalometric analysis. Compared to traditional CT scans, CBCT has a ower radiation dose. CBCT can obtain images in upright positions and allows for easy editing, such as rotation nd zoom views.

Disadvantages:

The process can be very time-consuming. It requires significant experience to perform accurate reconstructions.

Author	Year of study	Location	Ancestry	Sample size	Mean age	Method	CBCT machine	Sample type	Measurements analyzed in the study
Hwang et al..,	2010	Korea	Korean	20	28.1	Computerized 3D CT images	Alphard Vega; Asahi Roentgen Co., Kyoto, Japan	Department data base	31 landmarks (10 midline and 21bilateral) were identified according to De Greef et al..,
Zacharias Fourie et al.,	2010	Netherlands	Netherlands	7	-	Computerized 3D CT images	KaVo 3D exam (KaVo Dental GmbH, Bismabring, Germany) CBCT scanner	Cadaver	Facial soft tissue thickness at 11 different sites (soft tissue landmarks were measured)
Won-Joon Lee	2012	Korea	Korea	3	28.4	Combination methods	Alphard Vega; Asahi Roentgen Co., Kyoto, Japan	3 student volunteers	the deviation errors between the reconstructed and target faces were measured.
Wuyang Shui	2019	China	Han Chinese	140	-	Computerized 3D CT images	A Konica Minolta VIVID 910 laser scanner	140 living individuals (70 females and 70 males)	skull digitization, geometric measurements, sex classification and computerized CFR
Yang Wen et al..,	2020	China	Han Chinese	200	17-75	Region fusion strategy	CT scanner	Volunteers	-
Clemente Maia S. Fernandes	2012	Brazil	Brazilian female	1	-	Computerized 3D CT images	-	Volunteer	10 midline points and 11 bilateral points
Geraldo Elias Miranda et al..,	2017	Brazil	Brazilian Caucasian	4	21-49	Computerized 3D CT images	-	4 volunteers donated existing CT Data	Geometric comparison of the CCFR to the subject 3D face model (obtained from the CT data)

1. CR-GAN (Craniofacial Reconstruction using Generative Adversarial Networks) [33]

This method is used for personal identification. The method aims to reconstruct craniofacial images from 2D CT scans of skulls, which can be used for identifying human remains when other data (e.g., fingerprints, DNA) are unavailable.

CR-GAN uses deep learning, specifically GANs, to translate skull images into facial images. It incorporates conditional codes for age and gender to improve accuracy. The model was trained on 4551 paired skull-face images from 1780 CT head scans of the Han Chinese population. The method was evaluated using five deep learning face recognition algorithms, achieving high accuracy in identifying reconstructed faces. The paper presents a different method for craniofacial reconstruction using 2D CT scan skull images. This technique is crucial for identifying human remains when other data like fingerprints or DNA are unavailable. The core of the method is based on GANs, specifically designed to translate 2D skull images into related craniofacial images. This approach apprehends the multifaceted relationship between the skull and face.

To improve accuracy, the model uses conditional codes for age and gender, ensuring that the reconstructed faces uphold accurate age and gender characteristics. The method was verified using five profound learning-based face detection algorithms, resulting high accuracy in recognizing reconstructed faces from a vast dataset.

Advantages:

The method can use partial head CT scan data, making it easier to collect training data. The model attains high accuracy in face recognition tasks, with a top-1 accuracy of 80.39% and top-5 accuracy of 94.12%. The technique is completely automated, reducing the necessity for manual interference and rising reproducibility. Exploits a huge dataset of 4551 paired skull-face images, enhancing the model's training and performance.

Disadvantages:

Initial versions of the model demonstrated spot-like leftovers in the reconstructed images, which required supplementary techniques to exclude. The method involves complicated deep learning algorithms and needs significant resources. The model was primarily guided on the Han Chinese population, which may limit its generalizability to other traditional groups.

2. Face Warehouse and Celebrity Face Recognition datasets [34]

The paper uses regression trees to align facial landmarks from 2D images. Two structures were tested: a 68-landmark and a 74-landmark structure. The 68-landmark structure showed greater accuracy. These models are used to create 3D facial reconstructions from the aligned landmarks. The Surrey Face Model was used for this purpose. The accuracy of the models was evaluated using Root Mean Square, 75th Percentile, and Arithmetic Mean metrics. The 68-landmark regression tree produced better results. The Face Warehouse and Celebrity Face Recognition datasets were used for preparation and evaluation The study uses regression trees for facial landmark alignment and 3D morphable models to reconstruct 3D facial images from 2D portraits. The 68-landmark regression tree reached higher accuracy (85% and 90%) compared to the 74-landmark regression tree (82% and 83%)

Advantages:

The 68-landmark regression tree completed higher accuracy (85% and 90%) compared to the 74-landmark tree (82% and 83%). The method is efficient, requiring only a single 2D image for reconstruction. Applicable in various fields like facial rehabilitation, forensic investigations, and locating missing people.

Disadvantages:

The process is intensive, especially during the training phase. The accuracy heavily depends on the quality and type of input images and landmarks used.

Table 8: Accuracy and Precision Results of each model set.

Name of Model Set	Accuracy	Precision
FW – 74-RT	82%	645 %
FW – 68-RT	85%	71%
Celeb – 74-RT	83%	69%
Celeb – 68-RT	90%	79%

1. Multi-PIE and ColorFERET databases [35]

The paper offers a regression-based method to reconstruct textured full 3D face models from multi-view mugshot images, improving the accuracy and effectiveness of face recognition techniques. The method uses both linear and nonlinear regressors to reconstruct 3D face shapes and an effective texture recovery system to map the texture from mugshot images to the 3D face shape. The mentioned method significantly reduces the 3D face reconstruction error and improves recognition accuracy compared to existing methods, as proven through experiments on various databases. The reconstructed 3D faces are used to generate realistic multi-view face images, enhancing the gallery for arbitrary view face recognition and improving the performance of advanced deep learning-based face matchers The method uses linear and nonlinear regressors to reconstruct 3D face shapes from frontal and profile mugshot images. This approach effectively uses the complementary information from multiple views. The texture from the mugshot images is mapped onto the reconstructed 3D face shape using an all-in-one texture recovery scheme, ensuring accurate and detailed facial textures. The method was assessed on several databases, showing better-quality 3D face reconstruction accuracy and improved face recognition performance compared to existing methods.

Advantages:

The method lowers 3D face reconstruction error from 2.31mm to 1.88mm. It improves recognition accuracy by up to 4% on multi-PIE and 2% on ColorFERET databases. Uses 2D face images, which are more cost-effective than 3D scanners.

Disadvantages:

Some methods may fail if input face images are not standardised. The method may show problems with recovering fine facial details, mainly under large perspective changes.

Rank-1 identification rate (%) for different methods at different poses of probe images on Multi-PIE. The best results at each pose are highlighted in bold.

Method	Pose of Probe Images						Average
	±90°	±75°	±60°	±45°	±30°	±15°	
TP-GAN [40]	64.0	84.1	92.9	98.6	99.9	99.8	89.9
3D-PIM [43]	76.1	94.3	98.8	99.3	99.5	99.8	94.7
PIM [44]	75.0	91.2	97.7	98.3	99.4	99.8	93.6
Original Gallery	65.4	88.6	98.3	**99.8**	**100.0**	**100.0**	92.0
Ours without Fusion	66.2	86.0	97.0	99.5	99.9	99.9	91.4
Ours with Fusion	**81.5**	**94.1**	**99.1**	**99.8**	**100.0**	**100.0**	**95.8**

2. Photogrammetry and Blender + MakeHuman software [36]

The paper states on the successful application of 3D forensic facial approximation using free software in a real forensic case in Brazil. The process involved photogrammetry to create a 3D model of the skull, followed by facial reconstruction using Blender and MakeHuman software. The reconstructed face helped narrow down the search for the victim's identity, leading to faster DNA confirmation and reducing the number of genetic tests needed. Although not a primary identification method, 3D facial approximation is valuable in forensic investigations for individual recognition and reducing the number of potential victims. The skull was scanned using photogrammetry to create a full 3D model. This involved taking 22 digital photographs from various different angles and processing them to generate a 3D point screen and mesh. Free software like Autodesk 123D, MakeHuman, and Blender were used for image processing, model creation, and facial approximation. This approach is cost-effective and accessible. The digital replica of the skull was aligned with soft tissue markers, and individualized facial features were modelled and sculpted in Blender. The final face was rendered for recognition. The reconstructed face was compared with images in a civil identification database, leading to the identification of the individual through DNA testing.

Advantages:

Uses open-source software, reducing costs associated with exclusive tools. Helps narrow down possible matches, speeding up the identification process. Produces 3D facial reconstructions with reliable levels of accuracy and similarity.

Disadvantages:

Should be supported by other methods like DNA or dental records. Depend on the individual assessment of the observer, which can introduce bias.

3. Statistical Shape Model (SSM) [37]

The paper presents a system called CFRTools, which uses a statistical shape model (SSM) for craniofacial reconstruction, improving accuracy compared to traditional methods. The technique achieves a 97.14% accuracy rate in sex classification using skull SSM and centroid size, rather than geometric measurements. A fusion registration algorithm improves the accuracy of skull registration by aligning a template skull with the unidentified skull. The system allows for interactive face editing, enabling experts to modify and refine the reconstructed face based on their knowledge and experience. The method selects a geometrically similar template skull from a dataset to improve registration accuracy. Uses a hybrid registration algorithm combining ICP and NICP to align the template skull with the unidentified skull. Employs SSM and centroid size for sex classification, achieving a high accuracy rate of 97.14%. Utilizes a regression-based approach to reconstruct the face, allowing for multiple variations by adjusting PC coefficients

Advantages:

The computerized approach is faster and more flexible compared to traditional manual methods. The use of statistical shape models (SSM) and non-rigid registration improves the accuracy of skull registration and face reconstruction. High accuracy in sex classification (97.14%) using skull SSM and centroid size. Allows experts to create multiple variations of reconstructed faces, enhancing the usability for forensic purposes.

Disadvantages:

Requires sophisticated computer-based techniques and a large dataset of skull and face models. The accuracy of the reconstruction heavily depends on the quality of the digital skull generated from CT or laser scanning. Users need anatomical knowledge and skills in modelling software to effectively use the system.

4. Face-pool comparison and Geometric surface comparison [38]

The study examines how different average facial soft tissue depth datasets effect the accuracy of craniofacial reconstructions using two accuracy assessment methods: face-pool comparison and geometric surface comparison.

Face-Pool Comparison: This method involves matching a target craniofacial reconstruction (CFR) to a variety of faces in a photograph collection. Evaluators select the face they think resembles to the CFR. The recognition rates are then calculated based on the assessors' choices.

Geometric Surface Comparison: This method uses 3D modelling software to align CFRs with actual facial scans in a computer-generated space. Differences in skin thickness are measured and visualized as deviation charts. The accuracy is measured by the percentage of the CFR surface that differs from the actual facial scan by no more than ±2.5 mm.

Six craniofacial reconstructions were created using 3D modelling software from 3D skull images of live Korean adults . These were divided into two groups based on previous and recent facial soft tissue thickness datasets.

The recent dataset presented a higher recognition rate and better quantitative accuracy compared to the previous dataset. A positive correlation was found between the two accuracy assessment methods. The study suggests that using appropriate averaged facial soft tissue depth datasets improves the accuracy of craniofacial reconstructions, and that geometric surface comparison can be an alternative to face-pool comparison for evaluating accuracy.

Advantages: Using recent datasets significantly improves the accuracy of craniofacial reconstructions. Higher recognition rates were achieved with updated datasets, enhancing identification reliability.

Disadvantages: The face-pool comparison method is time-consuming and involves multiple steps, including acquiring and selecting facial photographs. Requires advanced 3D modelling software and cone-beam computed tomography, which may not be accessible to all researchers.

Table 7: Results of the geometric surface and face-pool comparative methods

CFR		Geometric surface comparison (%)*	Recognition rate in face-pool comparison (%)
Lee *et al.* (2012) (outdated FSTT)	CFR-A	54.3	23.2
	CFR-B	64.6	34.2
	CFR-C	76.7	9.2
	Mean	65.2	22.2
Lee *et al.* (2015) (recent FSTT)	CFR-D	87.5	26.2
	CFR-E	79.3	45.1
	CFR-F	86.8	20.7
	Mean	84.5	30.7

*Geometric surface comparison: Distribution (%) of the deviation error (minimum range within 2.5 mm) between the surfaces of the CFR and the face of the corresponding subject

5. Regression equations using CT-based 3D models [39]

The study aims to develop regression equations to predict nasal profiles using CT-based 3D models, considering sex and age factors. Researchers analysed craniofacial models from 389 Korean adults, using 18 measurements from 16 craniometric landmarks. Multiple regression equations incorporating sex and age provided better predictions of nasal profiles than those considering only sex.

These equations are useful for forensic facial approximation and can aid in human identification and facial reconstruction surgeries The study evaluated head CT images of 389 Korean adults (188 males and 201 females) to measure nasal profiles using 3D craniofacial models. Simple and multiple linear regression analyses were conducted to develop equations predicting nasal profiles based on the piriform aperture, considering sex and age. The accuracy of the regression equations was tested using a separate set of 30 Korean cadavers, showing improved predictability when both sex and age were included. The study found that including sex and age as independent variables improved the accuracy of predicting nasal profiles. The equations had coefficients of determination (R^2) ranging from 0.314 to 0.724, indicating varying levels of prediction accuracy. The models were validated using a test set, showing no significant differences between measured and predicted values for certain variables.

Advantages:

Utilizes CT-based 3D craniofacial models, providing more accurate and detailed measurements compared to 2D images. Employs multiple regression analyses considering sex and age, improving the prediction accuracy of nasal profiles. Includes a wide age range and both sexes, enhancing the applicability of the findings.

Disadvantages:

The study is based on a specific population (Koreans), which may limit the relevancy to other ethnic groups. Measurements were taken from horizontal position CT scans, which might not perfectly reflect the natural standing position of facial tissues.

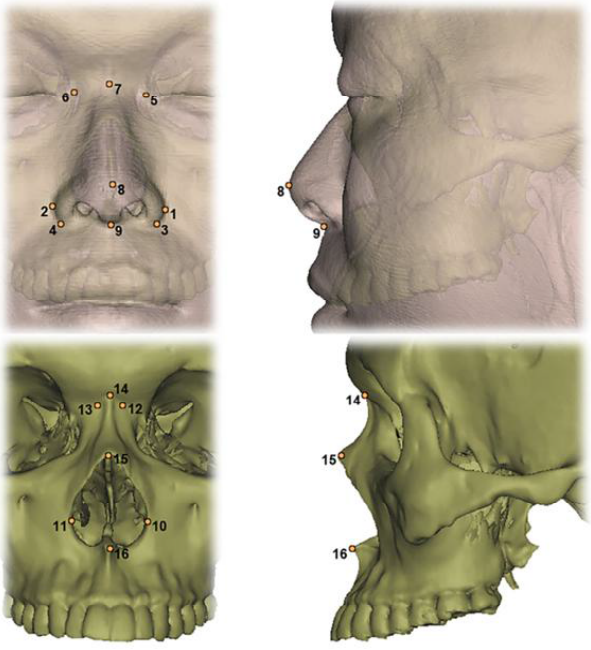


Fig. 2. Anatomical landmarks of soft and hard tissue of nose. 1, Left alare; 2, Right alare; 3, Left alar base; 4, Right alar base; 5, Left maxillofrontale; 6, Right maxillofrontale; 7, Sellion; 8, Pronasale; 9, Subnasale; 10, Left bony alare; 11, Right bony alare; 12, Left maxillofrontale; 13, Right maxillofrontale; 14, Nasion; 15,

Table 3
Equations conducted from multiple regression analysis for predicting nasal profiles.

Equations	Correlation (r)	Adjusted R^2	SEE
For known sex and unknown age			
C-01 = 0.89 (P-03) + 0.84 (Sex[a]) + 4.05	0.840	0.704	2.339
C-02 = 0.10 (P-03) + 3.08 (Sex[a]) + 17.66	0.617	0.377	2.291
N-01 = 0.86 (P-01) + 3.18 (Sex[a]) + 15.96	0.707	0.498	2.937
N-04 = 0.64 (P-03) + 1.49 (Sex[a]) + 14.69	0.638	0.404	3.532
N-05 = 0.63 (P-03) + 1.63 (Sex[a]) + 7.94	0.661	0.434	3.322
For known sex and age			
C-01 = 0.90 (P-03) + 0.80 (Sex[a]) + 0.39 (Age group[b]) + 2.46	0.852	0.724	2.260
C-02 = 0.10 (P-03) + 3.10 (Sex[a]) − 0.17 (Age group[b]) + 18.36	0.624	0.384	2.278
N-01 = 0.73 (P-01) + 3.32 (Sex[a]) + 0.57 (Age group[b]) + 17.64	0.736	0.537	2.818
N-03 = 0.75 (P-02) + 0.26 (Sex[a]) − 1.21 (Age group[b]) + 17.60	0.565	0.314	3.695
N-04 = 0.65 (P-03) + 1.47 (Sex[a]) + 0.16 (Age group[b]) + 14.06	0.640	0.406	3.529

SEE: Standard Error of Estimate. a = 0 for female and 1 for male. b = 1 for 20–29 yrs, 2 for 30–39 yrs, 3 for 40–49 yrs, 4 for 50–59 yrs, 5 for 60–69 yrs, and 6 for over 70 yrs. All equations were statistically significant at the level of 0.001.

6. GP-LVM and LSSVR [40]

The paper proposes a new method for craniofacial reconstruction using a region fusion strategy to estimate a person's face model from their skull. The skull and face are divided into five regions. Each region is plotted to a low-dimensional latent space using the Gaussian process latent variable model (GP-LVM). The least square support vector regression (LSSVR) model is then trained to establish the plotting relationship between skull and face regions. The method also incorporates characteristics like age and BMI for more detailed reconstructions. This segmentation helps in managing the complexity of the reconstruction process by focusing on smaller, more manageable areas. This step reduces the dimensionality of the data while preserving essential features. The Least Square Support Vector Regression (LSSVR) model is chosen for its ability to handle non-linear relationships and provide accurate predictions. The reconstructed regions are fused together to form a complete facial model. This fusion strategy ensures that the individual regions align correctly to produce a coherent and realistic facial appearance.

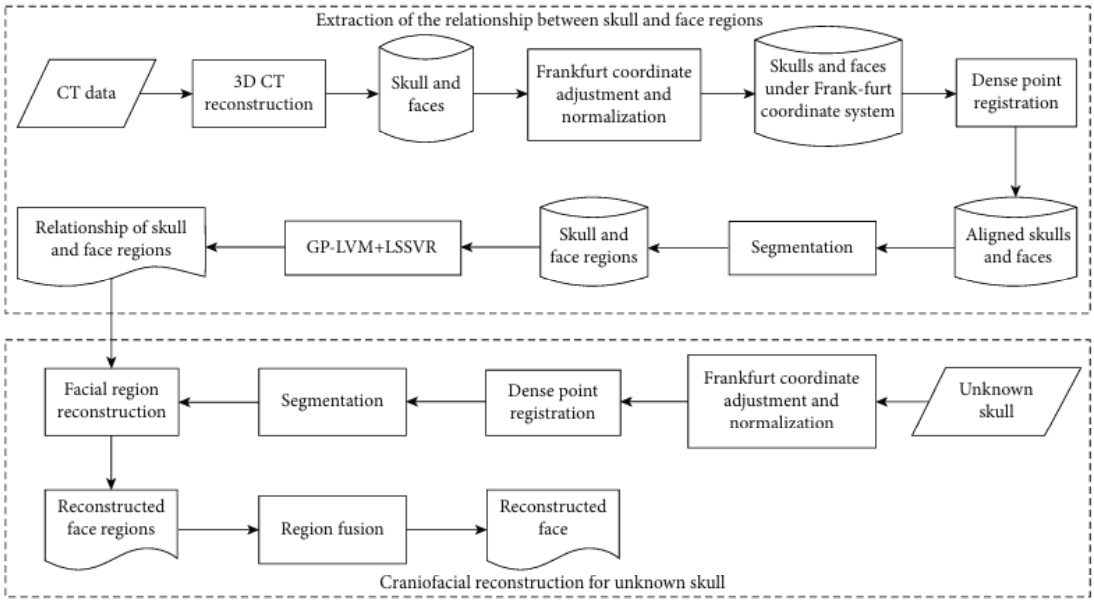
Advantages:

The use of GP-LVM and LSSVR allows for precise recording between skull and face regions, leading to high accuracy in the reconstructed faces. The region-based approach provides flexibility in handling different parts of the face separately, which can be particularly useful in dealing with complex cases. The method can incorporate additional attributes like age and BMI, enhancing the realism and detail of the reconstructions.

Disadvantages:

The method involves multiple steps, including segmentation, recording to latent space, and regional fusion, which can be intensive and complex to implement. The accuracy of the method heavily relies on the quality and quantity of the training data. Inconsistent or insufficient data can lead to less accurate reconstructions. Errors in individual regions can affect the overall reconstruction quality, especially if the frame region has significant inaccuracies. Although the method aims to

minimize manual intervention, some steps still require manual adjustments, which can introduce



7. 3D Scanners and Softwares [41]

The study explores the use of 3D technology for the reconstruction and remodelling of fragmented and missing elements of human skeletal remains, focusing on two forensic case scenarios. Utilized 3D laser scanning and 3D printing to digitally reconstruct missing and fragmented skeletal parts, specifically the zygomatic process and mandible. The reconstructed models were accurate based on anatomical features and digital analysis, demonstrating the potential of 3D technology in forensic practice. 3D reconstruction offers a non-invasive, accurate method for skeletal analysis, beneficial for forensic investigations and court presentations. The study utilized the NextEngine® 3D Laser Scanner and Faro® 8-Axis Design ScanArm 2.5C Laser Scanner for capturing detailed digital scans of skeletal remains. These scanners provide high accuracy and resolution for digital restoration. The Geomagic Studio 13® software was employed for precise reproduction of surfaces. This involved defining objects, manual alignment, global registration, replacement of missing elements, and bridging gaps using various methods. The reconstructed models were printed using a Flashforge™ Guider 2 3D printer. This printer uses Fused Deposition Modelling (FDM) technology to create accurate physical models from the digital reconstructions .The reconstructed models were compared with original data and literature to ensure accuracy, with an overall morphological error of 1.5501 ± 2.00 mm.

Advantages: High precision in reconstructing skeletal remains, ensuring anatomical correctness. Negligible physical contact with remains, reducing the risk of damage and contamination. Applicable to various forensic scenarios, including court presentations and trauma analysis.

Disadvantages: Requires significant digital skills and anatomical knowledge. High cost of 3D scanning and printing equipment. Potential for minor errors in reconstruction, though generally within acceptable limits.

8. MSCT imaging [42]

The study aimed to reconstruct the appearance of Saint-Nicolosa Bursa using a 3D printed skull model based on MSCT imaging. This method was validated by comparing the reconstructed face with the mummy's remains. The Manchester method was used, combining the Russian and American techniques. This involved layering clay on a 3D printed skull model to recreate facial features based on anthropometric data. MSCT was used to scan the mummified remains of Saint-Nicolosa Bursa to create a detailed 3D model of the skull. The body was scanned in two parts due to its length, using specific parameters to ensure high-resolution images. The images were processed to visualize the body, extract the head, and eliminate artifacts. The 3D model was then printed and used for facial reconstruction This non-invasive method was crucial for preserving the remains while allowing detailed analysis and presentation of the findings.

Advantages:

The Manchester method combines both Russian and American techniques, providing a more accurate facial reconstruction. Using MSCT imaging and 3D printing avoids damaging the mummified remains. This method allows for realistic models that can be used for exhibitions and presentations, enhancing public engagement.

Disadvantages:

Facial reconstruction is still an approximation and may not perfectly represent the actual appearance. The process is complex and requires careful planning, especially when dealing with sensitive materials like mummified remains.

Fig. 5. a: forensic facial reconstruction; b: photography of the mummified remains and; c: MSCT image of Saint-Nicolosa Bursa.

9. ABS filament and Ultimaker Software [43]

The study explores the use of 3D printing for forensic facial reconstruction, creating accurate skull replicas from CT scans. ABS filament was used due to its rigidity and surface quality. The printing process was optimized using rheological data and slicing software. The skull was scanned using X-ray computed tomography (CT) to create a 3D model in the form of a Stereo Lithography (STL) file. The 3D model was sliced using Ultimaker Cura software, optimizing the print position to ensure a larger contact surface and minimize defects. The model was sliced with a layer thickness of 0.2 mm and the optimal position for printing was determined using this software. The melt flow rate and shear viscosity of the ABS filament were analysed to determine optimal printing temperatures and ensure good printability. The skull was printed at a 1:1 scale using an in-house designed 3D printer with specific parameters, resulting in an accurate replica for facial reconstruction.

Advantages:

The 3D printed skulls provide detailed anatomical features, such as eye and brain cavities, which are superior to traditional gypsum casts. The method avoids handling the original skull, reducing the risk of damage to forensic evidence. 3D printing allows for the creation of complex geometries and is highly customizable.

Disadvantages: The surface of the 3D printed skull is rougher compared to gypsum casts, which might affect the final reconstruction. Issues like warping and layer delamination can occur, requiring careful optimization of printing parameters.

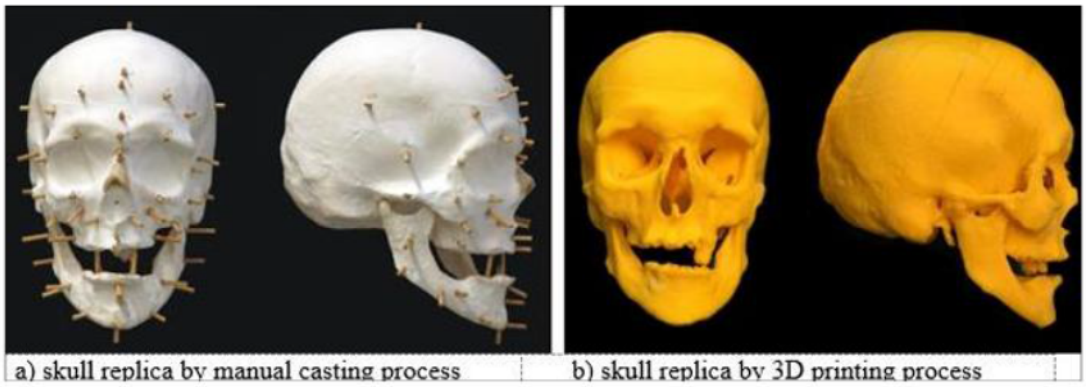


Figure 10. Replicas of the skull at 1:1 scale

4. Sassouni cephalometric analysis [44]

The study aimed to determine if the Sassouni cephalometric analysis could predict the two-dimensional shape of the human mandible using cephalometric planes and landmarks. A retrospective analysis of 100 lateral cephalometric radiographs was conducted, focusing on the positions of gonion and pogonion A retrospective observational exploratory study using 100 lateral cephalometric radiographs from Kingston Hospital Orthodontic Department. The Sassouni cephalometric analysis was adapted to estimate the positions of cephalometric points gonion (Go) and pogonion (Pog). Digital tracing of cephalometric points using Dolphin Imaging Plus™ software, with measurements taken for both linear and angular dimensions. The method showed reasonable accuracy in estimating the positions of Go and Pog, particularly in skeletal class I cases.

Advantages:

The method shows reasonable accuracy in estimating the positions of cephalometric points like gonion and pogonion, especially in skeletal class I cases. Useful in craniofacial anthropology, forensic science, and archaeological reconstruction for predicting mandibular shape.

Disadvantages:

The method is based on two-dimensional radiographic imaging, which may not capture the full complexity of the mandible's three-dimensional shape. The study's sample may not be representative of the general population, as it includes individuals with more complex malocclusions.

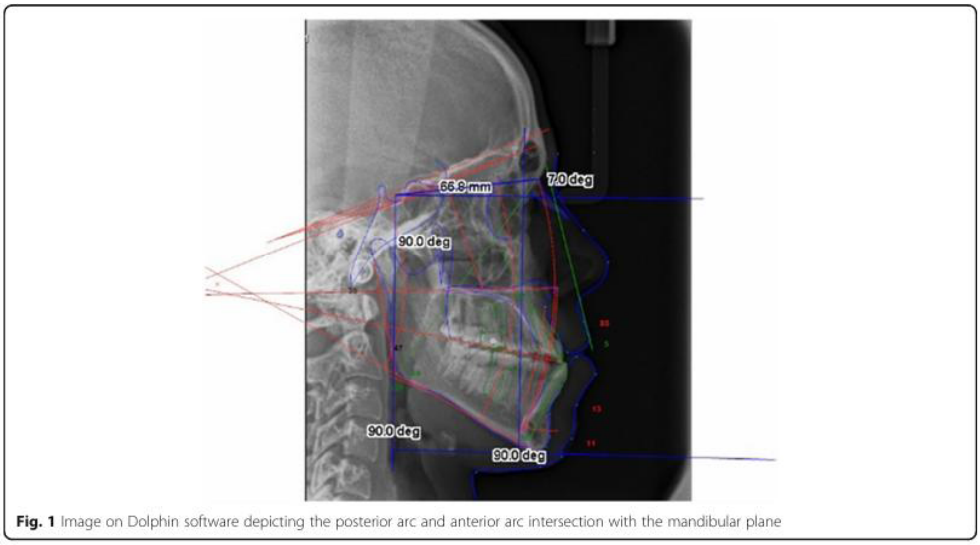


Fig. 1 Image on Dolphin software depicting the posterior arc and anterior arc intersection with the mandibular plane

5. Photogrammetry and Zbrush Software [45]

The study compares the accuracy of photogrammetry and CT scans in creating 3D models of skulls for forensic facial approximation. Photogrammetry involved taking multiple photographs of skulls and using software to create 3D models. These were compared to CT scan-based models. Photogrammetry slightly overestimated measurements compared to CT scans, but the differences were minimal, indicating photogrammetry's potential as a cost-effective alternative. Photogrammetry is highlighted as a useful tool in forensic science, especially when CT equipment is not accessible. Photogrammetry involved taking multiple photographs of skulls from different angles and processing them with Zephyr Lite software to create 3D models. These were compared to CT scan models using ZBrush software.

Zephyr Lite (3DFlow©) is a photogrammetric software used to create three-dimensional meshes from photographs. The software processes images to generate a dense point cloud, which is then used to create a 3D mesh . It performs automatic camera calibration and allows for manual adjustments. Users can choose from various categories (e.g., aerial photographs, close objects) and presets (e.g., fast, default, deep analysis) to optimize the processing. The final 3D mesh can be exported in .obj format, compatible with other 3D graphic software like ZBrush .

Advantages: Photogrammetry is cheaper and easier to use than CT scans. It does not involve radiation, making it safer for repeated use. Requires only a camera and software, making it more accessible.

Disadvantages: Photogrammetry tends to slightly overestimate measurements compared to CT scans. The accuracy can be affected by errors in the photographic process or software settings.

TABLE 3—*Results obtained from lateral and frontal measurements.*

Frontal												
Skull	ct ec-ec	ph ec-ec	ct d-d	ph d-d	ct go-go	ph go-go	ct ft-ft	ph ft-ft	ctfmt-fmt	ph fmt-fmt	ct n-pr	ph n-pr
14	92.6811	91.9091	22.7598	22.4602	92.7012	92.0106	111.4329	111.552	107.2287	107.6321	66.0727	66.6043
37	108.3795	105.9286	25.2019	25.6567	123.3012	122.4202	114.9559	113.8023	126.2641	124.8753	80.4972	80.2398
41	85.6925	87.4669	21.9719	22.9479	102.0271	102.9694	92.8701	94.018	104.369	105.241	70.1595	71.2905
42	91.1032	90.6583	24.5534	24.1376	86.1548	86.8723	95.1626	95.1208	100.9073	101.3738	57.1869	57.0273
54	92.247	92.996	21.2699	20.4665	104.5393	107.6605	81.6164	82.379	105.0731	106.026	82.5426	83.9895
57	87.62	93	35.02	40.42	112.65	111.33	93.87	100.13	112.31	118.23	76.77	82.01
58	99.2075	100.9338	27.4638	26.1735	107.8923	108.7616	105.8364	108.4415	113.6556	115.0095	72.6061	75.7914
59	97.9	102.4	28.93	29.2	119.9	124.3	129.44	131.6	130.78	135.6	59.54	62.88
Lateral												
Skull	ct b-g	ph b-g	ct n-ns	ph n-ns	ct gn-go	ph gn-go	ct pr-id	ph pr-id	ct id-gn	ph id-gn	ct b-gn	ph b-gn
14	111.3519	110.2155	48.6057	49.2696	79.1415	80.2536	23.1494	20.0144	25.5775	25.9097	214.9885	215.2413
37	128.1625	132.3508	64.9974	64.2338	104.0897	103.3116	25.1322	24.8956	31.8695	30.3281	255.0487	253.4209
41	107.8004	108.4174	50.652	50.4955	88.1708	89.2825	23.4131	23.2133	29.8	29.2884	210.5557	209.6244
42	108.6963	109.6506	42.2387	42.7226	76.9255	78.7318	28.7188	25.1795	24.5464	24.297	206.06	206.5294
54	104.7532	103.7884	57.2649	59.8809	98.8868	101.9661	30.2792	30.4315	29.0934	19.6161	217.2921	216.7931
57	102.77	104	61.5	69.03	89.92	100.66	30.01	27.9	28.53	29.24	205.12	210.9
58	117.4821	115.8324	57.284	57.6336	86.3814	85.0352	33.9892	21.8468	28.2351	30.911	221.3923	219.7924
59	97.9	100.2	57.16	52.47	0	0	0	0	30.5	28.53	220.9	217.21

FIG. 3—*Bland–Altman graph showing frontal measurements results.*

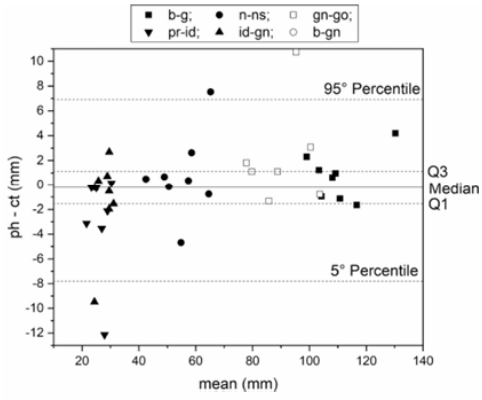


FIG. 4—*Bland–Altman graph showing lateral measurements results.*

6. Deep Convolutional Generative Adversarial Networks (DCGAN) [46]

The paper discusses using Deep Convolutional Generative Adversarial Networks (DCGAN) to convert forensic sketches into realistic facial images, aiding in crime investigations. GAN Architecture: It explains the Generative Adversarial Network (GAN) architecture, where the generator creates fake images and the discriminator distinguishes between real and fake images.

Various methods and datasets used for sketch-to-face transformation are reviewed, highlighting the accuracy and limitations of each approach. The paper suggests that more advanced GANs could further improve the efficiency of facial recognition and verification systems It describes how DCGAN can generate high-resolution, photorealistic images from low-quality sketches by training neural networks with forensic sketches as input. This technique is particularly useful in forensics, law enforcement, facial recognition systems, and security The generator creates images from sketches, while the discriminator differentiates between real and generated images. DCGAN is used to convert hand-drawn forensic sketches into high-resolution, realistic facial images, aiding in criminal identification.

Advantages:

The method can generate realistic facial images from low-quality sketches, improving crime investigation efficiency. Reduces human intervention and speeds up the process of image enhancement and recognition. Useful in various domains like forensics, law enforcement, and security systems.

Disadvantages:

Requires extensive training and resources. GANs can face issues with non-convergence, making the training process challenging. Performance may degrade with significant deviations in input sketches, such as different poses or lighting conditions.

Author	GAN model	Dataset	Performance	Drawbacks
Jian Zhao etal.	Generator has two networks i)face extraction network ii)face reconstruction network	Celebrity Face Attributes (CelebA)	Accuracy in terms of (PSNR) / SSIM is 16.306.	Used only with skip connections
Wengling etal.	Sketchy GAN	TU-Berlin dataset contains seventy five thousand real existing human sketches paired with photos and edge maps	Accuracy is compared with MRU, CRN, ResNet and DCGAN among this MRU performs better than all other models	Faithfulness and Realism test results are not photo realistic
Jun etal.	Composition–aided GANs (CA-GAN) and stacked CA-GANs (SCA-GAN)	CUHK, CUFS with 606 photos and CUFSF with 1194 face photos.	Accuracy is 99.7 %.	It does not give considerable accuracy if photos/sketches are with great deviations.
Mingming etal.	The colour image is altered into face sketch image by using xDOG filter	Dataset: Celeb A with 202,599 celebrity images, taking 40 binary attributes.	Good performance.	Difficult facial attributes is not considered.
Chaofeng etal.	Semi-supervised learning method	Datasets: the CUFS (consisting of the CUHK student dataset, the AR dataset, and the XM2VTS dataset) and the CUFSF are used.	Good accuracy.	Matching the features with input image and small set of photo-sketch pairs.
Shengchuan etal.	pGAN	CUFSF, CUHK face sketch dataset (CUFS), the CUHK face sketch FERET (CUFSF) dataset.	pGAN's accuracy for NLDA is 82.93% for CUHK dataset and FSIM Accuracy for AR is 73.02%, for XM2VTS is 67.32%.	Time-consuming, which restricts its real-world use in effective circumstances.
Lisa etal.	CGAN (Conditional Generative Adversarial Neural Network).	Sketchy Database large-scale collection of sketch-photo pairs created by Georgia Tech. It contains 12,500 images from 125 categories from ImageNet.	Evaluated by inception scores 79.05% for ground truth photos	Needs to work on it to fully understand the potential of this approach.

7. Database Forensic Investigation Metamodel (DBFIM) [47]

The paper aims to validate the Database Forensic Investigation Metamodel (DBFIM) using the qualitative face validity method to ensure its applicability in the field of database forensic investigation (DBFI). Six experts were selected to validate the DBFIM through structured interviews. The experts assessed the metamodel's completeness, coherence, logicalness, scalability, interoperability, and usefulness. The experts found the DBFIM to be complete, coherent, logical, scalable, interoperable, and useful for the DBFI field. Some modifications were suggested to improve the metamodel.

The validated DBFIM is considered a valuable tool for the DBFI field, with future work suggested to implement it in real scenarios to further ensure its applicability. Structured interviews were conducted to gather their feedback on the metamodel. The DBFIM helps practitioners derive solution models for DBFI, making the investigation process more systematic and standardized. Based on expert feedback, the DBFIM was refined to include new concepts and relationships, enhancing its applicability and effectiveness in real-world scenarios.

Advantages:

Ensures the model is reviewed by knowledgeable individuals, enhancing its credibility. Provides detailed feedback on the model's applicability, completeness, and usefulness . Validates the model's real-world applicability and logical coherence.

Disadvantages:

Relies on the opinions of selected experts, which can introduce bias. Typically involves a small number of experts, which may not represent the broader field. Conducting and analysing expert interviews can be lengthy and resource-intensive.

8. 3D Morphable Model (3DMM) [48]

The paper explores five main techniques for 3D face reconstruction: deep learning, epipolar geometry, one-shot learning, 3D morphable model, and shape from shading methods. It highlights challenges such as occlusion removal, makeup removal, expression transfer, and age prediction.

The paper provides a detailed performance analysis of different techniques in terms of software, hardware, pros, and cons . The authors discuss the future scope and potential applications of 3D face reconstruction, including facial puppetry, video dubbing, and virtual makeup .

3D Morphable Model (3DMM): This method generates facial appearances and shapes using dense point-to-point correspondence1. It is effective but can struggle with large pose variations and occlusions. Advantages: Good at disentangling facial colour and shape; works well with dense correspondence. Disadvantages: Requires face registration; struggles with large pose variations.

Deep Learning-based Reconstruction: Techniques like 3DGANs and 3DCNNs offer high fidelity and accuracy but require significant training time and resources. They are also sensitive to occlusions and lighting conditions. Advantages: High fidelity and accuracy; handles various expressions and lighting conditions. Disadvantages: Time-consuming training; struggles with occlusions and extreme poses.

Epipolar Geometry-based Reconstruction: This approach uses multiple perspective images to create a 3D image. It provides good geometric fidelity but requires calibrated cameras and orthogonal images. Advantages: Good geometric fidelity; effective with multiple perspective images. Disadvantages: Requires calibrated cameras; struggles with occlusions.

One-Shot Learning-based Reconstruction: Utilizes a single image to recreate a 3D model3. It is quick to train but struggles with pose variations and generalization to videos. Advantages: Quick training; good generalization from single images. Disadvantages: Limited to single images; struggles with pose variations.

Shape from Shading-based Reconstruction: Recovers 3D shapes from shading and lighting cues4. It works well under specific lighting conditions but has difficulty with occlusions. Advantages: Good shape modelling from shading cues; effective under specific lighting. Disadvantages: Struggles with occlusions; dependent on predefined facial geometry knowledge.

Hybrid Learning-based Reconstruction: Combines various techniques to leverage their strengths. It offers improved performance but can be complex and resource-intensive.

Advantages:

Combines strengths of multiple methods; effective in various scenarios.

Disadvantages:

Complex implementation; dependent on synthetic data.

9. Conditional Generative Adversarial Networks (CGAN) [49]

The paper proposes a novel approach for 3D craniofacial reconstruction using Conditional Generative Adversarial Networks (CGAN) based on depth maps. This method treats craniofacial reconstruction as a recording problem from skull to face. The use of depth maps to represent 3D craniofacial shapes is highlighted. Depth maps include most craniofacial features and are easy to generate and apply to neural networks. The proposed model consists of a generator, discriminator, and classifier. The generator translates skull images to face images, the discriminator enhances the realism of the generated images, and the classifier ensures the generated faces match the correct body mass index class (BMIC).

The paper demonstrates the superiority of the proposed method through comparative experiments with existing end-to-end GANs, showing improved accuracy and realism in craniofacial reconstruction results. 3D craniofacial shapes are represented with depth maps, which include most craniofacial features and are easy to generate and apply to neural networks. An end-to-end CGAN-based neural network is designed, incorporating Body Mass Index Classes (BMIC) to improve reconstruction accuracy . The model is trained with paired craniofacial data to learn the complex nonlinear relationship between skull and face, showing accuracy and verisimilitude in results through comparative experiments.

Advantages:

The method uses depth maps and Conditional Generative Adversarial Networks (CGAN) to achieve high accuracy and retain high-frequency details in 3D craniofacial reconstruction. The use of 2D convolutional networks with depth maps simplifies the process and makes it more efficient. Incorporating Body Mass Index Classes (BMIC) improves the accuracy of facial geometry reconstruction.

Disadvantages : The method involves complex neural network architectures and requires significant resources . The accuracy of the model heavily depends on the quality and quantity of the training data.

10. 3D surface scanning (3DSS) [50]

The study introduces a novel digital technique using 3D surface scanning (3DSS) and rapid prototyping to reconstruct fractured teeth, aiming to improve forensic odontology practices.

The researchers used laser scanning and structured light scanning to create 3D models of fragmented teeth, which were then aligned and printed using stereolithography (SLA) and fused deposition modelling (FDM) techniques. The reconstructed models showed a morphological error variance of 0.0526±0.05 mm, indicating high precision and potential for various forensic analyses. This technique offers a non-destructive method for handling fragile dental remains, aiding in identification, DNA extraction, and forensic facial reconstruction. Ten teeth were fractured using a mortar and pestle. Fragmented pieces were scanned individually using a structured light scanner to create 3D models. The scanned pieces were aligned using Geomagic Studio software, and the reconstructed models were converted into STL files. The STL files were printed using poly lactic acid (PLA) material by fused deposition modelling (FDM) technology and photo polymerizing resin using stereolithography (SLA) technique.

Advantages:

The method achieves an accuracy range of ±0.05mm, making it highly precise for forensic analysis. Digital scanning and 3D printing allow for the reconstruction of fragile fragments without further damaging them. The reconstructed models can be used for various forensic analyses, including morphometric and metric analyses, aiding in identification and investigation.

Disadvantages:

Requires advanced technology and expertise, which may not be readily available in all forensic labs. The setup and materials for 3D scanning and printing can be expensive. The choice of printing material can affect the accuracy and usability of the reconstructed models.

Table 1. Linear odontometric measurements of the reference teeth and 3D printed replicas to evaluate (mm).

Crown-root dimensions		Maxillary central incisors					Maxillary lateral incisors				
		SP:1	SP:2	SP:3	SP:4	SP:5	SP:1	SP:2	SP:3	SP:4	SP:5
Crown length											
	N	11.71	11.15	12.40	10.95	11.21	9.10	9.86	9.79	8.51	8.43
	SLA	11.44	11.01	12.09	10.65	11.17	9.12	9.65	9.67	8.51	8.14
	FDM	11.49	11.06	12.27	10.21	11.05	9.07	9.77	9.58	8.76	8.35
Mesio-distal width at incisal edge											
	N	9.29	8.74	9.34	8.36	8.87	5.20	6.26	6.30	6.35	6.87
	SLA	9.30	8.68	9.29	8.23	8.78	5.13	6.24	6.32	6.29	6.66
	FDM	9.25	8.54	9.11	8.19	8.65	5.21	6.27	6.24	6.21	6.54
Mesio-distal width at cervix											
	N	7.67	6.92	5.73	5.91	6.12	4.80	4.44	5.18	4.47	4.69
	SLA	7.64	6.84	5.45	5.47	6.23	4.76	4.34	5.12	4.45	4.56
	FDM	7.64	6.67	5.21	5.34	6.11	4.83	4.43	5.21	4.32	4.72
Bucco-lingual width at incisal edge											
	N	4.34	2.28	2.47	2.07	3.18	2.15	1.38	2.12	2.25	2.43
	SLA	3.76	2.12	2.23	2.11	3.16	2.02	1.31	2.10	2.21	2.32
	FDM	4.02	2.34	2.43	2.12	3.21	2.10	1.24	2.09	2.16	2.41
Bucco-lingual width at cingulum											
	N	6.67	7.46	7.07	6.87	6.91	6.09	5.49	6.02	6.25	6.34
	SLA	6.63	7.34	7.01	6.65	6.85	6.04	5.34	6.04	6.19	6.21
	FDM	6.68	7.24	7.12	6.66	6.87	6.02	5.26	6.10	6.28	6.11
Root length											
	N	15.88	13.10	13.25	12.54	13.54	12.19	12.12	12.20	13.35	13.34
	SLA	15.33	13.12	13.22	12.50	13.28	12.08	12.12	12.24	13.36	13.38
	FDM	15.29	13.09	13.19	12.47	13.34	12.10	12.07	12.10	13.12	13.38

SP: specimen; N: natural tooth; SLA: tooth printed by stereolithography technique; FDM: tooth printed by fused deposition modelling technology.

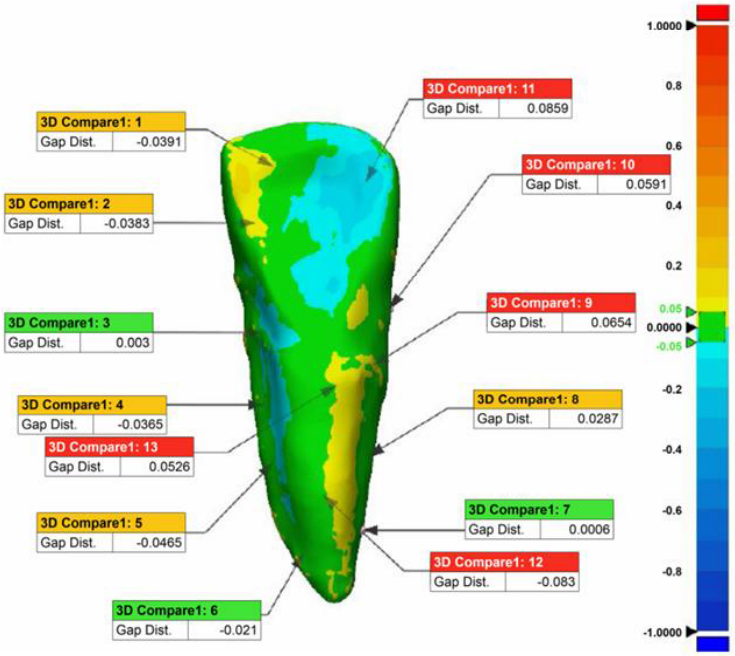


Figure 5. Qualitative congruency analysis performed on images of a reference tooth and a reconstructed tooth specimen.

11. Soft Tissue Thickness (STT) and Multi Detector row Computed Tomography (MDCT) [51]

The study aimed to collect soft tissue thickness (STT) values from 12 bone landmarks in an Italian population to improve facial approximation for identification purposes. Head CT scans of 100 Italian adults (50 males and 50 females) were analysed. Measurements were taken using a Multi Detector row Computed Tomography (MDCT) system. The study provides valuable data for forensic facial reconstruction, highlighting the importance of considering population-specific STT values . Measurements were taken from 100 Italian adults using head CT scans. Twelve osteological landmarks were used to measure STT. Significant differences in STT were found based

on sex and age. Females had higher values for the para-zygomaxillary landmark, while age differences were significant for the upper incisor and pogonion landmarks.

Advantages:

The study used CT scans taken for clinical purposes, avoiding additional radiation exposure. CT scans provide precise measurements of soft tissue thickness, crucial for forensic facial approximation. The study included 100 Italian adults, making it one of the largest datasets for this population.

Disadvantages:

The study lacked information on patients' BMI, which could affect soft tissue thickness. Some CT scans only included the upper half of the face, limiting the number of measurements for certain landmarks

12. Facial Soft Tissue Thickness (FSTT) and Cone Beam Computed Tomography (CBCT) [52]

The study aims to measure and compare facial soft tissue thickness (FSTT) in different Brazilian regions to determine if specific data sets are needed for forensic facial reconstruction. The researchers used cone beam computed tomography (CBCT) to measure FSTT in 101 subjects from the Midwest region and compared it to a previous sample from the Southeast. High compatibility was observed between the two regions, suggesting that different data sets for these regions are unnecessary. However, minor differences were noted, particularly in females. The study concludes that while regional differences exist, they are not significant enough to impact forensic facial reconstruction practices in Brazil. The study used 101 CBCT exams from Brazilian subjects in the Midwest region, ensuring no additional radiation exposure for participants. A standardized protocol was applied using Horos software to measure 32 craniometric landmarks. Data were analysed using SPSS with tests for normality, sex differences, and age-related changes. FSTT measurements were compared between the Midwest and Southeast regions of Brazil to assess regional differences.

Advantages:

The use of cone beam computed tomography (CBCT) ensures consistent and reliable measurements. The study found high compatibility in facial soft tissue thickness (FSTT) between different Brazilian regions, suggesting a single data set may be sufficient.

Disadvantages: Minor variances in FSTT were observed, which might not significantly impact forensic facial reconstruction. The study was limited to 101 subjects, which may not fully represent the diverse Brazilian population.

Table 3. Mean FSTT (millimeters and percentage), SD, and SE divided by sex.

	Female			Male					
	Mean	SD	SE	Mean	SD	SE	Mean difference (mm) **	Mean difference (%) ***	*P* value
Supraglabella	**4.5**	0.97	0.14	**5.5**	1.14	0.23	-1	18.1	0.00* T
Glabella	**4.9**	0.77	0.1	**5.9**	0.95	0.14	-1	16.9	0.00* T
Nasion	**6.2**	0.97	0.13	**8.1**	1.24	0.18	-1.9	23.4	0.00* T
Rhinion	**1.7**	0.43	0.06	**2.3**	0.58	0.09	-0.5	26.0	0.00* M
Mid-Philtrum	**13.1**	1.82	0.24	**15.7**	2.22	0.33	-2.7	16.5	0.00* T
Prosthion	**10**	1.58	0.21	**13.3**	2	0.3	-3.3	24.8	0.00* T
Infradentale	**9.8**	1.34	0.18	**11.9**	1.5	0.22	-2.1	17.6	0.00* T
Supramentale	**11.9**	1.53	0.2	**12.9**	1.85	0.28	-1.1	7.7	0.00* T
Pogonion	**9.8**	2.18	0.29	**11.4**	2.25	0.34	-1.6	14.0	0.00* T
Menton	**7**	1.73	0.24	**9**	2.08	0.33	-2	22.2	0.00* T
Frontal Eminence R	**4.1**	0.89	0.14	**5**	1.23	0.33	-0.9	18.0	0.00* T
Frontal Eminence L	**4.2**	1.11	0.18	**4.9**	1.2	0.32	-0.7	14.2	0.04* T
Mid-supraorbital R	**6.5**	1.27	0.17	**8.6**	1.33	0.2	-2.1	24.4	0.00* T
Mid-supraorbital L	**6.4**	1.37	0.18	**8.7**	1.33	0.2	-2.4	26.4	0.00* T
Mid-infraorbital R	**5.3**	1.47	0.2	**5.8**	1.51	0.23	-0.5	8.6	0.07 M
Mid-infraorbital L	**5.5**	1.58	0.21	**5.9**	1.65	0.25	-0.4	6.7	0.07 M
Malar R	**21.4**	2.57	0.34	**22.8**	2.75	0.41	-1.4	6.1	0.01* T
Malar L	**21.7**	2.38	0.32	**22.9**	2.8	0.42	-1.2	5.2	0.02* T
Lateral Orbital R	**9.1**	1.73	0.23	**8.3**	1.46	0.22	0.8	-9.6	0.02* M
Lateral Orbital L	**9.2**	1.74	0.23	**8.3**	1.36	0.2	0.8	-10.8	0.02* M
Zygion R	**7.8**	1.79	0.24	**9.2**	1.85	0.28	-1.4	15.2	0.00* M
Zygion L	**7.8**	1.9	0.25	**9.1**	1.87	0.28	-1.3	14.2	0.00* M
Supraglenoid R	**10.7**	1.9	0.25	**12.8**	1.57	0.23	-2.1	16.4	0.00* T
Supraglenoid L	**10.7**	1.84	0.25	**12.8**	1.6	0.24	-2.1	16.4	0.00* T
Gonion R	**12.8**	3.65	0.49	**18.3**	5.54	0.83	-5.5	30.0	0.00* M
Gonion L	**13**	3.88	0.52	**17.8**	5.49	0.82	-4.8	26.9	0.00* M
Ectomolare^2 R	**27**	3.62	0.48	**30.3**	3.29	0.49	-3.3	10.8	0.00* M
Ectomolare^2 L	**27.2**	3.64	0.49	**30.2**	3.44	0.51	-2.9	9.9	0.00* M
Occlusal Line R	**20.3**	2.82	0.38	**24.6**	3.17	0.47	-4.4	17.4	0.00* T
Occlusal Line L	**20.4**	2.86	0.38	**24.3**	3.21	0.48	-3.8	16.0	0.00* T
Ectomolare$_2$ R	**24.9**	3.06	0.41	**28.2**	3.96	0.59	-3.4	11.7	0.00* T
Ectomolare$_2$ L	**24.8**	3.2	0.43	**28.4**	3.48	0.52	-3.6	12.6	0.00* T

R–right; L–left; T–*t*-test; M–Mann Whitney.

* $P < 0.05$.

**; ***Differences were calculated between female means compared to male means.

13. Craniofacial Landmarks [53]

The study used 3D craniofacial models from CT scans of 180 Koreans, employing 18 craniofacial landmarks to analyse the morphometry of the eyebrow and orbit. Eyebrows are crucial for facial recognition, more so than colour or density. This study focuses on estimating the position and shape of eyebrows from the orbit The morphology of the orbit significantly influences the position of the superior margin of the eyebrow. The middle part of the eyebrow is more predictable than the medial and lateral ends Males generally had larger measurements for both eyebrows and orbits compared to females. Regression equations were developed to estimate eyebrow position from orbital measurements, with varying accuracy based on sex and side of the face.

Advantages:

The study showed very high intra- and inter-observer reliability with alpha coefficients of 0.999 and 0.998. The regression equations developed provide useful guidelines for estimating eyebrow positions, especially the middle part of the eyebrow.

Disadvantages:

The measurements and predictive accuracy differ between males and females, requiring different equations for each sex. The study was conducted on a Korean population, so caution is needed when applying these findings to other ethnic groups.

14. CT scanning and digital modelling techniques [54]

The study aimed to create 3D facial depictions of Ramesses II at different ages using CT scan data. Advanced CT scanning and digital modelling techniques were used to reconstruct the Pharaoh's face at age 90 (death) and age 45 (peak military activity).The reconstructions revealed detailed facial features, including the preservation of his prominent nose and the effects of . The mummified body of Ramesses II was scanned using a modified CT protocol suitable for ancient, desiccated bodies. This provided detailed 3D reconstructions of his head. 3D models of the cranium, mandible, and soft tissues were generated from the CT data. These models were used to create digital 3D facial reconstructions. The CT images helped estimate Ramesses II's age at death (around 90 years old) and provided insights into his dental health and embalming materials used. Two facial depictions were created—one at 90 years old and another at 45 years old, reflecting his appearance at the peak of his military activities. The study highlights the importance of including cultural representatives in research and the potential for such reconstructions to enhance public c understanding and interest in ancient history.

Advantages :

CT scans allow for detailed study without physically disrupting the mummified remains. Provides comprehensive 3D models of the skull and facial features. Enhances understanding of historical figures and promotes cultural heritage.

Disadvantages:

Reconstructions can be influenced by the researchers' cultural and subjective biases. The method may not fully capture the original appearance due to limitations in technology and interpretation.

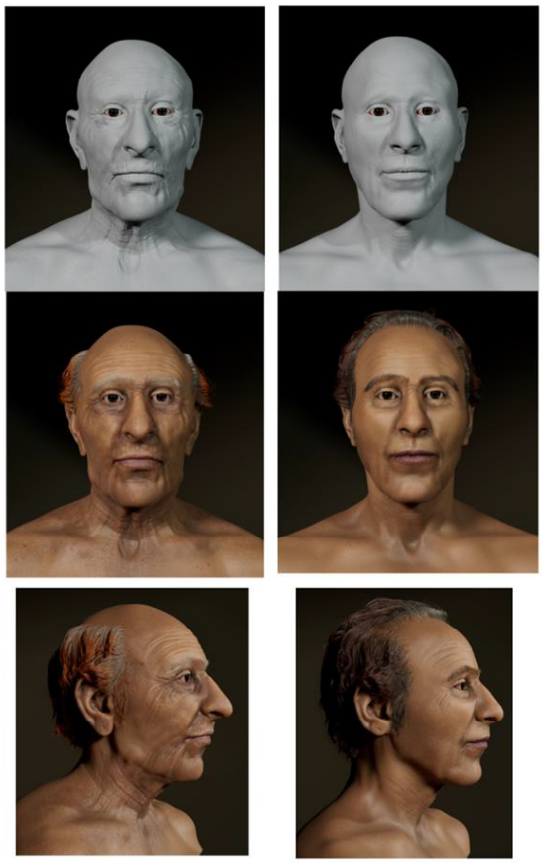


Fig. 6. Facial depictions of Ramesses II (2022) without (top) and with (middle/bottom) the application of colour and texture layers L: Age 90 years. R: Age 45 years. Images courtesy of Face Lab @ Liverpool John Moores University. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Discussion and Summary

CFR combines technology and medicine to reconstruct the facial features of individuals, frequently for forensic or medical purposes.

Computer-aided craniofacial reconstruction involves using specialized software and techniques to recreate the facial structure based on skeletal remains. One common approach is the use of CT scans or 3D scans of skulls to create a digital model. Software like Mimics or Blender are often working to manipulate these models and add tissue depth markers based on population averages. Various methods are applied in this process, such as the Manchester method, which involves placing tissue depth pegs on the skull based on anatomical landmarks.

Also, advancements in machine learning and artificial intelligence have improved the accuracy and efficiency of craniofacial reconstructions. These technologies can analyse large datasets to improve facial reconstructions and provide more reliable results.

In summary, computer-aided craniofacial reconstruction is an intricate and developing field that merges technology and medicine to recreate facial features. By using specialized software,

techniques like the Manual methods or computerized methods, and integrating machine learning, researchers and forensic experts can reconstruct faces with increasing accuracy and detail. This review article probably studies these software tools, techniques, and methods in detail and their recent development and significance in forensic science and other medical fields.

Conclusions

This study likely covered a range of advanced techniques and methods that are transforming the field. One key technology that has been discussed is 3D printing, which allows for the creation of precise implants and models. Software tools are often used to convert medical imaging data into 3D models, enabling practitioners to plan and practice complex procedures before operating.

Computer-aided design (CAD) software, plays a crucial role in designing customized models for craniofacial reconstruction. These tools enable to collaborate on intricate designs that fit each individual's unique anatomy perfectly. Moreover, virtual surgical planning software allows for detailed preoperative replications, enhancing surgical precision and outcomes.

By discussing these innovative techniques and software tools in this article, we provided a comprehensive overview of the innovative technologies driving progress in craniofacial reconstruction. Detailed analysis of these methods underscores the importance of technological innovation in shaping the future of craniofacial reconstruction in forensic science.

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"A Review on - Role of Chemistry for investigation in Forensic Science"

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ABSTRACT

Learning about crimes scenes can be interesting. This article will explain the premise of crime scene chemistry and how it is used to collect evidence for law enforcement agents. The knowledge of and technology associated with crime scene chemistry is one of the most important advances in criminal investigations. Firstly, the knowledge of chemistry allows law enforcement to find evidence which would previously have been completely hidden. Secondly, and more importantly, it let's them find evidence which is almost entirely accurate.

In this review it explains, how the forensic chemistry is helpful filed of the forensic science. It additionally explains the various fields of the forensic science and therefore the temporary introduction of forensic science and forensic chemistry. Chemical and physical expertise covers the widest area of work, which includes expertise of traces left after explosion, arson, traffic accidents, burglary, pollution environment, etc. In order to be as objective as possible, they apply a number of instrumental methods of analysis in their work. Which method will be applied depends primarily on the type and amount of trace, and it is always necessary to choose the method that will reduce the possibility of sample contamination.

Keyword- Law enforcement agents, Finger print, Chemical technique.

Introduction

Forensic chemistry is important because without it we wouldn't know the outcome of a crime. The forensic chemist's job is to examine evidence given to them from a crime scene, when it happened, and even who committed the crime at times. Forensic chemistry deals with the chemical analysis of a variety of types of physical evidence.

Every chemist is schooled in general, organic, and analytical chemistry, but forensic chemists also specialize in specific areas of expertise. For example, an inorganic chemist may examine traces of dust by using microchemistry to identify the chemical composition of tiny particles. Another chemist might employ thin-layer chromatography during the analysis of Forensic scientists examine evidence from crime scenes in an effort to solve crimes. This scientist is removing a piece of blood-stained material gathered at a crime scene for DNA testing.

The physical contact between a suspect and a victim, vehicle or a crime scene during the commission of a crime, can and often does result in the transfer of materials such as blood, semen, saliva, hairs, fibers, paint, plastic and adhesives. Also, in the investigation of fires, the analysis of fire debris samples for the identification of ignitable liquids is necessary to help determine the cause and origin of the fire. The Forensic Chemistry Section of the Crime Laboratory is responsible for the examination, identification and/or comparison of these types of materials which may be present at the crime scene, on the victim, the suspect, clothing articles, vehicles, weapons, tools and other objects. In order to conduct these examinations, various serological, chemical, microscopic, and/or instrumental techniques are utilized.[11]

The truth is that a forensic scientist is often a chemist. This because the analysis of gunshot residues, hair or traces of blood that can link a suspect to a crime scene, is above all a process that uses the techniques of chemistry, instruments developed for chemistry and (note!), the methods for solving problems of chemists!. In fact, modern criminal investigation puts the limits and capabilities of the so called Analytical Chemistry to the test: a branch of chemistry that focuses on identifying the quantities of substances present in a sample. What about blood tests? Analytical chemistry, of course!

The development of analytical chemistry made it possible to detect the presence of substances in miniscule quantities, through a variety of techniques capable of recognizing the specific characteristics of each substance.

For example, with chromatography (a technique that allows to separate the various components of a sample), it is possible to detect absolutely minute quantities of sample in the order of nanograms per milliliter. And how much is that? Less than a packet of sugar dissolved in an Olympic swimming pool!

In the case of metals, can go up to 10 times further. Using a technique of vaporization of the sample at 10 000 degrees Celsius, it's possible, for example, to detect the presence of a toxic metal in a hair in a proportion equal to one gram of metal into four Olympic pools!

But the great challenge of analytical chemistry applied to criminal investigation goes beyond identifying the presence of drugs, explosives or poisons. It's about how to characterize the materials found at the crime scene and trace them back to their origin.In fact, the composition of materials such as glass fragments, traces of paint, textile fibers, paper or even the ink used to write a letter, can provide very important clues in the investigation of a crime. The combined use of analytical

techniques allows identifying even the geographical origin or date of manufacture of many materials.[14]

MATERIALS AND METHODS

This work is the result of the expert knowledge of its author and the literature available to him. The paper processing desk method was used.

The Role of Forensic Chemists

Forensic chemistry encompasses organic and inorganic analysis, toxicology, arson investigation, and serology. Each method of analysis uses specialized techniques and instrumentation. The process may be as simple as setting up a density gradient column to compare soil samples or as complicated as using a mass spectrometer or neutron activation analysis to characterize an unknown substance. A wide array of laboratory techniques and instrumentation is used in forensic studies. This includes ultraviolet, infrared, and visible spectrophotometry; neutron activation analysis; gas chromatography and mass spectrophotometry; high pressure liquid chromatography; and atomic absorption spectrophotometry.

Some important techniques of forensic chemistry are following

1. **Trace Evidence**

   The physical evidence which is small in size or microscopic is known as trace evidence. So, trace evidence is a term that encompasses all small pieces of material that are collected from crime scenes and assists in the investigation of these incidents.

Trace evidence is also known as “Silent Witness” because it has the potential to tell what actually was happened and who has been involved.

Trace evidence services include:

- Explosives analysis
- Fibre examination
- Glass examination
- Ignitable liquid residue analysis
- Paint examination
- Soil examination
- Gunshot residue (GSR) analysis

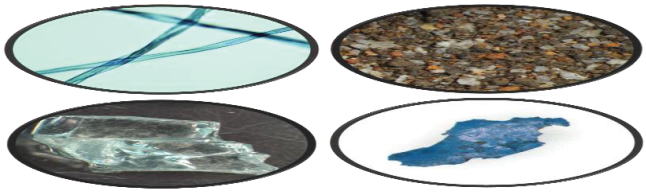

Fig.1

These types of materials which may be found and collected during investigations are compared to known or reference samples using various chemical, microscopic and instrumental techniques. The types of microscopes used include the stereomicroscope, the compound microscope, the comparison microscope and the polarized light microscope. Instruments used include the Fourier Transform Infrared Spectrophotometer (FTIR) and the micro spectrophotometer (MSP). The Forensic Chemistry Section participates in the Paint Debris Query (PDQ) system, sponsored by the Royal Canadian Mounted Police (RCMP) and Federal Bureau of Investigation (FBI). The PDQ database allows an examiner to process an automotive paint chip and potentially determine the make, model, and year of a vehicle. The PDQ database is especially important in difficult cases where investigators do not have a clear suspect vehicle.[17]

2. Fingerprinting

Fingerprints on smooth surfaces can often be made visible by the application of light or dark powder, but fingerprints on checks or other documents are often occult (hidden). Occult fingerprints are sometimes made visible by the use of ninhydrin, which turns purple due to reaction with amino acids present in perspiration. Fingerprints or other marks are also sometimes made visible by exposure to high-powered laser light. Some fingerprints can be treated with chemical substances, resulting in a pattern that fluoresces when exposed to light from lasers. Cyanoacrylate ester fumes from glue are used with fluorescent dyes to make the fingerprints visible. There's an older tactic that often comes in very handy in a crime scene. This tactic also relies on chemistry. It's the age old art of dusting for fingerprints. The days of dusting for prints is not gone, it is still a commonly used tactic for identifying fingerprints. However, there are also other methods. Some investigators use lasers, which react with the oils and chemicals that form a fingerprint. There is also work being done to attempt to extract enough DNA from a single fingerprint to properly identify someone. This would be extremely

helpful, as often fingerprints are very smudged and can not be used to properly identify someone.[14,16]

Fig.2

3. Testing for Alcohol

Accidents caused by intoxicated drivers kill nearly 15,000 persons a year in the United States alone (almost half of fatal auto accidents are alcohol-related), so a Breathalyzer kit is standard equipment in most police squad cars or state patrol vehicles. Breathalyzers are used to estimate the blood alcohol content of drivers suspected of being intoxicated; the driver may appear sober, but still have a blood alcohol level above the legal limit. Although it is impractical to take blood samples on the highway, research has shown that the concentration of ethanol in the breath bears a definite relationship to its concentration in blood. Many communities have now set a legal limit of 0.08 percent (meaning that 100 milliliters [3.38 fluid ounces] of blood would contain 0.08 grams [0.0028 ounces] of ethanol). In fact, authorities now consider that a person's driving ability is probably impaired at a blood ethanol level of 0.05 percent.

Several types of analytic devices are available to administer Breathalyzer tests. One test makes use of a portable infrared spectrophotometer, another uses a fuel cell, and the most common test employs several glass or plastic tubes and some common chemical reagents. The person being tested blows through a tube, which bubbles the breath through a solution of chemicals containing sulfuric acid, potassium dichromate, water, and silver nitrate. Oxidation of the alcohol results in the reduction of dichromate ion to chromic ion, with a corresponding change in color from orange to green. An electrical device employing a photocell compares the color of the test solution with a standard solution, giving a quantitative determination of the alcohol content. The test provides a quick and reproducible determination of the amount of alcohol in a person's breath and is a numerical measure of the amount of alcohol in the bloodstream. Use of a chemical test helps to avoid subjective opinions of sobriety and provides reliable evidence for court proceedings. The test can be readily and quickly administered by trained law enforcement personnel, but forensic chemists test and calibrate the equipment and testify to its accuracy.[1,9]

4. Serology

In homicide, sexual assault, aggravated assault, motor vehicle accident, burglary and other investigations, the Forensic Chemists routinely conduct serological examinations on clothing articles, weapons, vehicles, scene samples or other items in order to locate possible bloodstains, identify the blood by presumptive chemical testing, determine if the blood is of human origin and then select suitable and relevant samples for DNA analysis. The chemists also make observations of bloodstain patterns on evidence items and/or at crime scenes. When evidence is submitted in sexual assault cases, the Forensic Chemists examine the contents of sexual assault evidence kits, clothing articles, bedding or other items for the presence of semen. Possible semen stains are located visually or by alternate light source and then tested for a component of seminal fluid by presumptive chemical tests. Further testing is conducted microscopically for sperm cells. In the absence of sperm cells, further testing is carried out for an additional semen component in order to confirm the presence of seminal fluid. Suitable and relevant samples are then selected for DNA analysis. In some instances, saliva analysis is also requested. Stains are generally located visually or by alternate light source. Amylase, a chemical component of saliva, is identified to confirm the presence of saliva. Suitable and relevant samples are then selected for DNA analysis. [15,14]

5. Bloodstain Pattern Analysis

Bloodstain pattern analysis involves the examination and documentation of bloodstains, namely their size, shape and distribution. Bloodstain patterns are often indicative of the types of actions that produced or caused these patterns. This information is used to help reconstruct the sequence of events that occurred during the commission of a crime[9,14]

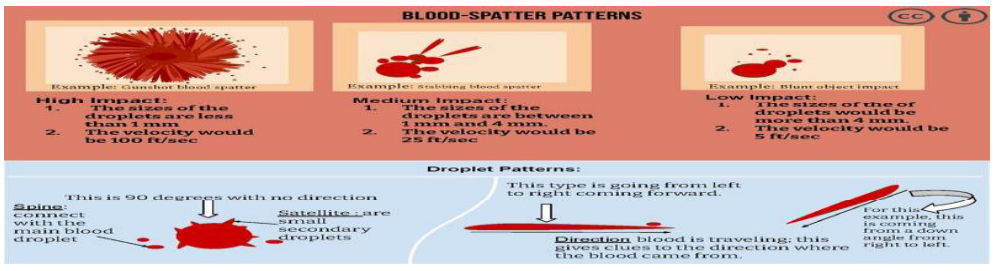

Fig.3

6. Fire Debris Analysis

The Fire Debris Analysis Unit examines evidence collected at fire scenes and is a part of the Forensic Chemistry Section of the laboratory. The purpose of this examination is to determine if an ignitable

liquid is present. Most ignitable liquids are petroleum products, however other non-petroleum products can be identified. Fire debris evidence is packaged in mason jars, paint cans, or fire debris evidence bags and generally consists of charred fire debris or clothing items. The examination procedure involves extracting ignitable liquids from the evidence using one of three extraction techniques or a combination of techniques. The three extraction methods used by this laboratory are Passive Diffusion Headspace, Simple Headspace, and Solvent Extraction. The extract is then analyzed on a Gas Chromatograph – Mass Spectrometer, which provides data that the examiner will then analyze. Based on the pattern, or appearance of the data, the examiner will identify the type of product, if any, in the extract. [14, 3]

7. Toxicology

Toxicologists examine a wide range of materials such as blood stains, urine, and blood gases for traces of poisons or drugs. Many businesses now require the drug screening of employees; it is the responsibility of the technician to distinguish between the presence of illegal drugs and metabolites from foods such as poppy seeds. Such tests may be as simple as paper or thin-layer chromatography or as complicated as gas chromatographic or electrophoretic and serological analysis of a blood sample. Following death by unknown cause, samples of the victim's lungs, blood, urine, vitreous humor, and stomach contents are examined for traces of poisons or medication. Insects found on or near corpses are also collected and examined; they may actually absorb traces of drugs or poisons from the body, and in fact, traces of poisons sometimes are found in the surrounding insects long after concentrations in the body have fallen below detectable limits.

Forensic biochemists perform blood typing and enzyme tests on body fluids in cases involving assault, and also in paternity cases. Even tiny samples of blood, saliva, or semen may be separated by electrophoresis and subjected to enzymatic analysis. In the case of rape, traces of semen found on clothing or on the person become important evidence; the composition of semen varies from person to person. Some individuals excrete enzymes such as acid phosphatase and other proteins that are seldom found outside seminal fluid, and these chemical substances are characteristic of their semen samples. The presence of semen may be shown by the microscopic analysis for the presence of spermatozoa or by a positive test for prostate specific antigen.

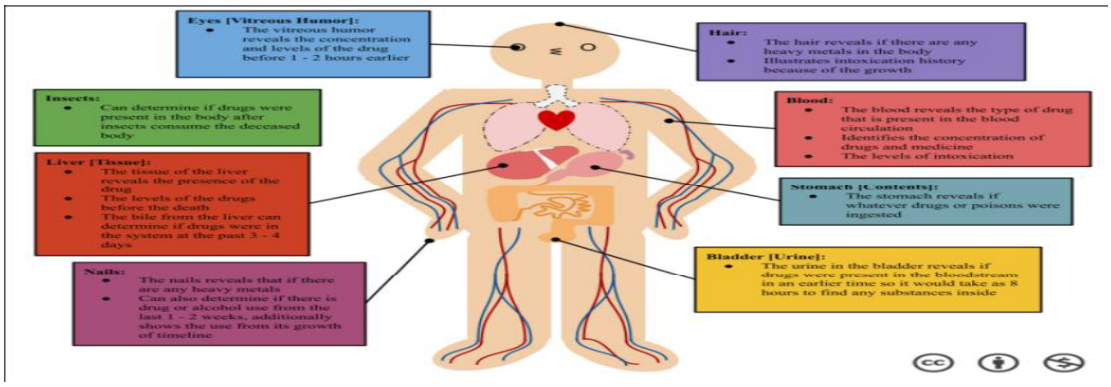

Fig.4

In cases of sexual assault, tiny samples of DNA in blood, semen, skin, or hair found on the victim may be purified and the amount of DNA increased by the use of a polymerase chain reaction to produce quantities large enough to analyze. Since DNA is as specific to a person as fingerprints, matching the DNA of a perpetrator to a sample found on a victim is considered to be proof of contact. The Federal Bureau of Investigation (FBI) is currently in the process of establishing a national Combined DNA Index System (CODIS) that will collect data from many states and law enforcement agencies and index it so that particular DNA patterns from evidence collected at many crime scenes can be compared and matched. Many perpetrators of crimes have been convicted and many innocent persons set free after years in prison as a result of DNA analysis.[6]

The Role of Analytical chemistry in Forensic Science

Analytical measurements are essential to everyday life, required to determine the composition and control the quality of many products, to protect the environment and to monitor health. Consequently Analytical Chemistry has a major impact, not only in chemistry, but also in fields such as biochemistry, and the forensic, food, environmental and pharmaceutical sciences. Forensic chemistry is the application of analytical chemistry to the law and involves the examination of physical traces, such as body fluids, bones, fibres and drugs. Success in analytical chemistry requires the ability to make rigorous measurements, an appreciation of the principles and practice of modern instrumentation, and a problem-solving approach. This course aims to develop these skills, with an emphasis on the use of coupled chromatography-mass spectrometry techniques, a powerful combination with applications in the analysis of complex mixtures relevant to forensic, atmospheric and biological systems. As technology infiltrates every aspect of our lives, it is no wonder that solving crimes has become almost futuristic in its advances. From retinal scanning to trace evidence chemistry, actual forensic technologies are so advanced at helping to solve crimes that they seem like something from a science fiction thriller.[1]

1. Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) :

When broken glass is involved in a crime, putting together even tiny pieces can be key to finding important clues like the direction of bullets, the force of impact or the type of weapon used in a crime. Through its highly sensitive isotopic recognition ability, the LA-ICP-MS machine breaks glass samples of almost any size down to their atomic structure. Then, forensic scientists are able to match even the smallest shard of glass found on clothing to a glass sample from a crime scene. In order to work with this type of equipment in conjunction with forensic investigation, a Bachelor's Degree in Forensic Science is usually necessary.[5]

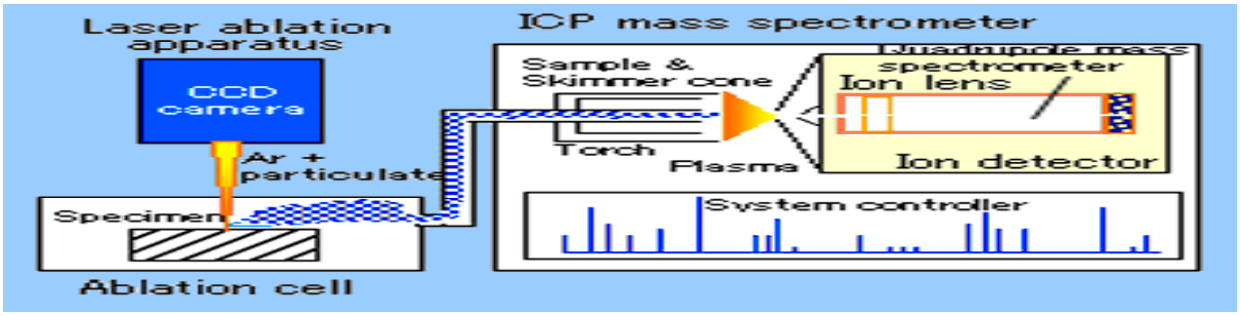

Fig.5

2. Alternative Light Photography :

For a forensic nurse, being able to quickly ascertain how much physical damage a patient has suffered can be the difference between life and death. Although they have many tools at their disposal to help make these calls quickly and accurately, Alternative Light Photography is one of the coolest tools to help see damage even before it is visible on the skin. A camera such as the Omnichrome uses blue light and orange filters to clearly show bruising below the skin's surface. In order to use this equipment, you would need a MSN in Forensic Nursing.[3]

3. High-Speed Ballistics Photography:

You might not think of it right away as a tool for forensic scientists, but ballistics specialists often use high-speed cameras in order to understand how bullet holes, gunshot wounds and glass shatters are created. Virtually anyone, from a crime scene investigator to a firearms examiner, can operate a high-speed camera without any additional education or training. Being able to identify and match bullet trajectories, impact marks and exit wounds must be done by someone with at least a Bachelor's of Science in Forensic Science.

4.Video Spectral Comparator 2000:

For crime scene investigators and forensic scientists, this is one of the most valuable forensic technologies available anywhere. With this machine, scientists can look at a piece of paper and see obscured or hidden writing, determine the quality of paper and origin and " lift " indented writing. It is sometimes possible to complete these analyses even after a piece of paper has been so damaged by water or fire that it looks unintelligible to the naked eye

It is sometimes possible to complete these analyses even after a piece of paper has been so damaged by water or fire that it looks unintelligible to the naked eye. In order to run this equipment, at least a Bachelors degree in Forensic Science or a Master's Degree in Document Analysis is usually required.

5. Digital Surveillance for Xbox (XFT Device):

Most people don't consider a gaming system a potential place for hiding illicit data, which is why criminals have come to use them so much. In one of the most ground-breaking forensic technologies for digital forensic specialists, the XFT is being developed to allow authorities visual access to hidden files on the Xbox hard drive. The XFT is also set up to record access sessions to be replayed in real time during court hearings. In order to be able to access and interpret this device, a Bachelor's Degree in Computer Forensics is necessary. [10]

6. 3D Forensic Facial Reconstruction:

Although this forensic technology is not considered the most reliable, it is definitely one of the most interesting available to forensic pathologists, forensic anthropologists and forensic scientists. In this technique, 3D facial reconstruction software takes a real-life human remains and extrapolates a possible physical appearance. In order to run this type of program, you should have a Bachelor's Degree in Forensic Science, a Master's Degree in Forensic Anthropology or a Medical Degree with an emphasis on Forensic Examination and Pathology. [3]

7. DNA Sequencer:

Most people are familiar with the importance of DNA testing in the forensic science lab. Still, most people don't know exactly what DNA sequencers are and how they may be used. Most forensic scientists and crime lab technicians use what's called DNA profiling to identify criminals and victims using trace evidence like hair or skin samples. In cases where those samples are highly degraded, however, they often turn to the more powerful DNA sequencer, which allows them to analyze old bones or teeth to determine the specific ordering of a person's DNA nucleobases, and generate a "read" or a unique DNA pattern that can help identify that person as a possible suspect or criminal. [2]

8. Forensic Carbon-14 Dating:

Carbon dating has long been used to identify the age of unknown remains for anthropological and archaeological findings. Since the amount of radiocarbon (which is calculated in a Carbon-14 dating) has increased and decreased to distinct levels over the past 50 years, it is now possible to use this technique to identify forensic remains using this same tool.

The only people in the forensic science field that have ready access to Carbon-14 Dating equipment are forensic scientists, usually with a Master's Degree in Forensic Anthropology or Forensic Archaeology.[10]

9. Magnetic Fingerprinting and Automated Fingerprint Identification (AFIS) :

With these forensic technologies, crime scene investigators, forensic scientists and police officers can quickly and easily compare a fingerprint at a crime scene with an extensive virtual database. In addition, the incorporation of magnetic fingerprinting dust and no-touch wanding allows investigators to get a perfect impression of fingerprints at a crime scene without contamination. While using AFIS requires only an Associates Degree in Law Enforcement, magnetic fingerprinting usually requires a Bachelor's Degree in Forensic Science or Crime Scene Investigation.[10,2]

10. High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) also know as high-pressure liquid chromatography is an instrumental system based on chromatography that is widely used in forensic science. The "HP" portion of the acronym is sometimes assigned to the words high pressure (versus high performance), but it refers to the same analytical system. HPLC is used in drug analysis, toxicology, explosives analysis, ink analysis, fibers, and plastics to name a few forensic applications. Like all chromatography, HPLC is based on selective partitioning of the molecules of interest between two different phases.

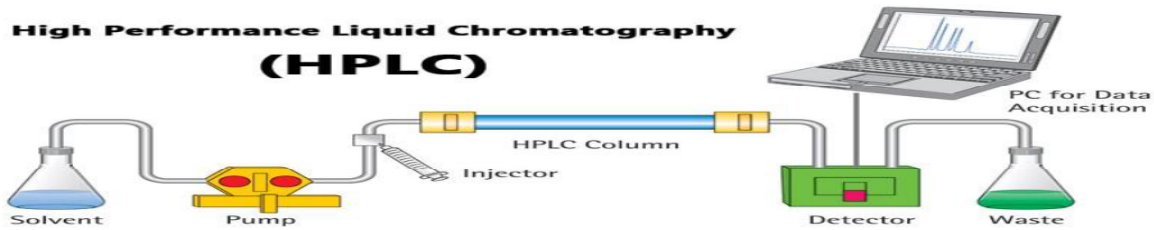

Fig.6

Here, the mobile phase is a solvent or solvent mix that flows under high pressure over beads coated with the solid stationary phase. While traveling through the column, molecules in the sample partition selectively between the mobile phase and the stationary phase. Those that interact more with the stationary phase will lag behind those molecules that partition preferentially with the mobile phase. As a result, the sample introduced at the front of the column will emerge in separate bands (called peaks), with the bands emerging first being the components that interacted least with the stationary phase and as a result moved quicker through the column. The components that emerge last will be the ones that interacted most with the stationary phase and thus moved the slowest through the column. A detector is placed at the end of the column to identify the components that elute. Occasionally, the eluting solvent is collected at specific times correlating to specific components. This provides a pure or nearly pure sample of the component of interest. This technique is sometimes referred to as preparative chromatography.[7,13

11. Gas Chromatography

Gas chromatography (GC) is an instrumental technique used forensically in drug analysis, arson, toxicology, and the analyses of other organic compounds. GC exploits the fundamental properties common to all types of chromatography, separation based on selective partitioning of compounds between different phases of materials. Here, one phase is an inert gas helium (He), hydrogen (H2), or nitrogen (N2) that is referred to as the mobile phase (or carrier gas), and the other is a waxy material (called the stationary phase) that is coated on a solid support material found within the chromatographic column. In older GC systems, the stationary phase was coated on tiny beads and packed into glass columns with diameters about the same as a pencil and lengths of 6 to 12 feet, wound into a coil.

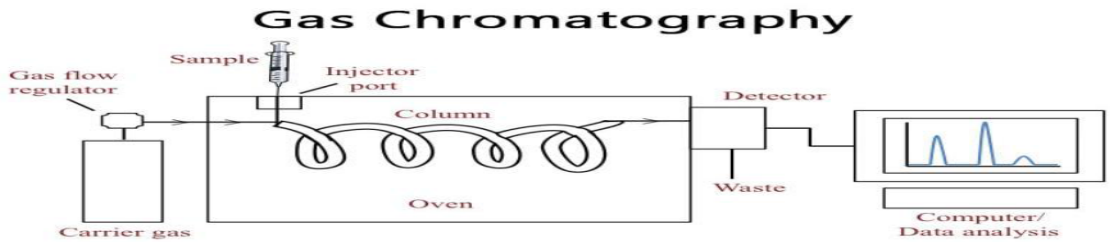

Fig.7

The heated gas flowed over the beads, allowing contact between sample molecules in the gaseous mobile phase and the stationary phase. Called “packed column chromatographs,” these instruments were widely used for drug, toxicology, and arson analysis. Around the mid-1980s, column

chromatography began to give way to capillary column GC, in which the liquid phase is coated onto the inner walls of a thin capillary tube (about the diameter of a thin spaghetti noodle) that can be anywhere from 15 to 100 meters long, also wound into a coil. Capillary column chromatography represented a significant advance in the field and greatly improved the ability of columns to separate the multiple components found in complex drug and arson samples.[8,18]

12. Ion Chromatography

Ion chromatography (IC) is an instrumental technique that can be used to detect anions (negatively charged atoms or molecules such as Cl-) and cations (positively charged species such as Na+). IC has been applied in forensic science for the analysis of gunshot residue (GSR) and explosives. The ions of interest include ammonium (NH4+), nitrate (NO3-), and chlorate (ClO4-), species that are often detected using color change or presumptive tests.

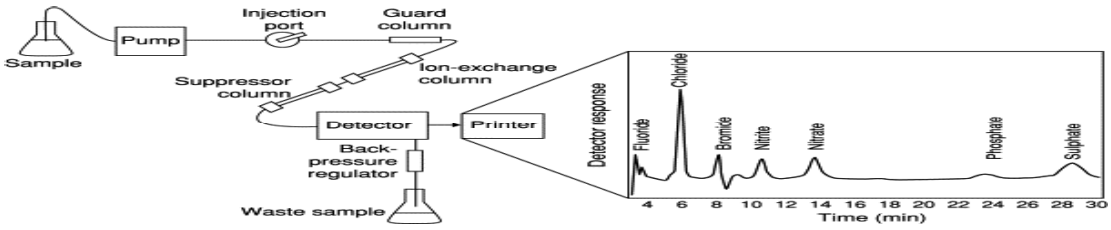

Fig.8

The advantages of IC in these cases include specificity (presumptive tests are subject to false positives and false negatives) and increased sensitivity, down to the part-per-billion (ppb) range. A part per billion is 1 microgram (μmg) per liter of water, and a microgram is 1/1,000,000 of a gram.[19]

Conclusion

Thanks to the chemistry of crime scene investigations, more guilty people get caught and more innocent people are freed. It is definitely one of the most important advances when it comes to criminal justice and as our technology and knowledge increase, it will only become more reliable.

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Applications of Transform Methods and Their in Forensic Science

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Abstract:

Transform methods are important tools in forensic science, offering robust techniques, this paper explores the use of Laplace Transform, Differential Transform Method (DTM), and Fourier Transform in forensic science. Laplace Transform aids in cleaning forensic audio and modelling toxicology dynamics. DTM helps recognize fingerprint patterns and predict blood droplet trajectories. Fourier Transform enhances forensic images and analyses audio frequencies. These methods improve forensic investigation accuracy and reliability.

Keywords: Laplace Transform, Differential Transform Method (DTM), Fourier Transform, ForensicScience, Signal Processing

Introduction:

Forensic science relies on advanced mathematical techniques to analyse and interpret complex data accurately. Transform methods such as Laplace Transform, Differential Transform Method (DTM), and Fourier Transform have proven invaluable in this field. These methods enhance various aspects of forensic investigations, including signal processing, pattern recognition, predictive modelling, image analysis, and spectral analysis. This paper explores the applications of these transform methods, highlighting their critical roles in improving the accuracy and reliability of forensic analyses.

Literature Review:

Mathematical transform methods play a crucial role in forensic science, enhancing data analysis and interpretation.

Laplace Transform	Differential Transform Method (DTM)	Fourier Transform
Used in signal processing to clarify forensic audio by converting signals to the s-domain (Harris, 2010; Brixen, 2007).	Applied in pattern recognition for fingerprint matching (Jain & Feng, 2011; Maltoni et al., 2009)	Enhances forensic images by transforming spatial data to the frequency domain (Roddy & Stosz, 1997; Lee & Gaensslen, 2014).
Models' dynamic behaviour in toxicology to predict substance concentrations (Chaturvedi & Rao, 1994; Liu & Kumar, 2011).	Assists in blood spatter analysis for crime scene reconstruction (Bevel & Gardner, 2008; Attinger et al., 2013).	Analyzes audio frequencies to detect hidden messages and electromagnetic signals (Koenig et al., 2004; Grigoras, 2005).

Methodology:

This study examines the applications of Laplace Transform, Differential Transform Method (DTM), and Fourier Transform in forensic science through a comprehensive literature review and practical case studies.

Literature Review:

- Collected and analysed existing research on the use of transform methods in forensic science.
- Reviewed key studies highlighting the applications and benefits of Laplace Transform, DTM, and Fourier Transform.

2. Practical Case Studies:
   - **Laplace Transform**: Analyzed case studies in forensic audio analysis and toxicology to demonstrate noise reduction and substance concentration modelling.
   - **DTM**: Evaluated pattern recognition in fingerprint analysis and predictive modelling in blood spatter analysis through documented forensic cases.
   - **Fourier Transform**: Examined the enhancement of forensic images and

spectral analysis of audio recordings in real-world investigations.

3. Data Analysis:
    - Compared the effectiveness of each transform method in improving the accuracy and reliability of forensic analyses.
    - Identified common challenges and limitations associated with the application of these methods.

This methodology provides a structured approach to understanding the impact and practical applications of transform methods in forensic science.

Result:

The study's analysis of transform methods in forensic science yielded significant findings:

1. Laplace Transform:
    - Improved clarity and noise reduction in forensic audio recordings.
    - Enhanced modeling of substance concentrations in toxicology, aiding in accurate time-of-administration estimations.
2. Differential Transform Method (DTM):
    - Increased accuracy in fingerprint pattern recognition, facilitating reliable matches.
    - Enhanced predictive modeling in blood spatter analysis, improving crime scene reconstructions.
3. Fourier Transform:
    - Superior enhancement of forensic images, allowing for clearer identification of features in fingerprints and ballistic marks.
    - Effective spectral analysis of audio recordings, uncovering hidden messages and identifying signal sources.

Case Study: Laplace Transform in Forensic Toxicology Introduction

Forensic toxicology often requires the analysis of how substances degrade in the body over time to determine the time and dosage of administration. The Laplace Transform is used to model the kinetics of drug absorption, distribution, metabolism, and excretion (ADME) processes, providing crucial insights into toxicology cases.

Case Background

In a forensic investigation, a deceased individual is found with suspicious circumstances suggesting possible poisoning. Forensic toxicologists are tasked with determining the concentration of the substance in the body at the time of death and estimating the time of administration.

Methodology

1. **Sample Collection**:
    - Biological samples (e.g., blood, urine, tissue) are collected from the deceased.
    - The concentration of the suspected toxic substance is measured in these samples.
2. Preprocessing:
    - Initial data on the substance's concentration over time is gathered from medical literature or experimental data.
    - The pharmacokinetics of the substance, including absorption rate, distribution volume, metabolism rate, and excretion rate, are established.
3. Application of Laplace Transform:
    - The differential equations describing the ADME processes are transformed using the Laplace Transform to convert them into algebraic equations.

For example, the concentration C(t) of the substance in the bloodstream can be modelled by the equation:

$$\frac{dC(t)}{dt} + k\ C(t) = De^{-kt}$$

where k is the elimination rate constant, and D is the dosage administered.

Applying the Laplace Transform:

$$(s) - C(0) + k \cdot C(s) = \frac{D}{s + k}$$

Simplifying, we get:

$$(s) = \frac{(0)}{s + k} + \frac{D}{(s + k)^2}$$

4. Solution and Inverse Laplace Transform:
    - Solve the algebraic equation in the s-domain to find (s).
    - Apply the inverse Laplace Transform to convert back to the time domain and obtain (t).

5. Postprocessing:
    - The time-domain concentration profile C(t) is analysed to determine the peak concentration and the time at which it occurred.
    - The data is used to estimate the time of administration and dosage.

Results

- **Concentration Profile**: The Laplace Transform provided a clear concentration-time profile of the substance in the body, showing how it degraded over time.
- **Time of Administration**: By analysing the concentration profile, forensic toxicologists estimated that the substance was administered approximately 12 hours before death.
- **Dosage Estimation**: The model indicated a high initial dosage, suggesting intentional poisoning.

Example:

Suppose the initial concentration (0) is known, and the dosage D and elimination rate k

are estimated. The transformed concentration (s) can be:

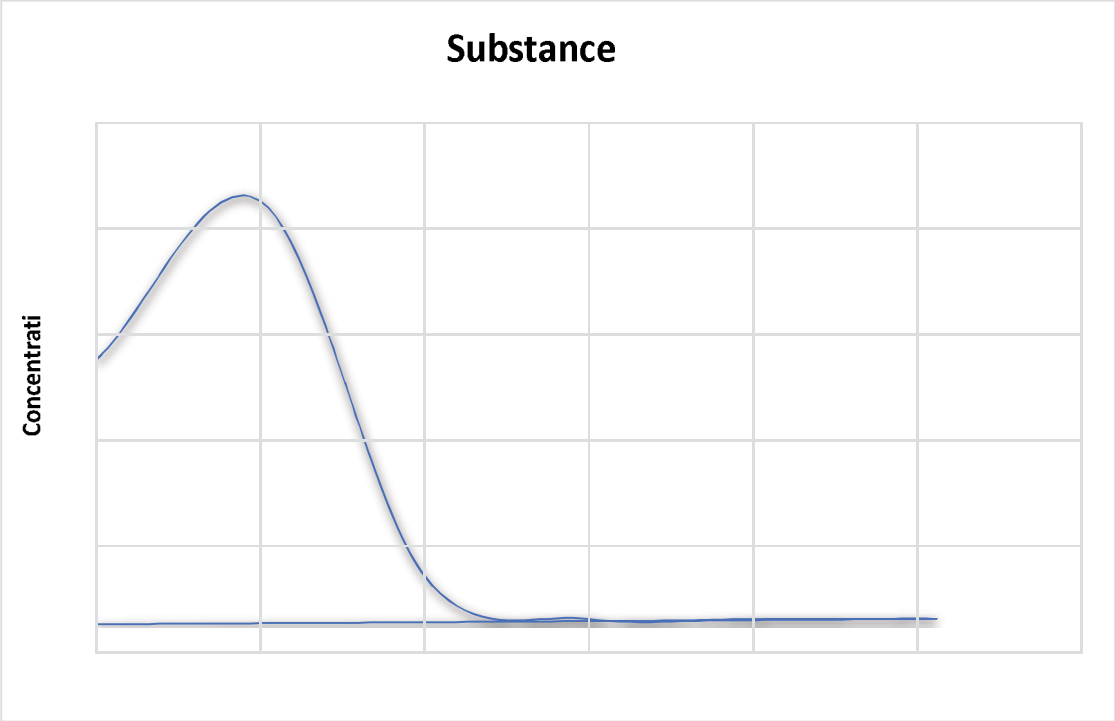
$$(s) = \frac{(0)}{s+k} + \frac{D}{(s+k)^2}$$

Taking the inverse Laplace Transform, we get:

$$\boldsymbol{C(t) = C(0)e^{-kt} + Dte^{-kt}}$$

This equation models the substance concentration in the body over time.

Graphical Representation for this example:



The graph above shows the concentration of a substance in the body over time using the modelled equation:

$$(t) = (0)e^{-kt} + Dte^{-k}$$

In this example:

- **Initial concentration** C (0) = 5 units.
- **Dosage** D= 10 units.
- **Elimination rate constant** k = 0.5 per hour.

This model illustrates how the substance's concentration changes over a 24-hour period, helping forensic toxicologists estimate the time and dosage of administration in forensic investigations

This case study illustrates the application of the Laplace Transform in forensic toxicology to model the kinetics of substance degradation. By transforming differential equations into algebraic ones, forensic toxicologists can accurately estimate the time and dosage of substance administration. This methodology provides vital insights in toxicology cases, aiding in the determination of cause and manner of death.

Conclusion:

This study highlights the crucial role of transform methods in forensic science. Laplace Transform enhances audio clarity and models toxicology dynamics, Differential Transform Method improves fingerprint recognition and blood spatter predictions, and Fourier Transform sharpens forensic images and analyses audio frequencies. These methods significantly boost the accuracy and reliability of forensic investigations, proving their indispensability in the field.

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"COMPARISION OF SOLUTIONS OF INTEGRAL EQUATIONS USING LAPLACE TRANSFORM METHOD AND SHORTCUT METHOD OF LINEAR DIFFERENTIAL EQUATIONS"

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Abstract –

We solve some higher order differential equations with initial boundary conditions using Volterra integral equation, Laplace method and shortcut method of linear differential equation. We compare the all solutions. We discuss some basic definitions and classifications of integral equations. We will solve some integral equations.

$(x)(x) = f(x) + \lambda \int_a^x k(x,t)u(t)dt$

Keywords - Integral equations, Volterra Integral Equation, Laplace Transform method, Successive Approximation

Introduction-

One of the most effective tools in mathematics' both in practical and theoretical situations is the integral equation [4].Various physical problems in physics & other applied field culminate into initial value or boundary value problems [1]. Integral equations have many applications in various areas, including mathematical, physics, electrochemistry, chemistry semi-conductors, heat conduction, metallurgy, fluid flow, scattering theory, chemical reaction and population dynamics [5]. It was also shown that Volterra integral equations can be derived from initial value problems [2].Volterra started working on integral equations in 1884, but his serious study began in 1896. The name integral equation was given by du Bois-Reymond in 1888. However, the name Volterra integral equation was first coined by Lalesco in 1908[2]. In this paper we apply Integral equation on linear differential equation on some example and the result obtained by it are compared with the result obtain by Laplace transform method, Shortcut method of differential equation which are exact solutions [7].

Volterra Integral Equation -

A linear integral equation of the form,

$$\int \qquad (1)$$

Where the upper limit of the integral is variable, $v(x)$, $f(x)$, $k(x,t)$ are known functions and $u(x)$ is unknown function, is said to be volterra integral equation of the third kind.If λ is a real or complex parameter and the function $k(x,t)$ is the kernel of the integral equation.[3]

First Kind of Volterra Integral Equation- If we set $(x) = 0$ in equation (1),

$$f(x) + \lambda \int_a^x k(x,t)u(t)dt = 0 \qquad (2)$$

Then it is called Volterra Integral equation of the The first kind.

Second kind of Volterra Integral Equation-

If $f(x) = 0$ then equation (1) becomes,

$$u(x) = \lambda \int_a^x k(x,t)u(t)dt \qquad (3)$$

is called homogeneous volterra integral equation of the second kind.

Method of Conversion of an initial value problem to a volterra integral equation [2] - Let a second order initial value problem be,

$\frac{d^2y}{dx^2} + (x)\frac{dy}{dx} + (x)y(x) = g(x)$ (4)

Subject to the initial conditions,

$(0) = \alpha, y'(0) = \beta$ (5)

Where α & β are constants. The functions (x) & (x) are analytic functions and $g(x)$ is continuous.

Let $\frac{d^2y}{dx^2} = u$ (x) (6)

Where u(x) is a continuous function. Integrating both sides of equation (6) 0 to x, weget

$\frac{dy}{dx} - y'(x) = \int_0^x u(t)dt$

$\frac{dy}{dx} = \beta + \int_0^x u(t)dt$ (7)

Integrating both sides of equation (7) from 0 to x, we get

$y(x) = a + \beta x + \int_0^x (x - t)u(t)dt$ (8)

Substitute equation (6), (7) & (8) in equation (4), and this solution of equation can be written in standard integral equation form is,

$(x) = f(x) + \lambda \int_0^x k(x, t)u(t)dt$ (9)

Successive Approximation Method-

Equation (9) is the integral equation.

Let f(x) be continuous is ,0, a- and $k(x, t)$ be continuous for $0 \le x \le a, 0 \le t \le x$.

We begin with some given function $u_0(x)$ Continuous in ,0. a- then replacing (t) on RHS of equation (4) by $u_0(x)$ we get,

$u_1(x) = f(x) + \lambda \int_0^x k(x, t)u_0(t)dt$ (10)

And so on.

The nth term is,

$u_n(x) = f(x) + \lambda \int_0^x k(x, t)u_{n-1}(t)dt$ (11)

Because of continuity of $f(x)$ and $k(x, t)$, the sequences $\{u_{n(x)}\}$ converges to $n \to \infty$ and thus the solution $u(x)$ is obtained [3].

Laplace Transform Method –

The transform of a function $f(t)$ is defined by

$L^*F(t)+ = \int_0^\infty f(t)e^{-st}\,dt = F(s), t \ge 0$ (12)

Where s is real or complex.[10]

Laplace transform of some basic mathematical functions [8]-

Sr.No.	F(t)	L[F(t)]=f(s)
1	1	$\frac{1}{s}$
2	t	$\frac{1}{s^2}$
3	t^2	$\frac{2!}{s^3}$
4	$t^n, n > -1$	$\frac{(n+1)}{s^{(n+1)}}$
5	e^{at}	$\frac{1}{s-a}$
6	$\sin at$	$\frac{a}{s^2 + a^2}$
7	$\cos at$	$\frac{s}{s^2 + a^2}$
8	$\sinh at$	$\frac{a}{s^2 - a^2}$
9	$\cosh at$	$\frac{s}{s^2 - a^2}$

Properties of Laplace Transform [9] **–**

Sr. No.	Name of Properties	Mathematical Form
1	Linearity	L,af(t) + bg(t)- = aL,f(t)- + b,g(t)-
2	Change of Scale	$L,f(at)- = \frac{1}{a} f(\frac{s}{a})$
3	Shifting	$L[e^{at}f(t)- = f(s-a)$
4	First derivative	$L,f'(t)- = sf(s) - f(0)$
5	Second derivative	$L,f''(t)- = s^2f(s) - sf(0) - f'(0)$
6	nth derivative	$L,f^n(t)- = s^nf(s) - s^{n-1}f(0) - s^{n-2}f'(0) - \cdots - f^{n-1}(0)$

Example I -

Volterra Integral Eqaution-

Reduce the following initial problems to volterra integral equations of the second kind and solve successive approximation method.

$$\frac{d^2y}{dx^2} + y = 0, y(0) = 0, y'(0) = 1 \quad (13)$$

Solution- Let $\frac{d^2y}{dx^2} = u(x)$ (14)

Integrating equation w.r.t. x from 0 to x, we get

$$\frac{dy}{dx} - y'(0) = \int_0^x u(x)dx$$

$$\frac{dy}{dx} = 1 + \int_0^x u(x)dx \quad (15)$$

Again integrating w.r.t. x from 0 to x, we get

$$y(x) - y(0) = x + \int_0^x u(x)dx^2$$

$$y(x) = x + \int_0^x u(t)dt^2$$

$$y(x) = x + \int_0^x (x-t)u(t)dt \quad (16)$$

Substitute equation (14) & (16) in equation (13) we get,

$$u(x) = -x - \int_0^x (x-t)u(t)dt \quad (17)$$

This is volterra integral equation of secondkind.

By using successive approximation method, Equation (17) comparing with,

$$u(x) = f(x) + \lambda \int_0^x k(x,t)u(t)dt$$

Here $f(x) = -x, \lambda = -1, k(x,t) = (x-t)$

The nth order successive approximation,

$$u_n(x) = f(x) + \lambda \int_0^x k(x,t)u_{n-1}(t)dt$$

$$u_n(x) = -x - \int_0^x (x-t)u_{n-1}(t)dt \quad (18)$$

Put $n = 1$ in equation (18), we get

$$u_1(x) = -x - \int_0^x (x-t)u_0(t)dt$$

$$u_1(x) = -x \quad (19)$$

Put $n = 2$ in equation in (18), we get

$$u_2(x) = -x - \int_0^x (x-t)u_1(t)dt$$

$$u_2(x) = -x - \int_0^x (x-t)(-t)dt$$

$$u_2(x) = -x + \frac{x^3}{3!} \quad (20)$$

Put $n = 3$ in equation (18) we get

$$u_3(x) = -x - \int_0^x (x-t)u_2(t)dt$$

$$u_3(x) = -x - \int (x-t)(-t + \frac{t^3}{3!})dt$$

$$u_3(x) = -x + \frac{x^3}{3!} - \frac{x^5}{5!} \quad (21)$$

$$u_4(x) = -x + \frac{x^3}{3!} - \frac{x^5}{5!} + \frac{x^7}{7!} \text{ \& so on.}$$

$$u_n(x) = -(x - \frac{x^3}{3!} + \frac{x^5}{5!} - \cdots + (-1)^n \frac{x^{2n+1}}{(2n+1)!})$$

$$u_n(x) = \sum_{n=0}^{\infty} (-1)^{n+1} \frac{x^{2n+1}}{(2n+1)!}$$

$$u(x) = \lim_{n\to\infty} \sum_{k=0}^{n} (-1)^{n+1} \frac{x^{2n+1}}{(2n+1)!}$$

$$u(x) = -\sin x \quad (22)$$

Laplace Transform Method-

Solve following initial value problem to Laplace transform method.

$$\frac{d^2y}{dx^2} + y = 0, y(0) = 0, y'(0) = 1$$

Solution- Taking Laplace transform both sides, we get

$$L[y''(x)] + L[y(x)] = 0$$

$$s^2Y(s) - sy(0) - y'(0) + Y(s) = 0$$

$$Y(s)(s^2 + 1) = 1$$

$$Y(s) = \frac{1}{s^2+1} \quad (23)$$

Shortcut Method of differential Equation-

Solution by shortcut method for differential equation $\frac{d^2y}{dx^2} + y = 0, y(0) = 0, y'(0) = 1$

Solution- Put $D = \frac{d}{dx}$ in given equation,

$$(D^2 + 1)y = 0$$

Auxiliary equation is,

$$(D^2 + 1) = 0$$

$$D = \pm i$$

Solution of differential equation is,

$$y(x) = c_1 \cos x + c_2 \sin x \quad (24)$$

But $y(0) = 0$ & $y'(0) = 1$ in equation (24), we get

$c_1 = 0$ & $c_2 = 1$

Equation (24) becomes,

$$y(x) = \sin x \quad (25)$$

Analytic Method-

Solution by general method for differential equation $\frac{d^2y}{dx^2} + y = 0, y(0) = 0, y'(0) = 1$

Solution- Given Differential equation is homogeneous.

We will assume that,

$y = e^{rx}$

$y'' = r^2e^{rx}$

Substitute in given equation, we get,

$r^2e^{rx} + e^{rx} = 0$

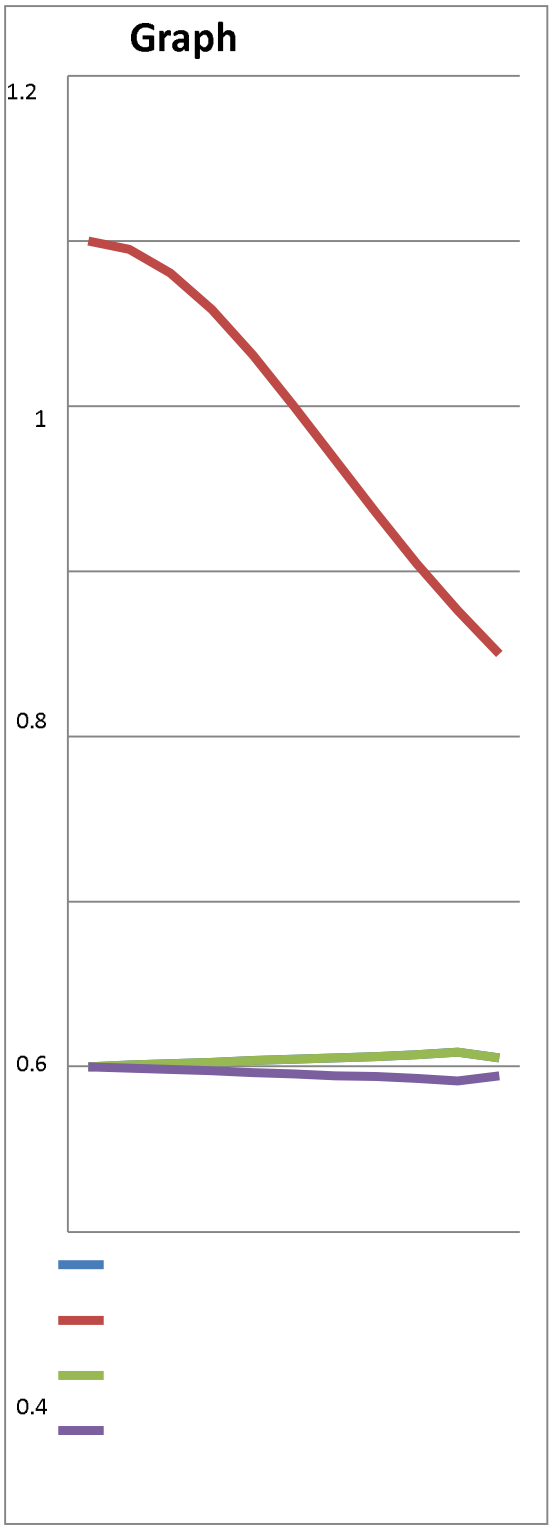
$r = \pm i$

The general solution of given differential equation is,

$(x) = c_1 \cos x + c_2 \sin x$ (26)

$y(0) = 0$ then $c_1 = 0$ $y'(0) = 0$ then $c_2 = 1$ Equation (26) becomes,

$(x) = \sin x$ (27)



Find out gap between given differential equation by using Laplace transform,Shortcut Method of Linear Differential equation and Volterra integral equation by using Successive Approximation Method

x	Exact Solution	Solution of Laplace Transform	Solution of Differential equation	Solution of Volterra Integral Equation by Successive Approximation Method
0	0	1	0	0
0.1	0.0017	0.9901	0.0017	-0.0017
0.2	0.0035	0.9615	0.0035	-0.0035
0.3	0.0052	0.9174	0.0052	-0.0052
0.4	0.007	0.8621	0.007	-0.007
0.5	0.0087	0.8	0.0087	-0.0087
0.6	0.0105	0.7353	0.0105	-0.0105
0.7	0.0122	0.6711	0.0122	-0.0122
0.8	0.014	0.6098	0.014	-0.014
0.9	0.0175	0.5525	0.0175	-0.0175
1	0.0105	0.5	0.0105	-0.0105

Example II -

Volterra Integral Eqaution-

Reduce the following initial problems to volterra integral equations of the second kind and solve successive approximation method.

$$\frac{d^2y}{dx^2} - y = \sin x, y(0) = 0, y'(0) = 0 \quad (28)$$

Solution- Let $\frac{d^2y}{dx^2} = u(x)$ (29)

Integrating equation w.r.t. x from 0 to x, we get

$$\frac{dy}{dx} - y'(0) = \int_0^x u(x)dx$$

$$\frac{dy}{dx} = \int_0^x u(x)dx$$

Again integrating w.r.t. x from 0 to x, we get

$$y(x) - y(0) = \int_0^x u(x)dx^2$$

$$y(x) = \int_0^x (x-t)u(t)dt \quad (30)$$

Substitute equation (29) & (30) in equation (28) we get,

$$u(x) = \sin x + \int_0^x (x-t)(t)dt \quad (31)$$

This is volterra integral equation of second kind.

The nth order successive approximation,

$$u_n(x) = f(x) + \lambda \int_0^x k(x,t)u_{n-1}(t)dt$$

$$u_n(x) = \sin x + \int_0^x (x-t)u_{n-1}(t)dt \quad (32)$$

Put $n = 1$ in equation (32) we get

$$u_1(x) = \sin x$$

$$u_2(x) = x$$

$$u_3(x) = \sin x + \frac{x^3}{3!}$$

$$u_4(x) = x + \frac{x^5}{5!}$$ And so on.

$$u(x) = \sin x + \sum_{n=1}^{n} \frac{(4n-1)}{(4n-1)!} + \sum_{n=0}^{n} \frac{(4n+1)}{(4n+1)!}$$

$$y(x) = \lim_{n\to\infty} u_n(x)$$

$$y(x) = \sin x + 2x^4 \quad (33)$$

Laplace Transform Method-

Solve following initial value problem to Laplace transform method.

$$\frac{d^2y}{dx^2} - y = \sin x, y(0) = 0, y'(0) = 0$$

Solution- Taking Laplace transform both sides, we get

$$L[y''(x)] - L[y(x)] = L[\sin x]$$

$$s^2\bar{y}(s) - sy(0) - y'(0) - \bar{y}(s) = \frac{1}{s^2+1}$$

$$\bar{y}(s) = \frac{1}{(s^2+1)(s^2-1)} \quad (34)$$

Shortcut Method of differential equation-

Solution by shortcut method for differential equation $\frac{d^2y}{dx^2} - y = \sin x, y(0) = 0, y'(0) = 0$

Solution- Auxiliary equation is,

$$(D^2 - 1) = 0$$

$$D = \pm 1$$

Solution of differential equation is,

$$y(x) = c_1 e^x + c_2 e^{-x} \quad (35)$$

Particular Integral is,

$$P.I = -\frac{1}{2} \sin x \quad (36)$$

Solution of Differential Equation is,

$$y(x) = c_1 e^x + c_2 e^{-x} - \frac{1}{2}\sin x \quad (37)$$

But $y(0) = 0$ & $y'(0) = 0$ in equation (37), we get $c_1 = \frac{1}{2}$ & $c_2 = -\frac{1}{2}$

Equation (37) becomes,

$$y(x) = \frac{1}{2} e^x - \frac{1}{2} e^{-x} - \frac{1}{2}\sin x \quad (38)$$

Analytic Method-

Solution by general method for differential equation $\frac{d^2y}{dx^2} - y = \sin x, y(0) = 0, y'(0) = 0$

Solution- Complimentary Function is,

$$C.F. = c_1 e^x + c_2 e^{-x} \quad (39)$$

Particular Integral is

$$P.I = -2\sin x \quad (40)$$

The general solution is

$$y(x) = c_1 e^x + c_2 e^{-x} - 2\sin x \quad (41)$$

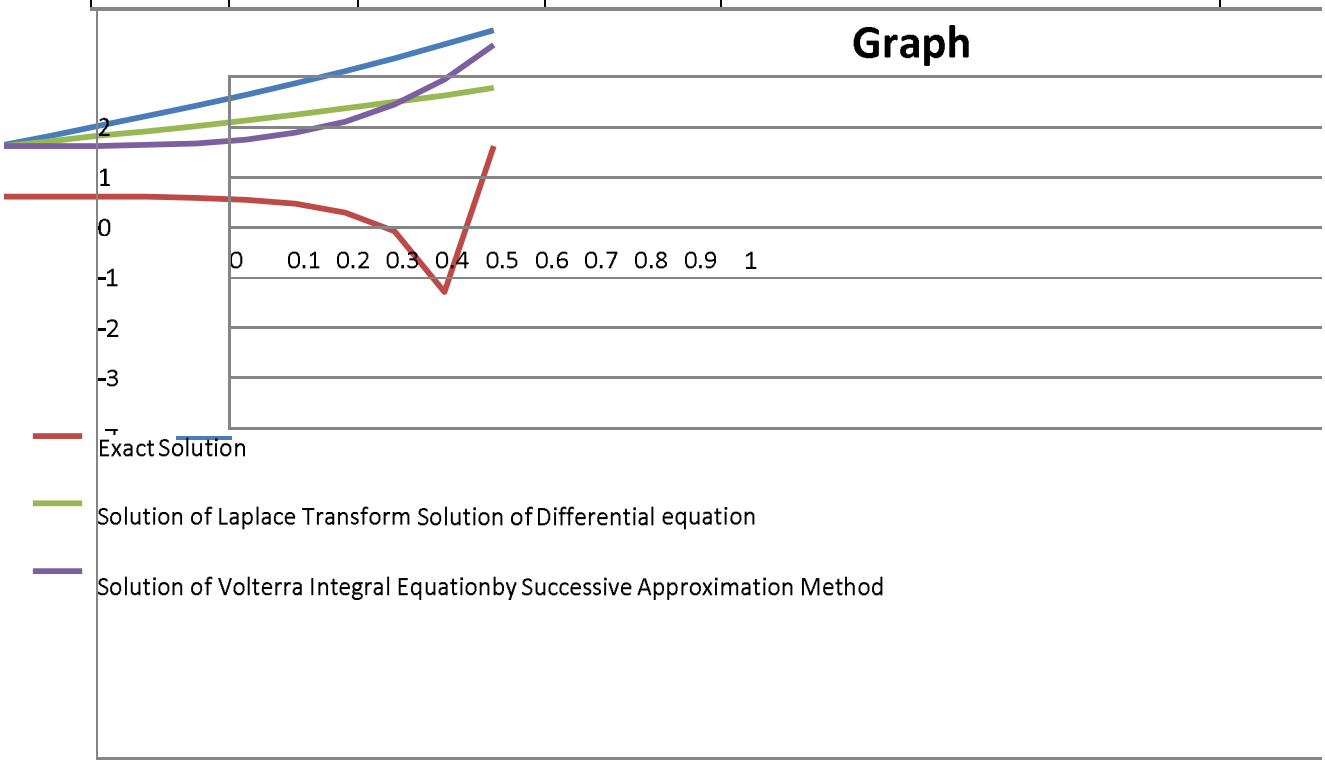
But $y(0) = 0$ & $y'(0) = 0$ in equation (41), we get $c_1 = 1$ & $c_2 = -1$

Equation (41) becomes,

$$y(x) = e^x - e^{-x} - 2\sin x \quad (42)$$

Find out gap between given differential equation by using Laplace transform, Shortcut Method of Linear Differential equation and Volterra integral equation by using Successive Approximation Method

x	Exact Solution	Solution ofLaplace Transform	Solution of Differential equation	Solution of Volterra Integral Equation by Successive ApproximationMethod
0	0	-1	0	0
0.1	0.1968	-1.0001	0.0993	0.0019
0.2	0.3957	-1.0016	0.1996	0.0067
0.3	0.5986	-1.0082	0.3019	0.0214
0.4	0.8075	-1.0263	0.4073	0.0582
0.5	1.0247	-1.0667	0.5167	0.1337
0.6	1.2524	-1.1489	0.6314	0.2697
0.7	1.4927	-1.316	0.7525	0.4924
0.8	1.7483	-1.6938	0.8811	0.8332
0.9	2.0216	-2.9078	1.0187	1.3279
1	2.3155	?	1.1665	2.0175



Conclusion-

In our review study of Integral Equations we solve some numerical with the help of integral equations and Laplace Transform method .We found that the solutions obtained by various methods are very closure at closure to exact solutions. An integral equation gives less error comparative to other method'

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Analytical approaches for prohibited drug profiling in Forensic Science - A Review

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Abstract: -

Drugs identifying illicit substances provides an overview of the range and complexity of forensic drug examination. Without accurate identification of suspected drugs, the law cannot be enforced. Unambiguous detection and identification of drugs and related compounds in solids, powders and liquids is a key area of forensic investigation and drug screening. Analytical approaches need to be accurate, detect drugs and other toxins at low levels in small samples, and provide results quickly.

Keeping pace with illicit drugs known as New Psychoactive Substances (NPS), also referred to as 'legal highs,' presents a huge challenge to border control authorities and police forces across the globe. The police, customs authorities, governmental and toxicology laboratories involved in forensic analysis require state-of-the-art scientific instruments for the unambiguous detection of suspicious materials that have been seized. Analytical methods to check suspicious substances must deliver unambiguous identification and proper quantification of seized compounds providing data robust enough for submission in legal proceedings. In the field of forensics, the chemical and physical identification of unknown substances like organic and inorganic impurities is an emerging challenge in illicit drug. Nowadays new advanced and powerful analytical techniques available for illicit drugs including such as AI, NMR, IR spectroscopy and chromatographic tools for illicit drug profiling.

An overview of illicit drug analysis, including an appreciation of drug profiling and clandestine laboratory analysis is provided. When identifying an illicit substance, it is essential to adhere to well accepted, and reliable methodologies to ensure that only robust, accurate and defensible analytical results are obtained. This practice uses a variety of chemical analysis methods to conduct both presumptive and confirmatory tests on seized material suspected to be or contain illegal substances.

Introduction:

According to the World Drug Report 2023, 36 million people had used amphetamines, 22 million had used cocaine, 20 million had used "ecstasy"-type substances and 60 million people engaged in non-medical opioid use in 2021, of whom 31.5 million used opiates, mostly heroin. After several years of steadiness, the number of new psychoactive substances (NPS) on the global market increased in 2021. Out of 618 NPS reported to be on the global market in 2021, 87 were recently identified. Additionally, as per the world drug report 2023, the market for "Captagon", an illicitly manufactured tablet commonly containing different concentrations of amphetamine, continues to grow in the near and Middle East. In parallel, an ethamphetamine market is developing in the near and Middle East shown through an increase in seizures of the drug [1]

During Covid-19 pandemic, illicit drug markets were resilient. Traffickers adapted to the pandemic context by changing their modes of transportation and trafficking. Since drug trafficking by air was entirely disrupted by restrictions imposed on air travel, there was an increase in the use of maritime routes to traffic heroin to Europe [2].

Furthermore, illicit drug trade continues to hold back economic and social development and constitutes a fundamental threat to security and stability in some parts of the world. In 2019, the most significant market growth was in synthetic drugs, mainly synthetic NPS, opioids (semisynthetic or synthetic opioids) and Amphetamine-Type Stimulants (ATS). Khat (seized in Arabian Peninsula, North America, Europe, and Africa) was the most seized plant based NPS, followed by kratom found in Malaysia and Thailand, then ayahuasca, kava and Salvia divinorum in descending order [3].

Previously, in Australia, illicit drugs such as heroin, cocaine, methamphetamine and 3, 4-Methylenedioxymethamphetamine (MDMA) were usually seized at the border via air transport (85–99% of seizure numbers for drugs). However, with the pandemic and reduced air travel markets, demand moved toward other transported, in expensive and advanced substitutes for heroin or diluted drugs with different and possibly hazardous chemicals [4].

Looking at all the new approaches in illicit drug trafficking mentioned above, law enforcement faces several challenges related to illicit drug seizures. Thus, the implementation of novel methodologies is needed to prevent crimes related to illicit drugs and identify suspects involved in

illicit drug manufacturing and trafficking. This paper firstly presents an overview of traditional process followed for seized illicit drugs profiling and discusses the information sought by traditional analysis, expected results and limitations.

The prohibited list of 2022 delineates performance-enhancing substances into multiple categories, such as "Prohibited at all times", "Prohibited in-competition", and "Prohibited in particular sports". The aforementioned categories further contain drugs of distinct classes, such as anabolic androgenic steroids, cannabinoids, and beta-blockers respectively.

Physical and Chemical Illicit Drug Profiling

Drug profiling is recognized as ''the extraction of a drug sample's chemical and physical profile, to be used in the application of policies against the illegal use of drugs (law enforcement, legislation, public health)'' [5]. Generally, chemical profiling provides evidence about the illicit substance along with adulterants, diluents, precursors, by-products, impurities, and solvents.8 In contrast, physical profiling of drugs includes packaging and appearance of a drug, thus adding complementary information to chemical profiles [6].

To generate chemical drug profiles, forensic chemists have the choice between several analytical techniques depending on the scope of analysis and the sample's characteristics. Since our manuscript focuses on analytical approaches for prohibited drug profiling, we will briefly mention some analytical techniques commonly applied for illicit drug chemical profiling. Among these, for inorganic profiling, inductively coupled plasma mass spectrometry (ICP-MS) offers an elemental profile of numerous illicit drugs comprising ATS, cocaine and heroin, thus revealing information regarding a drug's origin and synthesis route [7,8] For organic profiling, gas chromatography-mass spectrometry (GC-MS), the gold standard for illicit drug profiling, and gas chromatography-flame ionization detection (GC-FID) detect manufacturing byproducts providing evidence on trafficking paths, supply origin and link seizures [9-11]. Adulterants and diluent analysis can also be carried out by GC-MS and Fourier-transform infrared spectroscopy (FTIR) [12]. Moreover, ultra high-performance liquid chromatography (UHPLC), liquid chromatography-mass spectrometry (LC-MS or MS-MS) and isotope ratio mass spectrometry (IRMS) are considered as powerful tools in forensic investigations regarding drug profiling and determination of illicit drug's origin [6,13–17]. UHPLC is specifically suitable for heroin profiling where impurities as low as 0.02% could be detected allowing the determination of a

heroin sample's origin [18]. Through LC-MS, data on the origin of ephedrine and pseudoephedrine, precursors of MDMA, could be identified [19]. Furthermore, IRMS profile reflects the plants' environmental and growth conditions of natural illicit drugs collected from

Forensic Intelligence – The intelligent Use of Forensic Data

The intelligence cycle is the process of converting raw information into finished intelligence applicable for policy makers and law enforcement in building judgements.

This cycle starts with the collection of illicit drugs and ends with re-evaluation. Indeed, data can be collected from many sources (crime scenes, witnesses, and reports) then examined and evaluated in order to determine its validity and reliability and converts into information.

Following, information is combined and connected to existing information already recorded and stored. The structured collection of information is continuously analysed to add value, detect new patterns, and test hypotheses. This phase is critical, and its results add value to information, called intelligence [20].

For law enforcement and police, forensic intelligence's primary purpose is to link data to acquire a complete illustration of recurring criminal actions and stop a criminal from committing further crimes. Indeed, Cartier illustrated the procedure of forensic intelligence from data/trace to information/sign until intelligence. Data itself does not have any value and may be masked by other conflicting or interrupting data points. By extracting pertinent facts for analysis, these raw data are selected, processed, and converted into evidence that may reconstruct a case or identify possible connections between instances to detect potential links between seizures. Based on Cartier's illustration, intelligence's results yield to logical, Significant, timely and accurate conclusions [21].

Tactical intelligence (reactive micro-level of forensic intelligence) supports frontline enforcement officers to decide on special cases and is relevant to thorough investigations. In contrast, operational intelligence (meso-level) supports crime's decrease and decision-makers responsible for geographical areas [22]. Despite all that was mentioned, Ribaux et al., 2006, argued the contribution of intelligence in forensic science and initiated an intensive modelling program starting with a 'bottom-up' approach and ending with the identification of valuable primitive

inference entities (forensic conclusions such as a DNA profile or a suspect's confirmed visual description). Such entities can be identified and integrated into special

procedures of serial crime analysis to generate valuable and timely intelligence [23].

Forensic Intelligence Drug Profiling Among Countries

Several official international entities aim to develop the forensic examination of seized illicit drugs. Among these, since 1997, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has been dedicated for improving the practical recommendations for the examination of seized drugs [24].

Generally, Drugs' type and purity are determined through analysis to investigate whether seized

drugs at Australian borders are illegal. Based on AFP regulations, different drug characteristics are determined, including size, weight, color, logo, organic and inorganic impurities, and adulterants. Altogether, these extracted features constitute a drug profile, providing information

about the manufacturer, the distribution process, and the market's size. These profiles are incorporated into a database and compared to existing profiles in AFP to define relations among samples. Next, data can be interpreted in the circumstance of existing crime to acquire conclusive intelligence affecting illicit drugs' trade [25]. As an example of forensic intelligence for drug profiling, Morelato et al. examined the chemical profiling of MDMA. They realized a match between a related sample (inside seizures) and unrelated samples (among diverse seizures) using correlation coefficients [26]. Links among seizures were detected using GC-MS, a crucial technique for operational intelligence purposes.

Illicit drug profiling differs between countries and organizations. For example, in Australia, Australian Federal Police (AFP) is the organization handling the import or export of illicit drugs seized across Australian borders [27]. Moreover, in Switzerland, illegal drugs are analysed in different laboratories without having a centralized laboratory leading to a lack of harmonization since every laboratory has its own infrastructure and sample analysis requested by investigating magistrates [28]

Conclusion and Future work

In the present paper, we have first overviewed briefly the traditional physical and chemical illicit drug profiling in a forensic context. Drugs with similar profiles can be different regarding the chain of production, trafficking pathway, supply, and market distribution. Consequently, introducing new approaches for illicit drug profiling is required, and forensic intelligence application in this context is one efficient approach [29]

We discussed the forensic intelligence and its application in illicit drug investigations where the systematic linkage of illicit substances through their physical and/or chemical profiles could be associated with cases that were previously the object of separate investigations.

Indeed, each new specimen is compared with existing data in an intelligence cycle and organized in a memory built upon earlier seizures or known origin ref [30].

Given the level of novel psychoactive substances (NPSs) on the market, through iso- topic measurements, IRMS has been developed as a prospective procedure to assume the crop growing location of drugs, estimate trafficking means, and understand the synthetic pathways and chemicals used in clandestine laboratories. IRMS, in combination with developed chromatographic techniques such as GC, has provided developments in various areas of forensic science, and forensic chemistry is one of them. For trace amounts of sample, GC- IRMS is more appropriate than IRMS. One of the principal challenges in IRMS is the choice and use of suitable matrix harmonized and well- described reference materials (RMs). The gold standard for the chemical profiling of seized illicit drugs is GC- MS. Trace levels of drugs originating from different sources can be identified and links between seizures can be established [31]. The last few years have seen important advances in RMs for the calibration of δ^2H, $\delta^{18}O$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values [32].

There are several other spectroscopic techniques for the analysis of illicit drugs including ultraviolet–visible (UV- Vis), infrared (IR), Raman and scanning electron micro- scope coupled with energy- dispersive X- ray (SEM- EDX) spectroscopy that are well known in the literature. However, to be accepted in court, these techniques must be broadly acknowledged as appropriate for the particular type of drug in question. It must be noted that the development of suitable spectral databases remains a critical issue for the effective use of these spectroscopic techniques. Spectral libraries continue to expand and must accommodate new forms of materials likely to be

encountered. For example, the continuing evolution of illicit synthetic drugs means that the spectra of new chemicals must be included in databases to ensure effective identification. The myriad of techniques available can also pose another issue, as data may not be comparable if different methodologies are used. This can be the case with heroin and cocaine profiling for example, where samples are profiled differently depending on the region in which they are being analysed [33].

To help law enforcement and forensic investigators face all the challenges related to illicit drug trafficking and production, scientists should continuously develop new approaches to tackle the emerging types of illicit drugs. Finally, the authors suggest the emergence of an international collaborative initiative of forensic scientists to set standard profiling methodologies when investigating illicit drugs. This initiative could greatly enhance profiling efforts globally, especially since drug networks can span over a large area thousands of kilometres from the manufacturer's location.

Future work has discussed the application of AI to digital forensics with a particular focus on forensic drug testing. Overview of data-related challenges one may face when implementing an AI solution including many features (e.g. pieces of evidence), missing data, multiple conflicting decision criteria, and the need for interactive learning. Different techniques for dealing with these challenges were reviewed and applications in digital forensics were highlighted. A case study on a forensic science company to demonstrate real challenges of forensic reporting and the potential for AI to design a trustworthy automated system to present generated evidence in the court of law. The purpose important future directions for adopting AI techniques to address challenges in digital forensics. These include, first and foremost, the validation of the manually derived decision trees. It would be interesting to derive decision trees automatically using the available data. These trees could differ from the manually derived trees, and thus reveal alternative drivers and potential hidden biases. Another direction is the development of more advanced AI methods including belief or fuzzy rule-based models. To make these data-driven models more accurate, one can also investigate systematic ways of merging with knowledge base and rules by experts. Thus, updating the rules can be done in an interactive fashion, for example as and when new scientific insight from chemistry becomes available. Certainly, these directions of future research are relevant for forensics in drug testing but also for digital forensics in general.

Subclass of Bi-univalent Functions Associated With q-differential Operator

RENU PRAVEEN PATHAK[1], BALASAHEB BAPPAJI GADEKAR[2]

Abstract. In this present study, we employ the definition of q-differential operator, to establish novel subclasses of bi-univalent functions. Subsequently, we derive bounds for the initial Taylor-Maclaurin coefficients applicable to these newly defined subclasses.

Keywords: Analytic functions; bi-univalent function; q-differential operator.

1. Introduction

Let $\mathcal{H}(\mathbb{D})$ represent the class of all functions analytic in the unit disk $\mathbb{D} = \{z \in \mathbb{C} : |z| < 1\}$, and also denote by $\mathcal{A}$ the subclass of $\mathcal{H}(\mathbb{D})$ comprising of functions of the form

$$f(z) = z + \sum_{n=2}^{\infty} a_n z^n \quad z \in \mathbb{D}, \tag{1}$$

which are normalized by the condition $f(0) = f'(0) - 1 = 0$.

A domain $\mathbb{D}$ is an open connected subset of the complex plane $\mathbb{C}$. A complex-valued function f(z) of a complex variable is called univalent in a domain $\mathbb{D}$ if it does not take the same value twice, so that for z_1 and z_2 in $\mathbb{D}$, $f(z_1) \neq f(z_2)$ exists. A univalent function, also known as a one-to-one function or injective function, is a type of function in complex analysis that maps each point in its domain to a single point in the range. In other words a function is said to be univalent, if it never maps two different points in its domain to the same point in its range

The class of all analytic functions $f(z)$ defined on unit disk $\mathbb{D} = \{z \in \mathbb{C} : |z| < 1\}$ with normalization $f(0) = 0$ and $f^{'}(0) = 1$ refers by $\mathcal{S}$. Normalization transforms the family $\mathcal{S}$ into a compact normal family under uniform convergence topology. The Taylor's series expansion of functions in $\mathcal{H}(\mathbb{D})$ in the open unit disk $\mathbb{D} = \{z \in \mathbb{C} : |z| < 1\}$ is given by (1). The collection of all such functions is denoted by $\mathcal{S}$. For $f(z) = \sum_{n=1}^{\infty} a_n z^n$ and $g(z) = \sum_{n=1}^{\infty} b_n z^n$, $z \in \mathbb{D}$, which are two analytic functions in $\mathbb{D}$, the *Hadamard (or convolution) product* of f and g is defined by

$$(f * g)(z) = \sum_{n=1}^{\infty} a_n b_n \, z^n \quad z \in \mathbb{D}. \tag{2}$$

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1.1. Starlike and Convex Functions.

Definition 1.1. *(Duren 1983) A domain $\mathbb{D}$ of the complex plane $\mathbb{C}$ is considered starlike with respect to a point z_0 in $\mathbb{D}$ if, the line segment connecting z_0 to any point z in $\mathbb{D}$ lies within $\mathbb{D}$ itself. That is for any z in $\mathbb{D}$ with $0 \leq t \leq 1$, $tz_0 + (1 - t)z$ is in $\mathbb{D}$. If f in $\mathcal{H}(\mathbb{D})$ maps $\mathbb{D} = \{z \in \mathbb{C} : |z| < 1\}$ onto a starlike domain with regard to the origin, then f in $\mathcal{H}(\mathbb{D})$ is starlike with respect to the origin. S^* denotes the subclass of functions f in $\mathcal{H}(\mathbb{D})$ that are starlike univalent with respect to the origin.*

Theorem 1.1. *(Goodman 1983) A necessary and sufficient condition for a function f(z) to be in the class S^**

$$R\left\{\frac{zf'(z)}{f(z)}\right\} > 0, \tag{3}$$

where, z is in $\mathbb{D}$.

Definition 1.2. *(Robertson 1936) A function f in $\mathcal{H}(\mathbb{D})$ is called starlike of order δ, $(0 \leq \delta < 1)$, if*

$$R\left\{\frac{zf'(z)}{f(z)}\right\} > \delta,$$

z is in $\mathbb{D} = \{z \in \mathbb{C} : |z| < 1\}$.

Equivalently,

$$\frac{zf'(z)}{f(z)} = \frac{1 + (1 - 2\delta)z}{1 - z}.$$

All such functions is represented by $S^*(\delta)$. Also remember that, $S^*(\delta)$ is contained in S and $S^*(0) = S^*$.

Definition 1.3. *(Duren 1983) A domain $\mathbb{D}$ of the complex plane $\mathbb{C}$ is considered convex if,line joining any two points of $\mathbb{D}$ lies entirely in $\mathbb{D}$.That is for any two poins z_1,z_2 in $\mathbb{D}$ implies that $tz_1 + (1 - t)z_2$ also lies in $\mathbb{D}$ $(0 \leq t \leq 1)$. A function f in $\mathcal{H}(\mathbb{D})$ is called convex in $\mathbb{D}$ if it is function from $\mathbb{D}$ onto convex domain. C represents the collection of all functions of $\mathcal{H}(\mathbb{D})$ which are convex univalent in $|z| < 1$.*

Theorem 1.2. *(Robertson 1936)The following is a necessary and sufficient condition for a function f(z) to be in the class C*

$$R\left\{1 + \frac{zf''(z)}{f'(z)}\right\} > 0 \tag{4}$$

where, z is in $\mathbb{D}$.

Definition 1.4. *(Robertson 1936) A function f in $\mathcal{H}(\mathbb{D})$ is called convex of order δ, $(0 \leq \delta < 1)$, if*

$$R\left\{1 + \frac{zf''(z)}{f'(z)}\right\} > \delta,$$

z is in $\mathbb{D}$.

Equivalently,

$$1 + \frac{zf''(z)}{f'(z)} = \frac{1 + (1 - 2\delta)z}{1 - z}.$$

The class of all such functions of order δ is represented by $C(\delta)$. Note that, $C(0) = C$-class of convex univalent functions.

Theorem 1.3. *Let $f(z) \in \mathcal{S}$, for $(0 \leq \delta < 1)$, $f(z) \in C(\delta)$ if and only if $zf'(z) \in S^*(\delta)$.*

2. Close-to-Convex and Quasi-Convex Functions

In 1952 (Kaplan), introduced and investigated the class of close-to-convex functions. It is a larger univalent subclass of $\mathcal{H}(\mathbb{D})$.

Definition 2.1. *(Kaplan 1952) A function $f(z)$ in $\mathcal{H}(\mathbb{D})$ is called as close-to-convex univalent function if it satisfies following condition*

$$Re\left\{\frac{f'(z)}{g'(z)}\right\} > 0 \quad z \in \mathbb{D}, \tag{5}$$

for some $g(z) \in C$. This class of functions are represented by $\mathcal{K}$.

This definition does not forcing that the function $g(z)$ is normalized, but in most of the results derived for close-to convex functions are assumed that the function $g(z)$ is normalized, so here also we consider $g(z)$ is normalized, $g(0) = g'(0) - 1 = 0$.

Definition 2.2. *(Robertson 1936) A function $f(z) \in \mathcal{H}(\mathbb{D})$ is called as close-to-convex of order β and type δ , if there exists a function $g(z) \in S^*(\delta)$ such that*

$$Re\left\{\frac{zf'(z)}{g(z)}\right\} > \beta, \quad 0 \leq \beta, \quad \delta < 1, \quad z \in \mathbb{D}. \tag{6}$$

Definition 2.3. *A function $f(z) \in \mathcal{H}(\mathbb{D})$ is called as quasi-convex univalent function if there exists a function $g(z) \in C$ such that*

$$Re\left\{\frac{(zf'(z))'}{g'(z)}\right\} > 0, \quad z \in \mathbb{D}. \tag{7}$$

This class is represented as $\mathcal{K}^*$

Remark 2.1. *(Kaplan 1952) proved that every close-to-convex function is univalent. Also, observed that every convex function is starlike and every starlike function is close-to-convex. Therefore we get following inclusion relation*
$\mathbb{C} \subset S^* \subset \mathcal{K} \subset S$.

3. Bi-Univalent Function

Definition 3.1. *Let $f(z) \in \mathcal{H}(\mathbb{D}), f(z) = z + \sum_{n=2}^{\infty} a_n z^n$, $z \in \mathbb{D}$, then $f(z)$ in $\mathbb{D}$ is said to be bi-univalent if both $f(z)$ and $f^{-1}(z)$ are in unit disk $\mathbb{D}$.*

The class of bi-univalent function is denoted by $\sum$. A systematic study of the class of bi-univalent function in $\mathbb{D}$, which is introduced in 1967 by Lewin [1]. For a brief history and interesting examples of functions which are in (or which are not in) the class $\sum$ together with various other properties of the bi-univalent function class $\sum$ one can refer to the work of Kamble et al. [2].

Definition 3.2. *Let $f(z) \in \mathcal{H}(\mathbb{D}), f(z) = z + \sum_{n=2}^{\infty} a_n z^n$, $z \in \mathbb{D}$, , then $f(z)$ is strongly bi-starlike of order α in $\mathbb{D}$ if for $0 < \alpha \leq 1$,following properties should satisfied $f(z) \in \sum$ and*

$$\left|arg\left[\frac{zf'(z)}{f(z)}\right]\right| < \frac{\alpha\pi}{2}; \qquad (z \in \mathbb{D}) \tag{8}$$

and

$$\left| arg\left[\frac{\omega g'(\omega)}{g(\omega)}\right]\right| < \frac{\alpha\pi}{2}; \qquad (\omega \in \mathbb{D}) \tag{9}$$

where $h(z)$ is the inverse of $f(z)$.

4. q-Calculus

Recently many newsworthy results related to subclass of analytic functions and q-operators are meticulously studied by various authors ([3], [5], [4]).

Definition 4.1. *For $0 < q < 1$ the q-derivative of a function $f(z) = z + \sum_{n=2}^{\infty} a_n z^n$, $z \in \mathbb{D}$,*

$$D_q(z) = \begin{cases} \dfrac{f(qz) - f(z)}{(q-1)z} & for\ z \neq 0, \\ f'(z) & for\ z \neq 0. \end{cases} \tag{10}$$

Remember that $\lim_{q\to 1} D_q(z) = f'(z)$

From equation (11) we deduce that,

$$D_q(z) = 1 + \sum_{k=2}^{\infty} [k]_q\, a^k z^{k-1}, \tag{11}$$

where,

$$\lim_{q\to 1} [k]_q = \frac{1-q^k}{1-q} = 1 + q + ... + q^k \to k.$$

Definition 4.2. *The function $f(z) = z + \sum_{n=2}^{\infty} a_n z^n$, $z \in \mathbb{D}$, is in the class $f(z) \in S_q^*(\alpha)$, $0 < q < 1$, $0 \leq \alpha < 1$ if the following conditions satisfied, for $f(z) \in \Sigma$,*

$$f(z) \in \Sigma, \quad \frac{z(D_q f(z))}{f(z)} > \beta \qquad (0 \leq \beta < 1, z \in \mathbb{D}) \tag{12}$$

and

$$f(z) \in \Sigma, \quad \frac{z(D_q h(\omega))}{h(\omega)} > \beta \qquad (0 \leq \beta < 1, z \in \mathbb{D}), \tag{13}$$

where the function $h(z)$ is the extension of $f^{-1}(z)$ to $\mathbb{D}$.

Lemma 4.1. *If $f(z) \in \mathcal{H}(\mathbb{D})$ then $|B_k| \leq 2$ for each $k > 1$ and the inequality is sharp for the function $\dfrac{1+z}{1-z}$.*

5. Coefficients bounds

Definition 5.1. *The function $f(z)$ given by (1) to belong to the class $\mathcal{H}_{q,\varphi}$ $(0 < q \leq 1; 0 < \varphi \leq 1; \psi > 0)$, provided that it satisfies the following conditions:*

$$f \in \mathcal{H}_{q,\varphi}, \quad \arg\left(D_q f(z) + \varphi z (D_q f(z))'\right) < \psi\frac{\pi}{2}, \quad z \in \mathbb{D}, \tag{14}$$

and

$$\arg\left(D_q g(w) + \varphi w (D_q g(w))'\right) < \psi \frac{\pi}{2}, \quad w \in \mathbb{D}, \tag{15}$$

The function g denotes the extension of f^{-1} to $\mathbb{D}$.

Theorem 5.1. *For a function $f(z)$ given by (1) belonging to the class $\mathcal{H}_{q,\varphi}$, the inequalities for $|a_2|$ and $|a_3|$ are given by:*

$$|a_2| \leq \min\left\{\frac{2\rho}{(1+\varphi)\,[2]_p} \leq \frac{2\rho}{\left|2\alpha\,(1+2\varphi)\,[3]_p + (1-\alpha)\,(1+\varphi)^2\,[2]_p\right|}\right\},$$

and

$$|a_3| \leq \frac{4\rho^2}{(1+\varphi)^2\,[2]_p^2} + \frac{2\rho}{2\,(1+2\varphi)\,[3]_p}.$$

Proof. Let $f \in \mathcal{H}_{q,\varphi}$. Then we have

$$\arg\left(D_q f(z) + \varphi z (D_q f(z))'\right) = (M(z))^{\varrho}, \quad z \in \mathbb{D}, \tag{16}$$

and

$$\arg\left(D_q g(w) + \varphi w (D_q g(w))'\right) = (T(w))^{\varrho}, \quad w \in \mathbb{D}, \tag{17}$$

The function g denotes the extension of f^{-1} to U. For $M(z)$ we have

$$M(z) = 1 + M_1 z + M_2 z^2 + M_3 z^3 + \ldots \tag{18}$$

where $M_1, M_2, M_3, \ldots$ are coefficients. For $h(w)$ we have

$$T(w) = 1 + T_1 w + T_2 w^2 + T_3 w^3 + \ldots \tag{19}$$

These forms suggest that $M(z)$ and $T(w)$ are power series expansions around $z = 0$ and $w = 0$, respectively. From Equation (16) and (17), we get

$$(1+\psi)[2]_q a_2 = \varrho M_1 \tag{20}$$

$$(1+2\psi)[3]_q a_3 = \varrho M_2 + \varrho(\varrho-1)\frac{1}{2}M_1^2 \tag{21}$$

$$-(1+\psi)[2]_q a_2 = \varrho T_1 \tag{22}$$

$$(1+2\psi)[3]_q(2a_2^2 - a_3) = \varrho T_2 + \varrho(\varrho-1)\,\frac{1}{2}\,T_1^2 \tag{23}$$

Using equation (20) and (22), we get

$$M_1 = -T_1 \tag{24}$$

Also

$$2\,(1+2\varphi)\,[3]_q\,a_2^2 = \rho\,(M_2 + T_2) + \frac{\rho\,(\rho-1)}{2}\,\left(M_1^2 + T_1^2\right) \tag{25}$$

$$= \rho\,(M_2 + T_2) + \frac{\rho-1}{\rho}\,(1+\varphi)\,[2]_q^2\,a_2^2. \tag{26}$$

Then we have

$$a_2^2 = \frac{\rho^2 (M_2 + T_2)}{2\rho (1+2\varphi) [3]_p + (1-\rho)(1+\varphi)[2]_p^2} \tag{27}$$

Now using Lemma (4.1), we have

$$|a_2|^2 \leq \frac{\rho^2 \left(|M_1^2| + |M_2^2|\right)}{2(1+\varphi)^2 [2]_p^2} \leq \frac{4\rho^2}{(1+\varphi^2)[2]_p^2}.$$

and

$$|a_2^2| \leq \frac{\rho^2 (|M_2| + |T_2|)}{\left|2\rho (1+2\varphi)[3]_p + (1-\rho)(1+\varphi)^2 [2]^2\right|}$$

$$\leq \frac{4\rho^2}{\left|2\alpha (1+2\varphi)[3]_p + (1-\alpha)(1+\varphi)^2 [2]_p^2\right|}.$$

Now to gets the bounds of $|a_3|$ equation (21) from equation (23) , we get

$$a_3 = a_2^2 + \frac{\rho (M_2 - T_2)}{2(1+2\varphi)[3]_p} \tag{28}$$

By substituting the values of a_2^2 from (25) into (28), it follows that,

$$|a_3| \leq \frac{\rho^2 \left(M_1^2 + M_2^2\right)}{2(1+\varphi)^2 [2]_p^2} + \frac{\rho (M_2 - T_2)}{2(1+2\varphi)[3]_p}$$

$$\leq \frac{4\rho^2}{(1+\varphi)^2 [2]_p^2} + \frac{2\rho}{2(1+2\varphi)[3]_p}.$$

This evidently completes the proof. □

Definition 5.2. *The function $f(z)$ given by (1) to belong to the class $\mathcal{H}_{q,\varphi}$ $(0 < q \leq 1; 0 < \varrho \leq 1; \psi > 0)$, provided that it satisfies the following conditions:*

$$f \in \mathcal{N}_{q,\varphi}, \quad \arg\left(D_q f(z) + \varphi z (D_q f(z))'\right) > \beta, \quad z \in \mathbb{D}, \tag{29}$$

and

$$\arg\left(D_q g(w) + \varphi w (D_q g(w))'\right) > \beta, \quad w \in \mathbb{D}, \tag{30}$$

The function g denotes the extension of f^{-1} to $\mathbb{D}$.

Theorem 5.2. *For a function $f(z)$ given by (1) belonging to the class $\mathcal{N}_{q,\varphi}$, the inequalities for $|a_2|$ and $|a_3|$ are given by:*

$$|a_2| \leq \min\left\{\frac{2(1-\beta)}{(1+2\beta)[2]_q}, \sqrt{\frac{2(1-\beta)}{(1+2\beta)[3]_q}}\right\},$$

and

$$|a_3| \leq \frac{4(1-\beta)^2}{(1+\varphi)^2[2]_q^2} + \frac{2(1-\beta)}{(1+2\varphi)[3]_q}.$$

Proof. By definition 5.2 , we get

$$(D_q f(z)) + (\beta z (D_q f(z))') = (\beta) + ((1-\beta))(M(z)), \quad (z \in \mathbb{D},) \tag{31}$$

$$(D_q g(z)) + (\beta \omega (D_q g(\omega))) = (\beta) + ((1-\beta))(T(z)), \quad (\omega \in \mathbb{D}) \tag{32}$$

where $M(z)$ and $T(z)$ have the forms (18) and (19) respectively.
Now equating the coefficients in (31) and (32), we get

$$(1+\varphi)[2]_q a_2 = (1-\beta) M_1 \tag{33}$$

$$(1+2\varphi)[3]_q a_3 = (1-\beta) M_2 \tag{34}$$

$$-(1+\varphi)[2]_q a_2 = (1-\beta) T_1 \tag{35}$$

and

$$(1+2\varphi)[3]_q (2a^2 - 2 - a_3) = (1-\beta) T_2. \tag{36}$$

From (33) and (35), we get

$$M_1 = -T_1 \tag{37}$$

and

$$2(1+\varphi)^2[2]_q^2 a_2^2 = (1-\beta)^2 (M_1^2 + T_1^2). \tag{38}$$

From equation (34) and (36), we get

$$2(1+2\varphi)[3]_q a^2 - 2 = (1-\beta)(M_2 + T_2). \tag{39}$$

Now getting the absolute values of (37) and (38) and using lemma 4.1, we get

$$|a_2|^2 \leq \frac{(1-\beta)^2 \left(|M_1|^2 + |T_1|^2\right)}{2(1+\varphi)^2[2]_q^2} \leq \frac{4(1-\beta)^2}{(1+\beta)^2[2]_q^2}$$

and

$$|a_2|^2 \leq \frac{(1-\beta)(|M_2| + |T_2|)}{2(1+2\varphi)[3]_q} \leq \frac{2(1-\beta)}{(1+2\beta)[2]_q},$$

respectively.

Next in order to find the bound on $|a_3|$ by subtracting (36) from (34), we get

$$a_3 = a_2^2 + \frac{(1-\beta)(M_2 - T_2)}{2(1+2\varphi)[3]_q}.$$

Upon substituting the value of a_2^2 from (38) into (39) and observing that $M_1^2 = T_1^2$, it follows that,

$$a_3 = \frac{(1-\beta)^2 (M_1^2 + T_1^2)}{2(1+\varphi)^2 [2]_q^2} + \frac{(1-\beta)(M_2 - T_2)}{2(1+2\varphi)[3]_q}.$$

By using Lemma 4.1 , we readily get,

$$|a_3| \leq \frac{4(1-\beta)^2}{(1+\varphi)^2 [2]_q^2} + \frac{2(1-\beta)}{(1+2\varphi)[3]_q}. \tag{40}$$

This evidently completes the proof. □

Conclusions

we introduce interesting subclasses $\mathcal{H}_{q,\varphi}$ and $\mathcal{N}_{q,\varphi}$ of bi-univalent functions by q-derivative operator. We obtained the intial coefficient bounds for these subclasses.

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Original Research article

Generalization of Big Data Algorithms: Enhancing Data Visualization and Decision-Making through N. N. and C. P. Models

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Abstract:

In our manuscript, we generalized big data algorithms for different models, leading us to work with new generalizations of big data mathematical models such as the N. N. algorithm and the C. P. algorithm with various data sets. To validate our generalized results, we provided multiple numerical examples along with their error analysis through several iterations.

Furthermore, our research contributes to the field of data visualization, particularly in the context of big data. By employing various visualization techniques and machine learning algorithms, we facilitate improved decision-making processes. This study also focuses on analyzing big data visualization, demonstrating the broader applicability of our approach in data analysis.

Keywords:
Machine Learning, Big Data Analysis, (N.N. Algorithm)Neural Network Algorithm, Archetypoid Analysis (ADA), Educational Data Processing and modeling

1 Introduction

In today's digital age, rapid advancements in computer and network technologies have propelled us into an era of unprecedented data generation and utilization like cybersecurity given by [1] A. Sedik. While often termed the Information Age, it is perhaps more fitting to describe it as the Data Age. Among the myriad forms of data, curves represent a particularly complex subset Z. Huang et. al. [23], necessitating sophisticated tools for visualization, exploration, and structural analysis more we can see [2, 5, 9, 14, 20, 21].

In various applications, especially those involving the analysis of anatomical structures in medicine, it is crucial for metrics to distinguish not only the shape but also the size of curves from W. Cai et. al.[18]. This dual consideration leads to the differentiation between the shape space and the shape and size space. Elastic metrics, which cater to these spaces, are thoroughly discussed in recent literature. For instance, some researchers have proposed innovative metrics that separately account for shape and size contributions, providing a more nuanced analysis.

Paper from W. Khushboo et. al. [7] introduces a scalable methodology to address the

challenges of curve analysis. Our approach integrates two distinct distances within the shape and size space one well-established and the other recently introduced by L. I E. Feng et. al. [8] each designed to differentiate the contributions of shape and size in distance computation. Additionally, we leverage Archetypoid Analysis (ADA) B. Su [2], a novel technique in unsupervised statistical learning, to enhance elastic shape analysis. ADA identifies a set of archetypal curves, enabling the representation of complex data sets through convex combinations of these archetypes, thereby simplifying interpretation even for non-experts.

The analysis of planar shapes often involves selecting landmarks to characterize shapes and align them across different instances. However, this process can be cumbersome and imprecise. By parameterizing the boundary curves of objects, we transform every plane shape into a Riemannian manifold, facilitating more accurate and flexible shape characterization.

To illustrate the efficacy of our methodology, we apply it to both simulated and real-world data sets. A notable application X. Ning [20, 21] involves analyzing foot shape distributions to design ergonomically suitable footwear for the Spanish population. This practical example underscores the utility of our approach in addressing real-world challenges.

The exponential growth of raw data, especially in educational contexts, highlights the importance of effective data processing methods. By extracting valuable insights from vast data sets R. Gupta et. al. [15], we can transform raw data into actionable resources. the study from C. Yadav [3] addresses the need for visualizing college students' career paths (CP), a crucial aspect given the abundance of information yet the lack of clarity in its utilization.

Visualization techniques play a pivotal role in knowledge extraction M. Jiang [9], particularly in the context of big data. Effective data visualization not only aids in processing large volumes of information but also enhances decision-making processes given by K. U. Jaseena [6]. This study contributes to the field of data visualization by focusing on crime data analysis, employing various visualization techniques and machine learning algorithms to facilitate informed decision-making for more we can see [18, 11, 14, 22, 5, 16, 18].

In summary, of our work we divide our research work and presents significant advancements in the analysis and visualization of complex data, with applications ranging from anatomical studies to educational data processing and crime data analysis. By integrating innovative methodologies and visualization techniques, we aim to provide comprehensive tools for data interpretation and application across diverse fields.

2 Supporting Work

College students play a critical role as the future workforce, and their career development has been a significant area of study. Tavabie and Simms analyzed the essential skills and characteristics needed for nonclinical roles, categorizing this information into a comprehensive job description framework with four key levels, which serve as the foundation for career pathways from P. duggel et. al. [13]. Jackson underscored the importance of effective career planning (CP) amid the competitive graduate labor market and prevalent underemployment. His study highlighted how work-study programs positively influence students' career trajectories and improve engagement in CP.

Nordin and Hong explored the impact of career coaching on children's career development. Their research, conducted with 12-year-olds facing career planning challenges, identified four critical themes: understanding occupations, accessing occupational information,

making career choices, and learning about employment through parental occupations [16]. Hung et al. investigated the motivations behind Vietnamese students' decision to study in Taiwan, finding a strong correlation between motivation, career planning, and decision-making processes.

Ying et al. reviewed the application of deep learning (DL) methods in processing clinical data, noting the significant potential of DL in precision medicine despite various challenges. Deep learning, a subset of machine learning focused on multilevel data representation, plays a vital role in big data analytics (BDA). Hordri et al. identified key features that impact the effectiveness of DL methods in BDA, demonstrating that DL remains a vibrant research area within big data[15].

Duncan et al. examined the future directions of biomedical imaging and analysis, particularly the roles of electrical engineers in advancing the field. They highlighted the significance of biomedical imaging as a component of big data and discussed its promising developments M. chen et. al. [10].

Despite extensive research on the career paths of college students, most studies concentrate on course design and teaching experiences, with limited attention given to visualizing CP systems. Our research aims to bridge this gap by developing a visualization system for college students' career pathways, utilizing advanced data analytics and visualization techniques to offer detailed insights and support for career planning.

3 Big Data Algorithms

3.1 Mathematical modeling: N. N. Algorithm:

The structure of a Long Short-Term Memory (LSTM) network is akin to that of a Recurrent Neural Network (RNN). LSTM networks incorporate a "gate" mechanism and cell state concept within their hidden layer calculations. The gate mechanism controls the amount of information input at each time step, decides the extent of state information retention or discarding, while the cell state captures the state information at the current time step. These components ensure the retention of earlier sequence information and facilitate learning long-term dependencies in sequence features [8, 9]. Additionally, the cell state aids in preserving the gradient during training, thus mitigating the issue of gradient vanishing [10].

The hidden layer computation structure of an LSTM is depicted. The formulas for computing the hidden layer at time step t in LSTM include the forget gate ϕ, input gate ι, output gate ω, cell state update λ, and the hidden layer output η.

The equations are as follows:

$$\phi_t = \xi(\Phi_\phi[\eta_{t-1}, \chi_t] + \alpha_\phi)$$

$$\iota_t = \xi(\Phi_\iota[\eta_{t-1}, \chi_t] + \alpha_\iota)$$

$$\omega_t = \xi(\Phi_\omega[\eta_{t-1}, \chi_t] + \alpha_\omega)$$

$$\tilde{\lambda}_t = \tanh(\Phi_\lambda[\eta_{t-1}, \chi_t] + \alpha_\lambda)$$
$$\lambda_t = \phi_t \circ \lambda_{t-1} + \iota_t \circ \tilde{\lambda}_t$$

$$\eta_t = \omega_t \circ \tanh(\lambda_t)$$

where, $\xi(k)(1+\frac{1}{e^k}) = 1$

The sigmoid function in equation (2) is denoted by ξ, $\circ$ represents element-wise multiplication, and Φ and α are weights and biases respectively.

Position embedding, a matrix representing time-step information in the same shape as the input feature, can be a set of trainable variables or a custom matrix to denote differences between time steps. The common method is shown in Formulas (3) and (4).

$$(\text{csc}).\mathbb{PEM}_{\bar{p}os,2i} = \left(\frac{\bar{p}os}{10000^{2i/n}}\right)$$

$$(\text{sec}).\mathbb{PEM}_{\bar{p}os,2i+1} = \left(\frac{\bar{p}os}{10000^{2i/n}}\right)$$

where PEM denotes the position-embedding matrix, pos is the time step index in the PE matrix, i is the feature dimension index, and n is the total number of features.

Batch normalization, an optimization technique, normalizes neuron outputs within the same training batch, stabilizing the distribution of each layer's output and reducing inter-layer dependencies, thereby speeding up neural network convergence. Given a neuron value set $\{k_1, k_2, \ldots, k_n\}$ in a minibatch, the batch-normalization calculation is as follows:

$$\psi = \frac{1}{m}\sum_{i=1}^{m} k_i$$

$$\xi^2 = \frac{1}{m}\sum_{i=1}^{m} (k_i - \psi)$$

$$\hat{k}_i = \frac{k_i - \psi}{\sqrt{\xi^2 + \varepsilon}}$$

$$\text{BN}_{\gamma,\alpha}(k_i) = \gamma\hat{k}_i + \alpha$$

3.2 Mathematical modeling: C.P. Algorithm:

Before entering into the Canopy algorithm, let's define a few key concepts: Let $\Phi = \{d_1, d_2, \ldots, d_n\}$ be a dataset with n data elements. For any $d_i \in \Phi$:

$$\{\zeta_k | \exists \| \zeta_k - d_i \| \leq \Xi_2, \Xi_2 < \Xi_1, \zeta_k \in \Phi, i \neq k\}$$

the set ζ_k is called the non-Canopy candidate center point set.

If:

$$\{\zeta_i | \exists \| \zeta_i - d_i \| \leq \Xi_1, \zeta_i \in \Phi, i \neq j\}$$

then d_i is defined as a Canopy, with Ξ_1 being the Canopy set radius and ζ_i as the center point.

The Canopy algorithm involves two stages: The first stage uses an approximate distance metric to efficiently divide the dataset into multiple subsets. These subsets, called Canopies, may intersect but not entirely overlap. The second stage applies a more precise clustering algorithm on the data within each Canopy. This algorithm, which balances coarse and fine sets, is

particularly suitable for preliminary analysis of high-dimensional data [12, 13].

In the first stage, multiple Canopies are generated, each containing sample data. The algorithm sets a threshold for measuring differences.

As the first stage allows intersection between Canopies, a data object can belong to more than one Canopy, but must be in at least one. The second stage uses conventional clustering algorithms, like k-means, to cluster data within the same Canopy. It is generally assumed that the distance between data points in different Canopies is infinite. An extreme case would be if all data objects are grouped into the same Canopy in the second stage.

3.3 N. N. Algorithm data

The Long Short-Term Memory (LSTM) network, an advanced type of Recurrent Neural Network (RNN), incorporates a gate mechanism and cell state within its hidden layer calculations. This gate mechanism, which includes the forget gate, input gate, and output gate, regulates the flow of information at each time step. The cell state, on the other hand, captures and retains state information across time steps, allowing the network to learn long-term dependencies in sequence data. This functionality ensures the retention of earlier sequence information and aids in mitigating the issue of gradient vanishing during training. The hidden layer computation structure of an LSTM involves several key equations, which together define the operations of the forget gate, input gate, output gate, cell state update, and the hidden layer output.

Position embedding is another crucial aspect, representing time-step information in a matrix that mirrors the input feature shape. This matrix, which can consist of either trainable variables or custom values, helps denote differences between time steps. Commonly used formulas for position embedding demonstrate how to encode time-step information into the model, thereby enhancing its ability to learn temporal patterns.

Batch normalization is a widely used optimization technique in neural networks. By normalizing the outputs of neurons within the same training batch, it stabilizes the output distribution of each layer, reduces inter-layer dependencies, and accelerates convergence. The batch normalization process involves calculating the mean and variance of neuron values, followed by normalization and scaling transformations.

3.4 C. P. Algorithm data

The Canopy algorithm is a two-stage clustering technique designed to handle large and high-dimensional datasets efficiently. In the first stage, the algorithm uses an approximate distance metric to divide the dataset into multiple overlapping subsets known as Canopies. Each Canopy is defined by a center point and a threshold radius, ensuring that data points within the Canopy are within a specified distance from the center. This stage balances computational efficiency and clustering precision by allowing intersections between Canopies, meaning that a data point can belong to multiple Canopies.

In the second stage, the algorithm applies a more precise clustering method, such as k-means, to the data within each Canopy. This approach ensures fine-grained clustering by focusing on subsets of the data rather than the entire dataset. The Canopy algorithm thus provides a balance between coarse and fine clustering, making it particularly suitable for preliminary analysis and clustering of high-dimensional data. This method is advantageous because it reduces the computational complexity of clustering by narrowing down the data points

that need to be clustered together, effectively handling the curse of dimensionality often encountered in large datasets.

By integrating these advanced techniques—LSTM networks for sequence learning, position embedding for temporal data representation, batch normalization for training stability, and the Canopy algorithm for efficient clustering—this research aims to enhance the capabilities of data mining and expert systems in educational and employment guidance applications. The combination of these methods allows for sophisticated data analysis, facilitating the development of robust and intelligent systems that can provide accurate and personalized recommendations for college's career planning and academic guidance.

To integrate the given linear regression formulas into the application of the LSTM and Canopy algorithms, we will use the linear regression as an additional tool for predicting specific values based on known variables. Here's how we can incorporate linear regression into the process:

4 Validation of generallised result

1. Data Preparation - Collect Student Data: Include academic records (grades, courses), extracurricular activities, internship experiences, job application statuses, and demographics (age, gender, major). - Preprocess Data: Normalize numerical data, encode categorical data, handle missing values.

2. Applying the LSTM Algorithm - Sequence Data Preparation: Organize student data into sequences. For example, each student's academic record is a time series of grades over semesters. - Define and Train LSTM Model:

$$\phi_t = \xi(\Phi_\phi[\eta_{(t-1)}, \chi_t] + \alpha_\phi)$$

$$\iota_t = \xi(\Phi_\iota[\eta_{(t-1)}, \chi_t] + \alpha_\iota)$$

$$\omega_t = \xi(\Phi_\omega[\eta_{(t-1)}, \chi_t] + \alpha_\omega)$$

$$\tilde{\lambda}_t = \tanh(\Phi_\lambda[\eta_{(t-1)}, \chi_t] + \alpha_\lambda)$$

$$\lambda_t = \phi_t \circ \lambda_{(t-1)} + \iota_t \circ \tilde{\lambda}_t$$

$$\eta_t = \omega_t \circ \tanh(\lambda_t)$$

- Position Embedding: Use position embedding to represent time-step information. - Batch Normalization: Normalize neuron outputs within the same training batch to stabilize output distribution and accelerate convergence. - Train the Model: Train the LSTM model on the historical data to learn patterns in academic performance and employment trends. - Prediction: Use the trained LSTM model to predict future academic performance and potential employment outcomes.

3. Applying the Canopy Algorithm - Initial Clustering with Canopy: - Define Canopy set radius Ξ_1 and Ξ_2.
- Use approximate distance metric to divide the dataset into multiple subsets (Canopies).
- Refined Clustering with K-Means: Apply k-means clustering within each Canopy to further segment students into more precise clusters.
- Cluster Analysis: Analyze clusters to identify common characteristics and provide personalized recommendations.
4. Applying Linear Regression - Define Variables: - Ξ (dependent variable) and Φ (independent variable).
- Formulas for Prediction: - Predicting Ξ: $\Xi = a + b\Phi$ - Predicting Φ: $\Phi = c + d\Xi$ - Determine Regression Coefficients: Calculate constants a, b, c, d based on the training data. - Use Linear Regression for Specific Predictions: Integrate linear regression to predict specific outcomes based on known variables, complementing the LSTM predictions.

Application
Data Example: - Student Data: - Student A: Φ = [3.5, 4.0, 3.8] (past GPAs), Ξ = 85 - Student B: Φ = [3.0, 3.2, 3.4], Ξ = 75 - Student C: Φ = [3.8, 3.9, 4.0], Ξ = 90 LSTM Application: - Sequence Input: Past GPAs for each student. - Output: Predicted future GPA and potential employment success rate.
Canopy Application: - Initial Clustering: Group students into Canopies based on GPA, demographics, etc. - Refined Clustering: Use k-means within each Canopy for precise student segmentation.

Linear Regression Application: - Predicting Employment Success Rate: - Use $\Xi = a + b\Phi$ to predict employment success rate based on known GPAs. - For Student D with Φ [3.6, 3.7, 3.8], predict Ξ.
- Predicting GPA Based on Employment Success: - Use $\Phi = c + d\Xi$ to predict GPA based on employment success rate. - For a student with expected employment success rate $\Xi = 80\%$, predict Φ.

Detailed Implementation:
“‘python import numpy as np from sklearn.linear_model import LinearRegression from sklearn.preprocessing import StandardScaler from keras.models import Sequential from keras.layers import LSTM, Dense, BatchNormalization

Sample data student_data = "GPA": [[3.5, 4.0, 3.8], Student A [3.0, 3.2, 3.4], Student B [3.8, 3.9, 4.0] Student C], "EmploymentSuccessRate": [85, 75, 90] Corresponding employment success rates
Linear Regression Model def linear_regression_model(X, y): model = LinearRegression() model.fit(X, y) return model

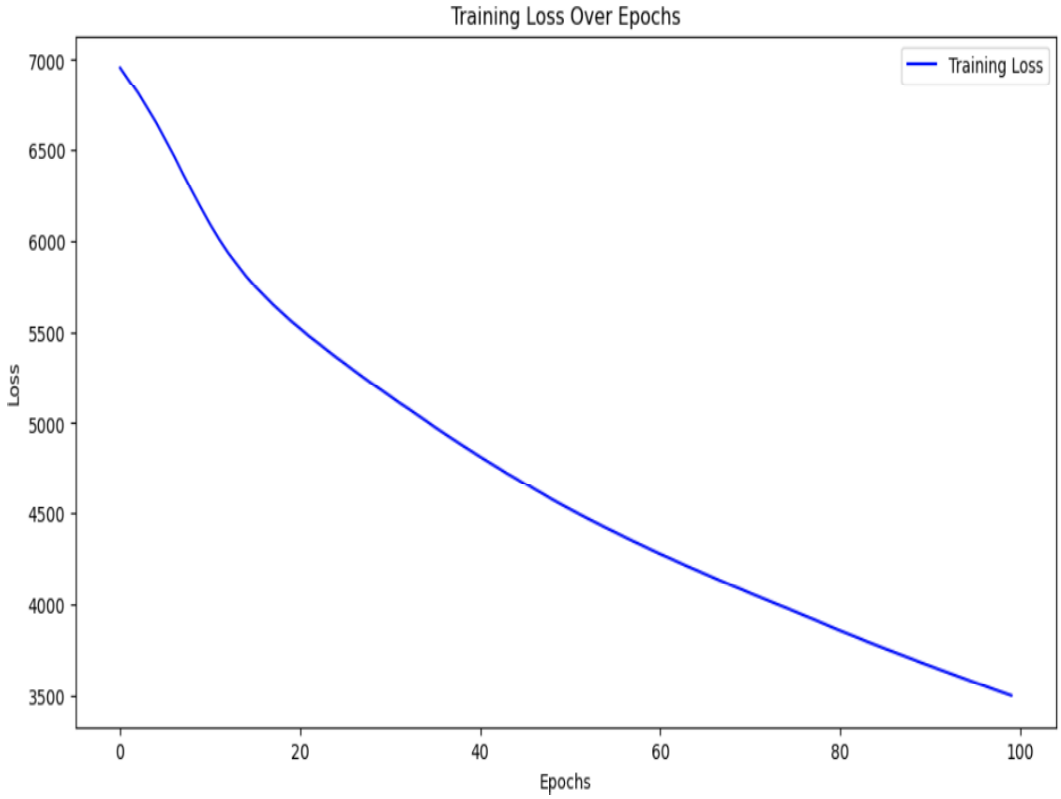
Preparing Data for Linear Regression X = np.array(student_data["GPA"]) y = np.array(student_data["EmploymentSuccessRate"])

Train Linear Regression Model lr_model = linear_regression_model(X, y)

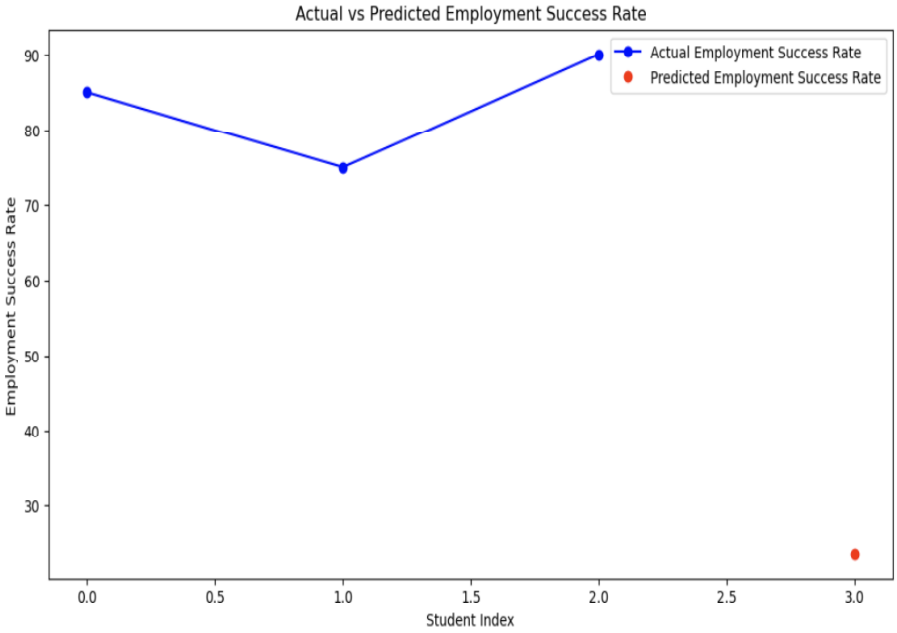
The table summarizing the relevant data and predictions based on your LSTM model:

Sr. No.	GPA 1	GPA 2	GPA 3	Predicted Employment Success Rate
1	3.5	4.0	3.8	85
2	3.0	3.2	3.4	75
3	3.8	3.9	4.0	90
4	3.6	3.7	3.8	24.274467

This table includes the original GPA data for three students and their corresponding actual employment success rates, as well as the predicted employment success rate for a new set of GPA values [3.6, 3.7, 3.8].



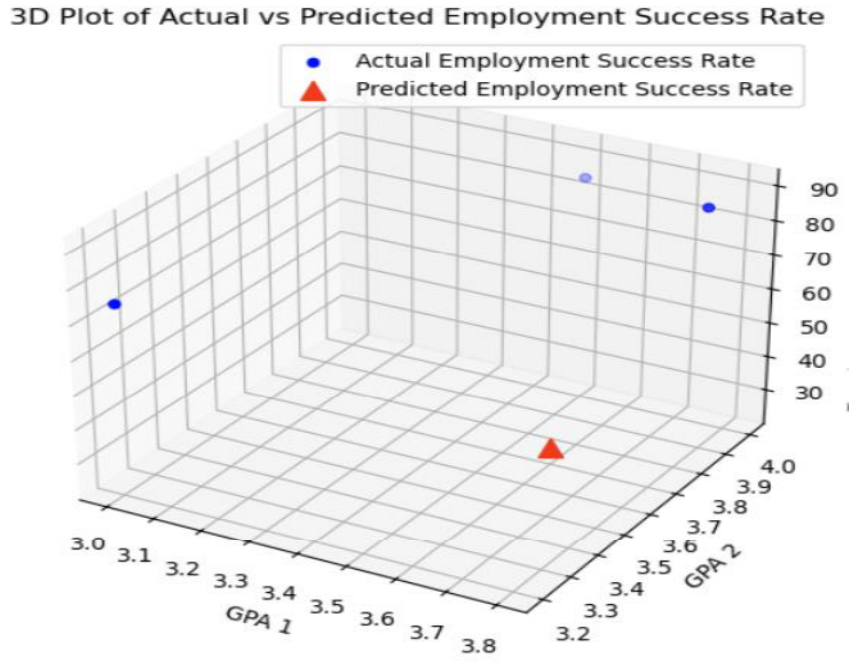
Predict Employment Success Rate for new data new_gpa = np.array([[3.6, 3.7, 3.8]])
predicted_employment_success = lr_model.predict(new_gpa)
print(f"Predicted Employment Success Rate: predicted_employment_success[0]")




```
Predicted Employment Success Rate: 23.610801696777344
```

LSTM Model for Sequential Data Prediction def lstm_model(input_shape): model = Sequential() model.add(LSTM(50, return_sequences=True, input_shape=input_shape)) model.add(BatchNormalization()) model.add(LSTM(50)) model.add(Dense(1)) model.compile(optimizer='adam', loss='mse') return model

Prepare Data for LSTM X_lstm = np.expand_dims(X, axis=2) Assuming X is in shape (samples, timesteps, features) y_lstm = np.array(y)




```
Predicted Employment Success Rate: 24.27446746826172
```

Define and Train LSTM Model lstm = lstm_model((X_lstm.shape[1], X_lstm.shape[2])) lstm.fit(X_lstm, y_lstm, epochs=100, batch_size=1)

Predict using LSTM Model predicted_gpa = lstm.predict(np.expand_dims(new_gpa, axis=2))
print(f"Predicted Future GPA: predicted_gpa[0][0]")
Summary for above data

By integrating linear regression with LSTM and Canopy algorithms, the College CP Expert System can offer comprehensive, data-driven recommendations for academic and career planning. Linear regression provides specific predictions for variables, complementing the sequence learning of LSTM and the clustering of Canopy, resulting in a robust and intelligent system for personalized guidance.

4 Application to the Visualization System Design and Realization CP Path

• Core Task of College CP Expert System

- Objective: Utilize an expert system approach for College Career Planning (CP) focusing on academic planning and employment guidance.

- Core Focus: Employment guidance for college students.

- Method: Data mining to gather student data and develop a reasoning mechanism for the expert system.

• System Requirements

- Functions: The system should facilitate knowledge acquisition, reasoning mechanisms, data processing, and management tasks akin to a management information system.

• System Functional Design

- Foreground Function: Business services provided to users.

- Background Function: Management and maintenance by system administrators.

• Overall System Design

- Modular Approach: Design the system workflow considering both functional and performance requirements.

- System Workflow: Basis for the realization of the system.

• Core Components of Expert System

- Knowledge Base: Stores professional knowledge and experience.

- Inference Engine: Simulates human expert thinking to provide answers based on stored knowledge and user queries.

- Key Design Elements: Construction of the knowledge base, inference mechanism, and algorithm selection.

• Functionality Overview

- Career Planning Page: Accessible via the "Career Planning" tab.

- Academic Planning: Recommends professional courses based on senior students' course selection data.

- Employment Guidance: Provides employment-related advice and resources.

• System Data Layer Design

- Data Types: Includes raw data, system data, and knowledge data.

- Raw Data: Unprocessed data.

- System Data: Generated and used by system users, stored in a MySQL relational database.

- Knowledge Data: Results from preprocessing and data mining of raw student data.

• Intelligent Wearable Devices: Classification and Function

- Usage Statistics: Wrist (30), upper body (26), head (22), other (15), feet (7).

- Primary Functions: Medical health, sports fitness, heart condition monitoring, exercise tracking, health evaluation.

• Types of Wearable Devices

- Life Sports Health Equipment: Heart rate monitoring, exercise measurement, sleep quality monitoring.

- Leisure and Entertainment Equipment: Watches and glasses for notifications, voice input, and enhanced experiences.

- Safety Protection Equipment: GPS-enabled devices for children and elderly safety.

- Medical and Healthcare Equipment: Monitors health indicators like heart rate, blood oxygen, and blood pressure.

- Smart Home Products: Controls household appliances and monitors home security.

• Value of Intelligent Wearable Devices in Sports

- Research Insight: 70 of wearable devices focus on sports and health, 23 on social communication, 7 on sleep monitoring.

- Functionality: Data collection and collation of human motion data.

• Equipment Categories

- Correct Movement and Efficient Training: Devices like LUMO LFT and Wa Hoo use AI to improve sports techniques.

- Injury Prevention: Real-time data transmission to monitor athletes' conditions and prevent injuries through data analysis and early warnings.

• System Testing

- Purpose: Verify functionality and performance under various conditions.

- Server Load Test: Increase user connections in batches (10 to 40) and record average response times to assess server performance.

• Linear Regression

- Definition: Supervised machine learning technique to predict values based on known variables.

- Formulas:

- Predicting Ξ: $\Xi = a + b\Phi$

- Predicting Φ: $\Phi = c + d\Xi$

- Variables: Ξ and Φ are dependent and independent variables respectively, with a, b, c, and d being constants and regression coefficients.

• Key Takeaways

- Expert System Design: Involves modular design, comprehensive knowledge base, and efficient inference engine.

- Wearable Devices: Crucial in sports for monitoring, training, and injury prevention.

- System Testing: Essential for ensuring performance and reliability under load conditions.

above all structured and concise design ensures a comprehensive understanding of the college CP expert system and the classification of intelligent wearable devices.

The C.P. algorithm adds a critical dimension to the system by efficiently clustering high-dimensional data into manageable subsets. This two-stage clustering approach balances computational efficiency and precision, enabling fine-grained analysis within each Canopy while maintaining scalability for large datasets.

5 Conclusions

Our manuscript presents significant advancements in the generalization of big data algorithms for different models, exemplified by the N. N. algorithm and the C. P. algorithm applied to

various data sets. The validation of our generalized results, through multiple numerical examples and their error analysis across several iterations, underscores the robustness of our approach.

Our research also makes noteworthy contributions to the field of data visualization in the context of big data. By integrating various visualization techniques and machine learning algorithms, we enable more effective decision-making processes. Additionally, our focus on analyzing big data visualization highlights the broader applicability and potential impact of our methods in data analysis. This comprehensive approach not only enhances our understanding of big data but also opens new avenues for future research and practical applications.

6 Conflict of interest

There are no conflicts interest by all authors.

7 Use of AI tools declaration

The author declares she has not used Artificial Inttauigence (AI) tools in the creation of this article.

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Covering Letter

Title

Determining the feasibility of fingerprints under water on different substrates and developing new powder for the development of the same

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Abstract:

The evidences that are found from the water bodies are of much importance. Improper handling of these evidences can destroy and contaminate them, thus making them unrecognisable and unfit for the retrieval of any evidential information. The common techniques chosen by culprits these days is to destroy the evidences either by burning it or discarding them into water bodies like river, lakes, streams, ponds, drainage areas and the wells. As their fundamental goal is not to leave any traces or the things they have used at the crime scene. Their intention is to hide the weapon or any other object in a place it will not be seen or suddenly noticed by anyone. The fingerprint will act differently when it is submerged in

water owing to the various components present in the residue of the prints. In most of the cases these fingerprints and traces meets a lot of destructive conditions which can be a big challenge for the experts during the collection and analysis of the samples. The aim of the study is to develop the prints that are submerged in water effectively with help of non-conventional powders which are used in our day to day lives. And to analyse the quality of developed prints to determine the approximate time period or how long the particular exhibit was kept in the fresh water, which would give a major clue in the investigation. Also, the study focuses to determine which of the non-conventional powder used here could be the most effective to develop the latent fingerprints from non-porous surfaces submerged at various time intervals after drying them in room temperature.

Keywords: Submerged fingerprints, water bodies, non-conventional powders, developing latent fingerprints.

Text

"Fingerprints are not just patterns and prints; they are the physiological full proof identities of an individual. DNA can be same for two persons, but the fingerprints won't."

This study is mostly concentrated on detecting and developing the invisible latent fingerprints by using various non-conventional powders on different substrates which can be found easily in our day-to-day life, and which do not require expensive laboratory setup to develop the latent submerged prints. This work aims to develop latent prints from the exhibits that are submerged in fresh water by using the non-conventional powders, to analyse the effectiveness of the non-conventional powders in the development of the latent prints that are submerged in water, to opine the best non-conventional powder for development of the submerged latent prints and to measure the effect of time of submersion of the exhibit on effectively developing the latent prints using non-conventional powders

As we know finger prints consists of various kind of endogenous secretions like amino acids, sebum, salts, urea, fatty acids, etc. The oily secretions among these are hydrophobic which cannot be dissolved by water. These substances will get preserved no matter whether it is wet or not. When the exhibits are deposited in water bodies the water-soluble constituents of fingerprint residues like amino acids, sodium/salts, proteins, etc., will be washed off by water by leaving only the non-water-soluble constituents like lipid. The deposited prints are also vulnerable to physical as well as the chemical reactions due to various parameters in water. They are p^H, turbidity, biochemical oxygen demand (BOD), etc., These parameters can accelerate the degradation process of deposited latent fingerprints.

Latent fingerprints are deposited when the embossed or raised friction ridge structure of our skin on fingers comes in contact with the suitable surfaces [1]. The sweat composition is a mixture of both organic and inorganic materials that are produced in a human body which gets transferred to a particular substrate and this will produce a latent fingerprint. The print that is left behind on a surface consists of oily sweat composition exuded from the pores of the skin,

The residue left by the latent fingerprint is typically either eccrine or sebaceous or the combination of both. Currently not many studies have been conducted with respect to the latent print detection and development on various surfaces that have been submerged in water for a period of time using non-conventional powders. This study is to search the possibility of developing and tracing out the invisible prints from the surfaces that are recovered from water. This will help to determine what we're the techniques that can be used to recover the latent prints from the submerged surfaces.

As per the general classification, there are three major types of fingerprints. The classification is based on their physical appearance as wells as their characteristics. These include;

- Latent prints
- Visible prints

- Plastic prints

Latent prints: Latent prints are not visible with our naked eyes. It is something like chance prints. It can be defined as the accidental prints left by the friction ridges on a surface. It is very rare to get complete and clear fingerprints [3]. We need some physical and chemical methods to develop these prints to render it visible and analyse it. These prints are formed when the oil and sweat composition on the skin of our body comes in contact with a surface.

Visible prints: Visible prints don't require any kind of extra methods to make it visible because they can be directly seen with our naked eye. Visible prints are also known as patent prints. These prints are visible due to the transfer of extra foreign materials on the surface of finger which gets transferred to the surface of the substrate rendering it visible. The above-mentioned foreign materials include blood, damp paint, dust, etc., with which the finger comes to contact [3]. The prints obtained are well defined and highly distinct ridge impression.

Plastic prints: Like the visible prints, plastic prints are also easily visible without the application of any technique to enhance it for viewing. The plastic prints are deposited when the finger comes in contact with soft, malleable surface which makes an indentation according to the flow or movement of friction ridges. These malleable surfaces include wax, soap, unfired clay, grease, freshly coated paint, gum, thick blood, etc. [3] to preserve such prints moulding is done with dental mass or Plaster of Paris (POP).

These kinds of latent, non-visible fingerprints can be detected, developed and analysed using various techniques that can be applied on the exhibits surfaces which stick to the ingredients of the chance fingerprints left behind. There are special powders that are used for fingerprint development like fingerprint powders, magnetic powders, or fluorescent powders. However, we cannot use these powders to detect and develop the prints on wet surface because these fine powders will accumulate due to water and stick to the surface and thus fingerprints won't be clear enough. The role of chemicals in such cases is of immense

importance as they are able to detect and visualize the latent fingerprints on the wet surfaces [24]. The commonly used chemical powders used till date for submerged fingerprints development are Small particle reagent (SPR), Cyano-acrylate fuming, chemical visualization methods, single and multi-metal deposition methods etc. As these chemicals are expensive and are not readily available in the crime scene the need to develop certain non-conventional powders that are cheap, readily available and easy to use is there by essential to retrieve such prints that are submerged under water.

Materials and Methods:

In this study samples the total sample size was 300 and it was taken on 10 different substrates at 6 different time intervals as follows:

- 1 hour
- 1 day
- 3 days
- 10 days
- 15 days
- 30 days

The substrates that were chosen included:

- White colored plastic
- Grey colored polished surface
- Transparent glass pieces
- White tiles
- Aluminum sheets

There were 10 non-conventional powders that were used in this study to develop the submerged latent prints are:

- Coal powder
- Incense stick ash
- Talcum powder
- Turmeric powder
- White cement
- Cement
- Dental stone
- Icing sugar powder
- Tea powder
- Mehndi powder

The instrument and other devices used for the detection, visualization and capturing are:

- Foster and Freeman Video Spectral Comparator 40
- Zeiss Stemi 508 Stereo Microscope
- Bodelin Technologies PS-EDU-100 Proscope EDU USB Digital Handheld Microscope (Black)
- Lenovo Laptop

Sampling:

Sample prints were collected in such a manner that same finger for the similar surface to keep the consistency throughout. All the substrates mentioned above are used for the print's deposition. The substrates are cleaned properly to make sure that no prints are deposited in the particular substrate other than the sample print. Prints are taken from the donor at the normal stage of daily activities (like walking, running) without washing the donor's hand. Print deposited surfaces are kept in two large plastic tubs which are filled with tap water to stimulate the fresh water condition. The surfaces are held by the donor for a while, to transfer

the print properly and the surfaces were kept as such in the room condition for 15 minutes to dry the fingerprints.

These prints were directly kept in water without disturbing the prints and more care were taken to avoid overlapping. Gloves were worn during the entire process to avoid the deposition of unwanted prints. The surfaces in which the latent prints were deposited were taken out at proper time intervals and air dried in the room temperature itself for 30-45 minutes. Suitable newly developed powders (non-conventional powders) were used to develop the prints from the particular surfaces. Details were noted and photographed using Video Spectral Comparator itself during visualizing.

(Insert Figure 1-15 here)

Results:

The results and observations are tabulated in Tables 1-5. The Table 1 shows the result of the fingerprint developed from Transparent Glass surface while the Table 2,3,4 & 5 show the results of polished surface, Aluminium sheets, White tiles and pieces of White Plastic square pieces respectively.

(Insert Table 1-5 here)

Discussion

Forensic significance of the study: Submerged fingerprints are fragile and there are not many techniques available to develop them. Conventional powders are not readily available at the crime scene; thus, this study aims to find cost effective alternatives that can be found conveniently. This study also aims to correlate the time since deposition of exhibits under the water with their quality of latent print developed using these powders. The study focuses on developing new powders for the development of submerged latent fingerprint.

Future scope of the study: The deposited prints are vulnerable to various factors like p^H, turbidity, biochemical oxygen demand (BOD), etc.; these parameters can accelerate the degradation process of deposited latent fingerprints. The powder size also affects the

efficiency of the development of latent prints submerged in water. Proper handling of the samples has to be done or else the results can be compromised. Comparative study of feasibility of fingerprints in fresh and seawater can be done. More substrates and non-conventional powders can be used. Development of latent fingerprints can be done after one-hour intervals to understand the gradual degradation of latent prints submerged in water.

Conclusion

The quality and clarity of submerged latent prints developed depend upon the time duration of the exhibit submerged under water. Thus, time plays a vital role in the effective development of the submerged latent prints. For prints submerged for 1-hour, white cement was the best powder, for one day dental stone was the best powder and for three days turmeric powder gave best results. As the time increases it becomes difficult to develop the latent fingerprints, as the latent prints start losing their viability and thus the efficiency to develop such prints reduces. Prints developed by these powders till 3-4 days gave good results and the latent prints developed were clear. Prints developed by the powders after a time interval of 10 to 12 days gave prints that were not clear, only their presence was conspicuous. Submerged prints could not be developed after 13 days using these powders.

We can conclude that the following list of non-conventional powders used during the research can be used to develop latent submerged fingerprints. We couldn't conclude the approximate time interval of the submersion of the exhibit based on the quality of the prints.

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Conflict Of Interest:

No conflict of interest exists.

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Tables

Table 1: Substrate used here was a Transparent Glass surface

Name of Powder / Time	Coal	Incense stick ash	Mehndi	Tea	Talcum	Dental stone	White Cement	Cement	Turmeric	Icing Sugar
1 hour	○	○	○	✓	○	○	✓	○	○	✓
1 Days	○	○	○	✓	○	✓	✓	○	○	✓
3 Days	○	○	○	○	○	○	✓	○	○	○
10	○	×	×	○	×	×	○	×	×	×

Days										
15 Days	×	×	×	×	×	×	×	×	×	×
30 Days	×	×	×	×	×	×	×	×	×	×

Abbreviations: The '○' indicates the **Presence of Prints** while '✓' and '×' indicates the **Best Prints and No prints** respectively.

Table 2: The substrate used here was a polished surface

Name of Powder / Time	Coal	Incense stick ash	Mehndi	Tea	Talcum	Dental stone	White Cement	Cement	Turmeric	Icing Sugar
1 hour	✓	○	○	○	○	○	✓	✓	○	○
1 Days	○	○	○	✓	✓	✓	○	✓	○	○
3 Days	×	×	×	×	×	×	×	×	○	○
10 Days	○	×	×	○	×	×	○	×	×	×
15 Days	×	×	×	×	×	×	×	×	×	×

30 Days	×	×	×	×	×	×	×	×	×	×

Abbreviations: The '○' indicates the **Presence of Prints** while '✓' and '×' indicates the **Best Prints and No prints** respectively.

Table 3: The substrate used here was Aluminium sheets

Name of Powder / Time	**Coal**	**Incense stick ash**	**Mehndi**	**Tea**	**Talcum**	**Dental stone**	**White Cement**	**Cement**	**Turmeric**	**Icing Sugar**
1 hour	✓	○	✓	✓	○	○	○	○	○	○
1 Days	○	○	○	○	○	✓	✓	○	○	○
3 Days	○	○	○	○	✓	○	○	○	○	○
10 Days	×	×	×	×	×	○	×	×	×	○
15 Days	×	×	×	×	×	×	×	×	×	×
30 Days	×	×	×	×	×	×	×	×	×	×

Abbreviations: The '○' indicates the **Presence of Prints** while '✓' and '×' indicates the **Best Prints and No prints** respectively.

Table 4: The substrate used here was White tiles

Name of Powder / Time	Coal	Incense stick ash	Mehndi	Tea	Talcum	Dental stone	White Cement	Cement	Turmeric	Icing Sugar
1 hour	✓	○	○	○	○	○	✓	○	○	○
1 Days	✓	○	○	○	○	✓	✓	○	○	○
3 Days	○	○	○	○	○	✓	○	○	✓	✓
10 Days	×	×	×	×	×	×	×	×	×	×
15 Days	×	×	×	×	×	×	×	×	×	×
30	×	×	×	×	×	×	×	×	×	×

Days										

Abbreviations: The '○' indicates the **Presence of Prints** while '✓' and '×' indicates the **Best Prints and No prints** respectively.

Table 5: The substrate used here was pieces of Whitte Plastic square pieces

Name of Powder / Time	Coal	Incense stick ash	Mehndi	Tea	Talcum	Dental stone	White Cement	Cement	Turmeric	Icing Sugar
1 hour	○	○	○	○	○	○	✓	✓	○	○
1 Days	○	○	○	○	○	○	✓	✓	○	○
3 Days	✓	○	○	○	○	✓	✓	○	✓	○
10 Days	×	×	×	×	×	×	×	×	×	×
15 Days	×	×	×	×	×	×	×	×	×	×

30 Days	×	×	×	×	×	×	×	×	×	×

Abbreviations: The '○' indicates the **Presence of Prints** while **'✓'** and '×' indicates the **Best Prints and No prints** respectively.

Figure Legends

Figure 1: Latent fingerprint on glass and aluminium sheets that are submerged under fresh water.

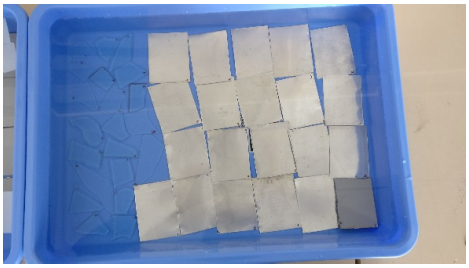


Figure 2: Latent fingerprints on tiles, plastic and polished surfaces submerged under fresh water.

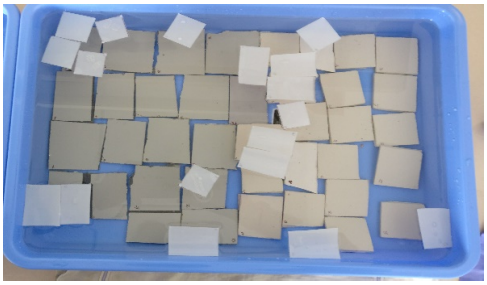


Figure 3: Latent fingerprints bearing surfaces (glass, tiles, plastic, aluminium sheet, polished surfaces) kept for drying after recovering from fresh water.

Figure 4: Submerged latent print developed by using tea powder on glass viewed under transmitted light**.**

Figure 5: Latent fingerprint developed using coal powder on polished surface and made visible under spot light.

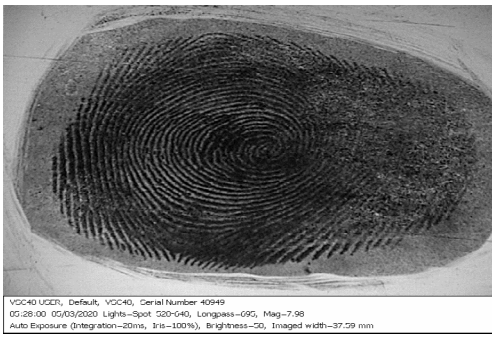


Figure 6: Submerged latent fingerprint developed using tea powder on Al sheet made visible under spot light.

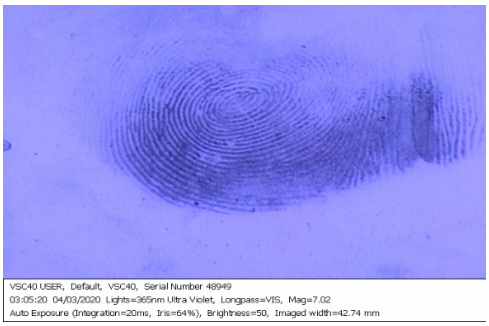


Figure 7: Submerged latent fingerprint developed on tiles using coal powder visible under spot light.

Figure 8: Submerged latent fingerprint developed on plastic by using cement visible under UV 365.

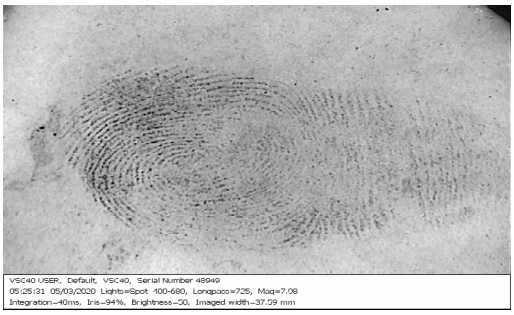


Figure 9: Submerged latent fingerprint developed on glass using tea powder made visible under spot light.

Figure 10: Submerged latent fingerprint developed on polished surface using cement, made visible under spot light. Submerged latent fingerprint developed on Al sheet using dental stone viewed under spot light.

Figure 11: Submerged latent fingerprint developed on Al sheet using dental stone viewed under spot light.

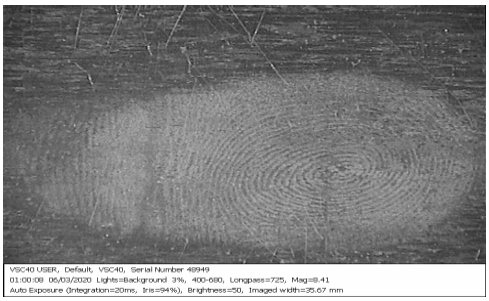


Figure 12: Submerged latent print developed using dental stone on tiles viewed under side light.

Figure 13: Submerged latent print developed on plastic using white cement viewed under UV.

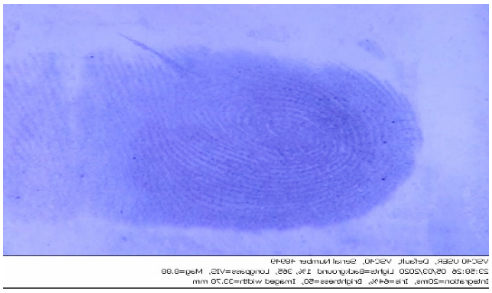
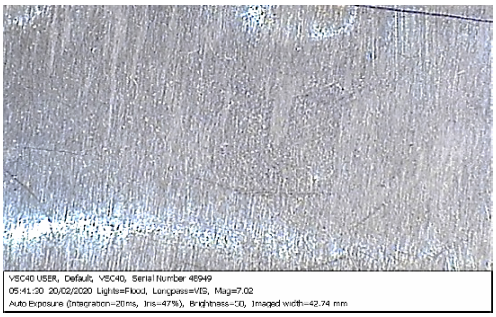


Figure 14: Submerged latent fingerprint developed using white cement viewed under flood light

Figure 15: Partially developed submerged latent fingerprint by using talcum powder viewed under flood light.



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