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Comparative Study of Fluorescent Dyes for TLC Detection of Arson Accelerants: Identifying a Safer Alternative to Rhodamine B

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Abstract

Thin Layer Chromatography (TLC) is a widely used analytical technique in forensic science for the detection of arson accelerants. Rhodamine B, a fluorescent dye commonly employed in TLC, is known for its effectiveness but poses significant chemical hazards and environmental risks. This study aims to identify safer and equally effective alternatives to Rhodamine B by comparing its performance with four other fluorescent dyes: Eosin Yellow, Fluorescein Sodium, Rose Bengal, and Coumarin. Experimental results showed that Eosin Yellow outperformed Rhodamine B and other tested dyes in sensitivity, clarity, and cost-effectiveness, while also addressing chemical hazard concerns due to its lower toxicity and biodegradability

Keywords: Thin Layer Chromatography, Rhodamine B, Eosin Yellow, arson accelerants, fluorescent dyes

CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION

Arson, the deliberate act of setting fire to property, poses a significant challenge to forensic investigators. The identification of accelerants used in arson cases is a critical step in determining the cause of the fire and linking suspects to the crime scene. Traditional methods, such as Gas Chromatography-Mass Spectrometry (GC-MS), are highly effective but may not always be feasible due to cost and time constraints. Thin Layer Chromatography (TLC) has emerged as a rapid and cost-effective technique for preliminary detection of accelerants. The addition of fluorescent dyes in TLC analysis enhances sensitivity and specificity, allowing for improved visualization of trace evidence.

This study aims to conduct a comparative analysis of various fluorescent dyes in TLC detection of common arson accelerants. By evaluating their fluorescence intensity, detection limits, and forensic applicability, the research will determine the most effective dye for use in forensic casework. This introduction provides a comprehensive background on TLC, fluorescent dyes, and their forensic relevance in arson investigations. These dyes, when exposed to ultraviolet (UV) light, produce visible fluorescence, allowing forensic experts to easily identify the presence of hydrocarbons and other flammable substances. The use of fluorescent dyes such as Rhodamine B has revolutionized arson investigations, making it possible to detect accelerants even in very small quantities. This project aims to conduct a comparative study of different fluorescent dyes used in TLC for the detection of arson accelerants, focusing on their efficiency and ability to detect a range of accelerants commonly used in criminal activities.

1.2 BACKGROUND AND SIGNIFICANCE

Arson crimes are often challenging to investigate due to the destruction of evidence by fire. Accelerants, such as gasoline, kerosene, and diesel, leave behind chemical residues that can be detected even after combustion. These residues play a crucial role in forensic investigations, helping to establish the presence of an accelerant and potentially identifying the source of the fire.

TLC is a well-established chromatographic technique used for the separation and identification of organic compounds. It offers several advantages, including simplicity, cost-effectiveness, and rapid analysis. However, the detection of arson accelerants using TLC can be challenging due to the low concentrations of residues. The application of fluorescent dyes addresses this limitation by enhancing the visibility of analytes under ultraviolet (UV) light.

Fluorescent dyes have unique chemical properties that allow them to absorb and emit light at specific wavelengths. When applied to TLC plates, these dyes interact with accelerant residues, producing fluorescence that can be detected using UV light sources. The choice of dye significantly influences the sensitivity and selectivity of detection. Therefore, it is essential to compare different dyes to identify the most suitable one for forensic applications.

1.3 THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is a widely employed analytical technique used to separate components of a mixture. In the context of arson investigations, TLC is used to identify accelerants in fire debris by separating and analyzing the chemical compounds present. The process involves applying a sample to a TLC plate coated with a thin layer of absorbent material, such as silica gel, and then using a solvent to move the sample up the plate. The individual components of the sample separate based on their interactions with the solvent and the stationary phase, allowing for the identification of accelerants. When combined with fluorescent dyes, TLC becomes even more effective, as the dyes enhance the visibility of the separated components under UV light. The dye interacts with accelerants, producing a fluorescent pattern that forensic scientists can use to identify specific accelerants, even at low concentrations. The technique is particularly valuable in arson investigations because it can provide a clear, reproducible method for detecting accelerants in fire debris. TLC also offers the advantage of being relatively simple and cost-effective compared to other more sophisticated analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), which is often more expensive and requires specialized equipment.

1.4 RHODAMINE B: A COMMONLY USED FLUORESCENT DYE FOR TLC

Rhoda mine B is one of the most widely used fluorescent dyes in forensic science, particularly for the detection of arson accelerants. This dye has a high sensitivity to light and is well-suited for use in TLC. When applied to a TLC plate, Rhodamine B interacts with hydrocarbons present in accelerants, allowing them to fluoresce brightly under UV light. This fluorescence is a clear indicator of the presence of accelerants, even in traces that are too small to be detected by other methods. Rhodamine B has proven to be particularly effective for detecting gasoline, kerosene, and diesel, which are some of the most commonly used accelerants in arson cases. Its ability to enhance the visualization of these substances makes it an indispensable tool in forensic investigations. However, while Rhodamine B is highly effective for detecting certain accelerants, it may not be as useful for identifying others, particularly those that do not fluoresce well. Also it poses significant chemical and environmental hazards. Its use is associated with toxicity, carcinogenicity, and mutagenicity, posing risks to laboratory personnel and the environment. Additionally, Rhodamine B's persistence in the environment and potential to contaminate water sources and soil further exacerbate its ecological impact .This limitations has led to research into alternative fluorescent dyes that might offer better or more comprehensive detection. Rhodamine B's strong performance in many arson cases has made it the standard choice for many forensic scientists, although it is by no means the only option available.

1.5 EOSIN YELLOW

Eosin Yellow (Eosin Y or Eosin B) is a versatile xanthene dye known for its bright fluorescence, high sensitivity, and excellent contrast properties. It is widely used in biological staining, forensic analysis, and fluorescence microscopy, making it an essential tool in both medical and forensic sciences. One of its key advantages is its strong affinity for proteins and cellular components, allowing for clear and detailed visualization of biological structures.

Eosin Yellow is moderately priced and readily available, making it an affordable and accessible option for laboratories. It is chemically stable, ensuring consistent and reproducible results in forensic and analytical applications. Unlike some fluorescent dyes, Eosin Yellow provides sharp and long-lasting fluorescence, making it ideal for evidence enhancement and microscopic examination.

With proper handling, Eosin Yellow is safe to use in controlled environments and does not pose significant risks when disposed of correctly. Its wide applicability, reliability, and efficiency make it a preferred choice in forensic investigations, histology, and various scientific fields requiring precise staining and fluorescence-based detection.

1.6 COUMARIN

Coumarin is a fluorescent benzopyrone compound that plays a crucial role in forensic science, ink analysis, and counterfeit detection due to its strong fluorescence and chemical stability. It is especially valuable in document examination where its UV fluorescence helps identify alterations and forgeries. Additionally, it is used in forensic toxicology for detecting trace substances in biological samples.

One of the main drawbacks of Coumarin is its high cost, which limits its widespread use in routine forensic applications. Additionally, certain synthetic derivatives of Coumarin have been associated with liver toxicity and potential carcinogenic effects when ingested or inhaled in large amounts. Although it is partially biodegradable, some Coumarin derivatives are persistent in the environment, necessitating careful handling and disposal.

Despite these concerns, Coumarin remains a highly effective and reliable dye in forensic and analytical sciences. Proper laboratory precautions, such as wearing gloves, using adequate ventilation, and avoiding direct inhalation, help mitigate health risks, making it a valuable yet cautiously handled forensic tool.

1.7 FLUORESCEIN SODIUM

Sodium Fluorescein is a highly sensitive, water-soluble fluorescent dye widely used in forensic fluid tracing, fingerprint detection, and contamination analysis. It exhibits strong yellow-green fluorescence under UV and blue light, making it an effective tool for detecting trace evidence. It is also used in ophthalmology for diagnostic purposes and environmental studies to trace water contamination.

However, Sodium Fluorescein is expensive, which can be a limiting factor for large-scale forensic use. Though it is generally non-toxic and biodegradable, exposure in high concentrations can cause mild skin and eye irritation. While it poses minimal environmental risks, excessive disposal into water bodies should still be avoided to prevent unnecessary accumulation.

Despite its cost, Sodium Fluorescein remains a preferred choice for forensic and environmental investigations due to its high fluorescence intensity, reliability, and non-toxic nature. Proper handling, including avoiding prolonged skin contact and storing it in a controlled environment, ensures safe and effective usage.

1.8 ROSE BENGAL (ACID RED)

Rose Bengal is a synthetic xanthene dye with strong fluorescence and excellent staining capabilities, making it a valuable tool in forensic investigations, biological staining, and latent fingerprint detection. It is particularly useful in bloodstain enhancement and forensic histology, where its high contrast properties aid in visualizing evidence.

Despite its effectiveness, Rose Bengal has some limitations. It can cause skin irritation, respiratory discomfort, and eye damage with prolonged exposure. Additionally, under light exposure, it generates reactive oxygen species (ROS), which may lead to cell damage if not handled properly. Environmentally, it is not easily biodegradable and can bioaccumulate in aquatic ecosystems, making proper disposal essential to prevent contamination.

Nevertheless, Rose Bengal is widely used in forensic and medical applications due to its high fluorescence

1.9 MIXTURE OF ACCELERANTS FROM CRIME SCENE

In many arson cases, multiple accelerants are used to create a more intense fire, making the investigation more complex. Fire debris collected from the scene may contain a mixture of hydrocarbons, including gasoline, diesel, kerosene, and other flammable liquids. Identifying each individual component of this mixture is crucial for establishing the origin and cause of the fire. TLC, combined with fluorescent dye detection, is highly effective in separating these mixed accelerants and identifying each substance individually. By analyzing the unique chromatographic patterns produced by each accelerant, forensic scientists can determine the specific mixture of substances used in the crime. Fluorescent dyes further enhance this process by allowing the accelerants to be visualized clearly, even when they are present in very small quantities. This ability to detect and identify complex mixtures is essential in arson investigations, as it can help link a suspect to the crime scene. Additionally, understanding the mixture of accelerants used can provide insight into the behavior of the fire, including its speed and spread, which are important factors in determining the intent of the perpetrator.

1.10 CHALLENGES AND LIMITATIONS

Despite its advantages, TLC-based detection of accelerants using fluorescent dyes has certain limitations:

- **Sensitivity Issues:** Some dyes may not exhibit strong fluorescence with all accelerants.
- **Interference from Background Substances:** Other fire byproducts may interfere with the detection process.
- **Environmental Factors:** Fluorescence intensity can vary under different lighting conditions and substrate compositions.
- **Validation with Real-Case Samples:** Further studies using real fire debris samples are necessary to confirm laboratory findings.

Addressing these challenges will be crucial in refining the TLC method for forensic applications.

1.11 FORENSIC SIGNIFICANCE

The forensic significance of this study lies in its potential to improve arson investigations. Law enforcement agencies rely on scientific evidence to establish the presence of accelerants at fire scenes. The ability to detect accelerant residues quickly and accurately enhances the investigative process. A more effective TLC method incorporating fluorescent dyes can:

- Provide rapid preliminary results before confirmatory tests like GC-MS.
- Aid in field investigations where portable detection methods are needed.
- Reduce costs associated with expensive analytical techniques.

The forensic significance of detecting accelerants at arson crime scenes cannot be overstated. The ability to positively identify the accelerants used in a fire can make a substantial difference in the outcome of an investigation, as it often helps to establish the presence of criminal intent. In arson cases, accelerants such as gasoline, diesel, or kerosene are typically applied to increase the speed and spread of fire, which makes it difficult to distinguish from accidental fires. This is where the expertise of forensic scientists comes in. TLC, when paired with fluorescent dye detection, allows for the separation and identification of chemical compounds, even those that are present in minute amounts. The identification of these accelerants is critical to proving whether a fire was accidental or intentionally set. The forensic significance of this research lies in improving detection techniques, providing more reliable evidence, and ultimately aiding law enforcement in solving arson cases. In addition to its role in criminal investigations, this technique also has applications in fire safety, helping to determine whether certain accelerants are being improperly or dangerously used.

1.12 CONCLUSION

The use of Thin Layer Chromatography (TLC) in forensic science for the detection of arson accelerants has long relied on Rhodamine B as a fluorescent dye due to its effectiveness in visualizing chemical compounds. However, the growing awareness of its chemical hazards and environmental risks has prompted the need for safer and more sustainable alternatives. This study seeks to address this concern by evaluating the performance of Rhodamine B against four alternative fluorescent dyes: Eosin Yellow, Fluorescein Sodium, Rose Bengal, and Coumarin. This research not only highlights the limitations of Rhodamine B but also underscores the potential of alternative dyes to enhance the safety and efficiency of forensic investigations.

CHAPETR 2: REVIEW OF LITERATURE

2.1 INTRODUCTION

Books and articles on thin-layer chromatography (TLC) detection methods is extensive, yet the comparative effectiveness of fluorescent dyes for identifying arson accelerants remains relatively unexplored. This study aims to evaluate and compare different fluorescent dyes used in TLC for detecting ignitable liquids in fire debris analysis. A total of 35 articles published between 2000 and 2025 were reviewed to understand the advancements in TLC techniques, the sensitivity of various fluorescent dyes, and their forensic applicability.

2.2 RELATED LITERATURE

Bertsch et al.(2000): The study compared chromatograms to identify potential accelerants. High boiling range distillates indicate sample preparation issues. Previous tests showed difficulties with gasoline and diesel fuel mixtures. Sample integrity is crucial to avoid cross-contamination. Modern chromatography and spectroscopy techniques enhance analysis accuracy. Dynamic headspace enrichment is a key sample preparation method.

McCurdy et al.(2001): The authors developed a vapour phase ultra-violet (UV) spectroscopy method for analyzing arson accelerants in fire scene debris. The technique is rapid, inexpensive, simple, and sufficiently sensitive for crime scene samples. It can complement gas chromatography–flame ionisation detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS) by providing additional information in some cases. The method is particularly advantageous when GC–MS analysis is impractical, making it a valuable tool in arson investigations. Applications to casework samples demonstrate its effectiveness.

Rodgers et al.(2001): This study demonstrated the use of ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) for identifying accelerants in fire debris. Accurate mass measurement enables definitive elemental composition analysis of accelerants like lighter fluid, kerosene, gasoline, diesel, turpentine, and mineral spirits. Unique molecular "fingerprints" for each accelerant were identified, even amidst hundreds of pyrolysis and matrix components in fire debris. Controlled burns with lighter fluid and turpentine confirmed the identification of most constituents, with 45 of 56 lighter fluid components and 126 of 133 turpentine components detected in the debris. This method proves highly effective for precise accelerant detection in complex fire scenes.

Sodeman et al.(2001): The authors detailed a forensic-based instrumental methods course experiment in this study that teaches students to determine arson accelerants using gas chromatography-mass spectrometry (GC-MS). Students analyze charred wood samples alongside known accelerants, learning key techniques such as headspace sampling, temperature programming in chromatography, and mass spectrometry analysis. The experiment reinforces the use of GC-MS in identifying unknown compounds and resolving challenges like isomer differentiation. The study effectively combines forensic science with traditional laboratory training to engage students and enhance practical skills.

Spangenberg et al.(2001): This study demonstrates the effectiveness of High- Performance Thin Layer Chromatography with a diode-array detector in forensic analysis. The method proves to be fast, inexpensive, and requires minimal pretreatment, making it an attractive option for forensic scientists. The innovative diode-array scanner enhances compound identification and purity testing by measuring spectra from 198 nm to 612 nm. The results show that High-Performance Thin Layer Chromatography is effective in detecting drugs in body fluids and allows for quick identification and quantification of substances, even in unresolved peaks. Overall, this method improves the efficiency of forensic analysis, and its advantages make it a valuable tool for forensic scientists.

Giang et al.(2003): This study explores the physical properties of accelerants and their detectability using techniques such as continuous steam micro-distillation and gas chromatography. Effective arson analysis requires a thorough understanding of accelerants' behaviours. The results indicate a high probability of detecting accelerants even after severe fires, highlighting the importance of proper sampling methods and matrix considerations in arson investigations**.**

Borusiewicz et al.(2006): The authors examined how various factors influence the detection of accelerant traces in fire debris. The results showed that the type of burned material had the greatest effect, with carpet retaining accelerant traces more effectively than wood or chipboard. Longer burning times reduced detection likelihood, especially for volatile accelerants, though carpet preserved traces better due to the formation of a protective char layer. Air availability had minimal influence on detection, suggesting that other factors, such as material arrangement, play a role. The study underscores the importance of selecting optimal sampling materials and using controlled analytical methods for reliable forensic investigations.

Pert et al.(2006): The authors reviewed advancements in techniques for detecting and analyzing arson residues. Accelerants like petrol and kerosene are key evidence but require precise analysis to distinguish criminal use from legitimate sources. Modern tools such as gas chromatography-mass spectrometry (GC-MS) and portable detectors have improved sensitivity and accuracy. Techniques like solid-phase microextraction (SPME) and two-dimensional gas chromatography enhance analysis of complex mixtures. Challenges include interference from pyrolysis products and the need for proper sampling and storage. Rigorous quality control ensures reliable results admissible in court.

Saitoh et al.(2006): The authors measured fluorescence of petroleum accelerants (kerosene, gasoline, diesel) using time-resolved spectroscopy with a pulsed Nd-YAG laser. They employed 266 nm and 355 nm excitation wavelengths and detected fluorescence with a cooled CCD camera. It is found that Kerosene's principal fluorescence component is dimethylnaphthalene and also there were significant differences in fluorescence spectra and lifetimes between accelerants and background materials. Heated kerosene can still be detected on burned materials. Thus time-resolved fluorescence imaging is effective for identifying petroleum accelerants in forensic investigations.

Bodle et al.(2007): The study present a novel approach for identifying petroleum- based accelerants using solid-phase microextraction (SPME) and gas chromatography with flame ionization detection (GC-FID). By employing multivariate data analysis techniques such as principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA), the researchers achieved high accuracy in classifying accelerants according to ASTM guidelines. The study highlights SPME as an efficient, solvent-free method for extracting accelerants, with optimized conditions ensuring reproducibility. The research demonstrates that this approach improves forensic fire investigations by enhancing the classification and recognition of ignitable liquids**.**

Whyte et al.(2007): The authors introduced a rapid method for detecting arson accelerants using Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-TOF- MS). Direct headspace analysis of burned materials without sample pre-treatment was used. Characteristic volatile organic compound (VOC) fingerprints identified for common accelerants (diesel, paraffin, petrol, white spirit) across various substrates.

Shaki et al.(2010): This study synthesized novel naphthalimide dyes containing amino and acetylamino groups and analyzed to understand their photophysical properties. Various characterization techniques, including DSC, TLC, FTIR, NMR, UV-visible spectroscopy, and fluorometry, were employed to assess their structural and optical behaviors. The dyes exhibited absorption maxima between 370 and 435 nm in solvents like DMF and THF, along with fluorescence characterized by Stokes shifts ranging from 4000 to 6600 cm^{-1}. This study underscores how different functional groups influence the optical performance of naphthalimide derivatives, providing valuable insights into their potential applications in fluorescence-based technologies.

Heath et al.(2011): This study highlighted the dangers of using accelerants like gasoline in arson. Here a case study is described, where a 49-year-old male was died in an explosion caused by gasoline vapors, which destroyed a commercial building. Autopsy findings revealed extensive burns and blunt trauma but no evidence of inhalation injuries, indicating death occurred during the explosion. Gasoline vapors are highly volatile and explosive within specific concentrations (1.1%–6%), a risk often underestimated by arsonists. It concludes that accelerant misuse poses significant risks to perpetrators and others nearby**.**

Frantz et al.(2012): The authors employed Principal Component Analysis (PCA) to statistically analyze gasoline as an accelerant, focusing on its composition, seasonal variations, and grade distinctions. Gasoline samples were subjected to Gas Chromatography- Mass Spectrometry (GC-MS) to examine differences between summer and winter blends, as well as regular and premium grades. Advanced distillation experiments and evaporation profiles were conducted to assess compositional changes under different conditions. PCA successfully differentiated gasoline samples based on seasonal and grade variations, even after significant evaporation. However, after a burn, gasoline residues were indistinguishable by grade, complicating forensic identification.

Sawicz et al.(2013): The authors evaluated the effectiveness of various epoxy cure accelerators using fluorescent molecular probes. Fluorescence Probe Technology (FPT) was employed to monitor cure kinetics of epoxy resin/anhydride compositions. DMA, DMP-30, and DBU were identified as the most effective accelerators. FPT offers a cost-effective and efficient alternative to traditional methods like DSC and FTIR for monitoring epoxy curing processes.

Deubel et al.(2014): The authors conducted Five fire tests to analyze the impact of fire accelerants on room fires and it shown that different scenarios included varying amounts and locations of accelerants. Key findings shown that accelerants significantly reduced time to flashover and increased heat release rates and ignitable liquids were detected in fire debris and soot samples from walls. A new headspace solid-phase microextraction method was developed for chemical analysis. Overall results support the use of soot sampling as an additional strategy in arson investigations.

Pyka (2014): This study explored known indicators and dyes as new visualizing reagents for detecting drugs and bioactive compounds. The effectiveness of these reagents depends on their chemical structure, the substance being detected, and the type of chromatographic adsorbent used. The method of application such as spraying or dipping methods affect the visualizing results of detected drugs. Also various indices assess the effectiveness of visualizing reagents in detection.

Overall, advancements in thin-layer chromatography are improving the detection of selected drugs.

Yao et al.(2014): The authors provides a comprehensive review of analytical techniques for detecting trace accelerants in fire investigations, highlighting advancements in sample pre-treatment and instrumental analysis through this study. It discusses various extraction methods, including Headspace, Solid Phase Micro Extraction (SPME), and Supercritical Fluid Extraction (SFE), which enhance the accuracy of detecting volatile and traceless accelerants like ethanol and acetone. The study also covers analytical tools such as UV-visible spectroscopy, Gas Chromatography (GC), and Gas Chromatography-Mass Spectrometry (GC-MS), emphasizing their role in forensic fire residue analysis

Dhabbah et al.(2015): The study highlighted the importance of analyzing fire debris and identifying accelerants in arson investigations, focusing on Saudi Arabia. It covers commonly used accelerants, including gasoline, kerosene, diesel, and paint thinner, as well as methods for collecting and storing evidence from fire scenes. Challenges include obtaining evidence after fire suppression and site release, making proper sampling crucial. Gas Chromatography with Headspace (GC-Headspace) is recommended for analyzing solid- phase residues, while Gas Chromatography-Mass Spectrometry (GC-MS) or Fourier Transform Infrared (FT-IR) are suitable for liquid samples. Study emphasize the difficulty of preserving evidence at arson scenes and suggest improvements in evidence handling to overcome these issues.

Scott (2015): The author critically examined the misuse of accelerant-detecting dogs in arson investigations, highlighting concerns over their reliability and the potential for wrongful convictions. The study emphasized issues such as handler bias, false alerts, and the subjective nature of canine responses, which often lead to unconfirmed accelerant alerts being admitted as evidence in court. The research pointed out several cases where unverified canine alerts contributed to wrongful arrests and convictions, underscoring the need for confirmatory laboratory analysis. The author advocated for restricting the use of unconfirmed canine alerts in legal proceedings while recognizing their potential utility in preliminary investigations.

Kwang et al.(2017): The authors emphasized the importance of managing flammable materials and understanding combustion characteristics to prevent and investigate arson. They analyzed statistical data on arsons and common flammable materials like wood, paper, synthetic textiles, and resins, alongside accelerants such as gasoline, diesel, and solvents. Thermogravimetric analysis was performed to evaluate the thermal properties of these materials. Additionally, burning and flame spread rate tests were conducted to compare and analyze their combustion characteristics. The findings aim to enhance arson prevention and investigative methods.

Alberca et al.(2018): This study explored the chemical alterations of ignitable liquids (ILs) such as thinners and bitumen of Judea when mixed with sulfuric acid, a modification that could affect forensic investigations of arson cases in this study. Using ATR-FTIR and GC–MS techniques, researchers identified significant compositional changes, including ester hydrolysis, Fischer esterification, and alkylation of aromatic compounds. Alcohols and ketones reacted to form new by-products, while alkanes remained mostly unaltered. Extended acid exposure led to similar chemical profiles among different ILs, potentially complicating forensic identification.

Jasper et al.(2018): This study explored the application of stable isotope analysis in arson investigations, using gas chromatography/isotope ratio mass spectrometry (GC- IRMS) to trace accelerants to their sources. Traditional gas chromatography/mass spectrometry (GC-MS) is commonly used for identifying ignitable liquids, but stable isotope analysis offers a highly specific fingerprint for accelerants. Through controlled fire experiments, the study demonstrates how isotopic compositions change due to evaporation and combustion, providing a potential method for linking fire debris to specific fuel sources. Results indicate that molecular isotopic signatures remain detectable despite moderate combustion, though significant degradation occurs at extreme conditions.

Harris et al.(2019): This study provide a comprehensive overview of key forensic science disciplines, emphasizing the complexities of investigating arson, explosions, drug- related crimes, and trace evidence. They highlight the challenges in proving arson, where identifying accelerants is crucial, and discuss solvent wash as a common technique for their recovery. Additionally, they explore the significance of trace evidence, such as fibers, paint, and glass fragments, in establishing connections between objects and suspects. With advancements like Fourier transform infrared spectroscopy, forensic science continues to evolve, enhancing the accuracy and reliability of investigations.

Yadav et al.(2020): The authors reviewed advancements in forensic techniques for arson investigations, particularly the application of vibrational spectroscopy. The authors emphasized the increasing use of infrared and Raman spectroscopy as non-destructive techniques, which allow for the identification of accelerants and fire-damaged materials with higher accuracy. Their study highlighted the advantages of vibrational spectroscopy over conventional methods, such as sensitivity, rapid analysis, and the ability to preserve evidence for re-evaluation.

O'Hagan et al.(2021): The authors reviewed the use of accelerant detection canines (ADCs) in arson investigations, highlighting their role in detecting accelerants like gasoline and diesel at fire scenes. Their use has limitations, including the potential for false alerts caused by background materials and handler bias. While they greatly reduce investigation time and costs, their alerts require confirmation through laboratory testing to be admissible in court. The review also discusses challenges in fire investigations, including the proper collection and packaging of evidence and the role of other detection methods, like photoionization detectors. Overall, the study underscores the value of ADCs but calls for further refinement to minimize errors and enhance their forensic reliability.

Sousa et al.(2021): This study successfully evaluated and optimized thin-layer chromatography (TLC) methods for detecting cannabinoids, achieving accurate and reliable results. The researchers identified the ideal solvent system, hexane:ethyl ether (8:2 v:v), and visualization reagent, Fast Blue RR, in acidified conditions. The study effectively detected and distinguished various cannabinoids, including CBD, Δ9-THC, CBN, and CBG, each exhibiting distinct colors. Overall, this research demonstrates the effectiveness of TLC in cannabinoid analysis and provides a valuable tool for forensic applications, improving the accuracy and reliability of cannabinoid detection.

Kaur et al.(2022): The authors provide an in-depth examination of fire investigation, The study outlines essential protocols followed at fire scenes, including methods for classifying ignitable liquids, proper sample collection, and the analytical techniques used for detecting accelerants. Furthermore, the author highlights the importance of quality control and assurance in forensic fire analysis to ensure accuracy and credibility in findings. This work contributes to the field by providing a structured approach to fire investigation, reinforcing the significance of standardized methodologies in forensic science.

Singh et al.(2022): This study utilized four fire accelerants and six mixtures applied to cotton and nylon fabrics to determine their persistence after burning. Analysis using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy confirmed the presence of accelerants based on similar spectral peaks, indicating common functional groups. These findings highlight the importance of detecting accelerants in arson investigations to determine whether a fire was intentionally set.

Kwon et al.(2023): The authors examined air sampling effectiveness for volatile accelerants at fire scenes. Traditional methods such as SPME, dynamic headspace struggle with highly volatile substances. It is found that air sampling using a portable pump and solid adsorbents (Tenax TA, Carbopack B) proved more efficient. Highly volatile accelerants diffuse into air, making air sampling crucial for accurate detection. The overall results indicate air sampling significantly outperforms fire debris sampling methods in recovering volatile components.

Park et al.(2023): The authors used a practical GC–MS dataset containing approximately 4000 suspected arson cases for devoloping three machine-learning based classification models and their performances were evaluated. All models trained for classifying the data from fire residue into six categories; no fire accelerants detected or else one of fire accelerants was used within gasoline, kerosene, diesel, solvents, or candle. The classification accuracies of the random forest, supporting vector machine, and convolutional neural network model were 0.88, 0.88, and 0.92, respectively. By calculating feature importance of the random forest model, several potential chemical fingerprints of fire accelerants were discovered.

Stevanovic et al.(2024): This comprehensive study highlights the versatility and advantages of high-performance thin-layer chromatography in forensic chemistry, particularly in drug analysis and toxin identification. High-performance thin-layer chromatography has emerged as a cost-effective and efficient analytical technique, offering advantages such as low sample requirements, high accuracy, and low solvent consumption. This study demonstrates its effectiveness in analyzing cannabinoids, synthetic cannabinoids, benzodiazepines, and prescription drugs, and shows that combining high-performance thin- layer chromatography with mass spectrometry and chemometrics can increase its effectiveness. Overall, high-performance thin-layer chromatography is a powerful, reliable and practical tool in modern forensic chemistry, suitable for routine analyses and complex forensic investigations.

Zhang et al.(2024): The authors developed a novel approach for species categorical authentication of accelerants through flame video analysis. The study demonstrated the flame characteristics, particularly the probability density function (PDF) of flame apex angle counts, can effectively distinguish accelerant species. The method proved to be independent of factors such as accelerant loading amount, ignition location, ventilation conditions, and substrate type, ensuring stability and reliability. Additionally, the temporal variation of flame area and the tangent of the flame cone angle effectively differentiated gasoline from diesel. These findings highlight the potential of flame characteristic analysis as a non- invasive and efficient technique for arson investigations.

Jais et al.(2025): This study conducted a forensic analysis of accelerants on different fabrics using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy and chemometric techniques. The study examined six fabric types burned with RON95, RON97 gasoline, and diesel to assess burning rates and chemical profiles. The results indicated that RON95 burned the fastest, while wool exhibited the slowest burning rate across all accelerants. ATR-FTIR analysis revealed the presence of functional groups such as alkanes, alcohols, alkenes, alkynes, aromatics, and amines.

2.3 RESEARCH GAP

The existing studies discuss the application of thin-layer chromatography (TLC) in forensic science and the role of fluorescence in analytical detection methods. Over time, TLC has evolved as a crucial tool for identifying ignitable liquids in arson investigations, yet the comparative effectiveness of different fluorescent dyes remains underexplored. While various studies have examined fluorescence-based detection techniques in other scientific fields, their forensic applications, particularly in fire debris analysis, require further investigation. As forensic science continues to advance with improved detection methods, it is essential to assess and refine the use of fluorescent dyes in TLC to enhance sensitivity, accuracy, and reliability in identifying arson accelerants.

CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

Thin Layer Chromatography (TLC) is a well-accepted technique for the separation and identification of chemical compounds. In forensic science, it is used for detecting accelerants in burning cases, which are important to identify the existence of flammable substances like kerosene, diesel, and petroleum. In most cases, fluorescent dyes are used to visualize these substances under UV light. This study aims at comparing the efficiencies of five fluorescent dyes on TLC plates in detecting accelerants. The outcome is expected to show the most effective dye with applications in forensic science.

3.2 STATEMENT OF THE PROBLEM

Detection of the accelerants in arson cases forms an integral part of forensic investigations. The selected fluorescent dye in the TLC assay may influence the sensitivity and the clarity of the result. Although Rhodamine B is the most used, no comparative studies are available on its effectiveness, with the exception of Eosin Yellow, Fluorescein Sodium, Coumarin, and Rose Bengal. This study attempts to fill this gap through a systematic comparison of these dyes in search of the best accelerants detector.

3.3 RATIONALE FOR THE STUDY

Detecting accelerants in arson cases directly results in an accurate cause of fire and an evidential report. The use of the best fluorescent dye improves the endeavors in the forensic discipline. The comparative performance of five fluorescent dyes will provide insight into which dye provides the best visibility, sensitivity, and specificity in forensic applications. The research will contribute to improvement in forensic methodologies and hence resolving cases of arson.

3.4 OBJECTIVE OF THE STUDY

- To evaluate the visibility and clarity of each dye under UV light.
- To determine the sensitivity of each dye in detecting trace amounts of accelerants.

- To identify the most effective fluorescent dye for forensic TLC applications.

3.5 HYPOTHESIS

- Despite its chemical hazard concerns, Rhodamine B remains the most widely used fluorescent dye in forensic Thin Layer Chromatography (TLC) for detecting accelerants.
- There are alternative fluorescent dyes that can be used in tlc for detecting accelerants, which are safer and equally or more effective than Rhodamine B.

3.6 OPERATIONAL DEFINITIONS

3.6.1 QUENCHING

A phenomenon where the fluorescence of a dye is reduced or eliminated due to interactions with other chemicals or environmental factors. In this study, quenching will be observed if any accelerants interfere with the fluorescence of the dyes.

3.6.2 SOLVENT FRONT

The furthest point reached by the mobile phase on the TLC plate. It is used as a reference point for calculating Rf values.

3.6.3 FORENSIC THRESHOLD

The minimum fluorescence intensity required for a result to be considered valid in a forensic context. This threshold will be established based on the clarity and reproducibility of the results.

3.6.4 BASELINE

The starting line on a TLC plate where the sample (accelerants) is applied using a capillary tube. It is typically drawn 1 cm from the bottom edge of the plate. The baseline ensures consistent application and accurate measurement of the retention factor (Rf value).

3.6.5 SOLVENT SYSTEM

The mixture of solvents used in the mobile phase to separate compounds on a TLC plate. The choice of solvent system affects the polarity and separation efficiency. In this study, the solvent system will be optimized to achieve clear separation of accelerants.

3.6.6 CAPILLARY ACTION

The ability of a liquid (solvent) to flow in narrow spaces, such as the pores of the silica gel on a TLC plate, without the assistance of external forces. Capillary action is the driving force behind the movement of the mobile phase up the TLC plate.

3.6.7 SPOTTING

The process of applying a small amount of sample (accelerants) onto the TLC plate using a capillary tube. Proper spotting ensures that the sample is concentrated in a small area, which is critical for achieving clear and distinct spots after development.

3.6.8 PHOTOBLEACHING

The degradation of a fluorescent dye due to prolonged exposure to UV light. This will be monitored to ensure consistent results across all experiments.

3.6.9 MOBILE PHASE

The solvent system that moves up the TLC plate, carrying the accelerants with it. The choice of mobile phase affects the separation efficiency.

3.6.10 STATIONARY PHASE

The solid adsorbent (silica gel in this case) coated on the TLC plate, which interacts with the accelerants to facilitate separation.

3.7 RESEARCH DESIGN

The study focuses on comparing the effectiveness of five fluorescent dyes such as Rhodamine B, Eosin Yellow, Fluorescein Sodium, Coumarin, and Rose Bengal in detecting accelerants such as petroleum, diesel, and kerosene using Thin layer chromatography. Accelerants are applied to silica-coated plates, developed in a solvent system, and sprayed with each dye. Plates are visualized under UV light, and fluorescence intensity, clarity, and separation are assessed.

3.8 INCLUSION CRITERIA

The study will include only the five specified fluorescent dyes (Rhodamine B, Eosin Yellow, Fluorescein Sodium, Coumarin, and Rose Bengal), silica-coated TLC plates, and standardized accelerants (kerosene, diesel, and petroleum). A consistent UV light source will also be used for visualization.

3.9 EXCLUSION CRITERIA

Non-fluorescent dyes, accelerants other than kerosene, diesel, and petroleum, damaged or inconsistent TLC plates, and experiments conducted under uncontrolled environmental conditions will be excluded from the study.

3.10 RESEARCH TOOLS

The tools include silica-coated TLC plates, capillary tubes for sample application, a solvent system for development, fluorescent dyes, a UV lamp for visualization, a spray bottle for dye application, and image analysis software (e.g., ImageJ) for quantifying fluorescence intensity.

3.11 PROCEDURE

The research began with the preparation of thin-layer chromatography (TLC) plates coated with silica gel as the stationary phase. Accelerant samples—kerosene, diesel, and petroleum—were applied using capillary tubes onto a baseline marked 1.5 cm from the bottom of each plate. After drying, the plates were placed in a development chamber containing a pre-saturated mobile phase solvent, which separated the accelerant components as it ascended. Once the solvent front reached a predetermined point, the plates were removed, and the solvent front was marked for retention factor calculations.

Next, five fluorescent dyes such as rhodamine B, eosin yellow, fluorescein sodium, coumarin, and rose Bengal were evenly applied to the plates using a fine mist sprayer. The plates were dried in a controlled environment to ensure uniform fluorescence under UV light. In a darkened room, the plates were exposed to UV light at different wavelengths, and high-resolution images were captured to analyze the fluorescence intensity, clarity, and spot distinctiveness of each dye.

The analysis revealed the relative performance of each dye in terms of sensitivity, contrast, and stability. This comparative evaluation will identify an alternative dye that outperform rhodamine B, offering improved detection of accelerants in arson investigations. The study's methodical approach provided valuable insights for refining forensic methodologies in accelerant detection.

3.12 DATA ANALYSIS

In the qualitative analysis, the visibility and clarity of the accelerant spots for each fluorescent dye will be compared to assess their effectiveness in detection. Any differences in fluorescence patterns, such as variations in brightness, diffusion, or interference from the background, will be carefully documented to identify the most suitable dye for TLC detection. For the quantitative analysis, image analysis software like ImageJ will be used to measure the fluorescence intensity of each spot. The average fluorescence intensity for each dye and accelerant combination will be calculated to provide an objective comparison of their detection capabilities.

3.13 ETHICAL CONSIDERATIONS

Chemical safety must be prioritized by handling all chemicals, including fluorescent dyes, accelerants, and solvents, with care while using appropriate personal protective equipment (PPE) such as gloves, lab coats, and safety goggles. Environmental safety is also essential, requiring proper disposal of chemical waste in accordance with local regulations to minimize environmental impact. Maintaining data integrity is imperative, ensuring that all experiments are conducted with precision and honesty, avoiding any manipulation of data or results. Additionally, transparency must be upheld by clearly documenting all procedures, observations, and results to ensure reproducibility and accountability.

3.14 SUMMARY

This study aims to compare the effectiveness of five fluorescent dyes—Rhodamine B, Eosin Yellow, Fluorescein Sodium, Coumarin, and Rose Bengal—in detecting accelerants (kerosene, diesel, and petroleum) using TLC. The methodology involves preparing TLC plates, applying accelerants, developing the plates, spraying them with fluorescent dyes, and visualizing the results under UV light. The study will evaluate the dyes based on fluorescence intensity, sensitivity, and clarity, with the goal of identifying the most effective dye for forensic applications. Ethical considerations, such as chemical safety and data integrity, will be prioritized throughout the study. The results will contribute to improving forensic methodologies for arson investigations.

CHAPTER 4: RESULT ANALYSIS

4.1 INTRODUCTION

Thin Layer Chromatography (TLC) is a widely used forensic technique for detecting arson accelerants, often employing fluorescent dyes for enhanced visibility. Rhodamine B is the most commonly used dye in forensic TLC due to its strong fluorescence properties. However, concerns regarding its chemical hazards, toxicity, and environmental impact necessitate the exploration of safer and equally effective alternatives. This study aimed to compare the performance of Eosin Yellow, Fluorescein Sodium, Rose Bengal, and Coumarin with Rhodamine B in detecting arson accelerants. The objective was to determine whether an alternative dye could provide comparable or superior results while addressing the health and environmental concerns associated with Rhodamine B.

4.2 FIGURES OF SAMPLES

4.2.1 RHODAMINE

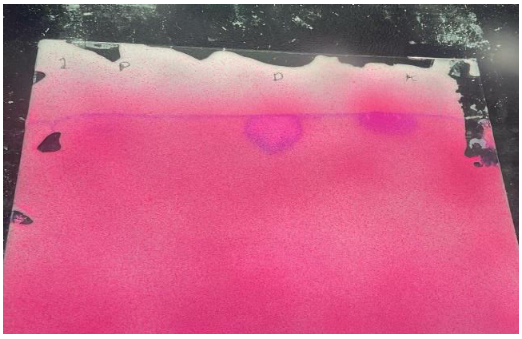

Fig 4.2.1.1 TLC plate treated with Rhodamine B, observed under fluo tube.

Fig 4.2.1.2 TLC plate treated with Rhodamine B, observed under short wave UV light.

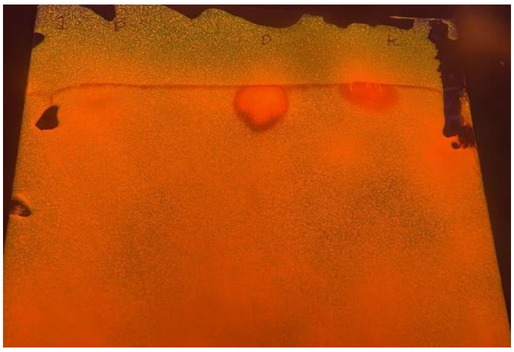

Fig 4.2.1.3 TLC plate treated with Rhodamine B, observed under low wave UV light.

4.2.2 EOSIN YELLOW

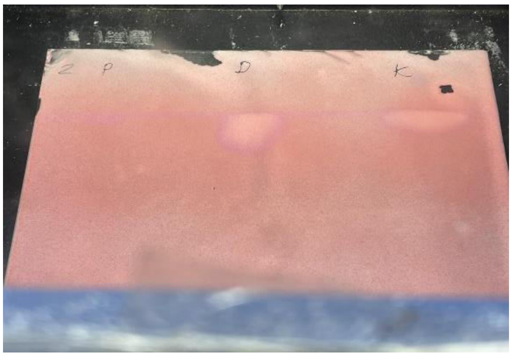

Fig 4.2.2.1 TLC plate treated with Eosin yellow, observed under fluo tube.

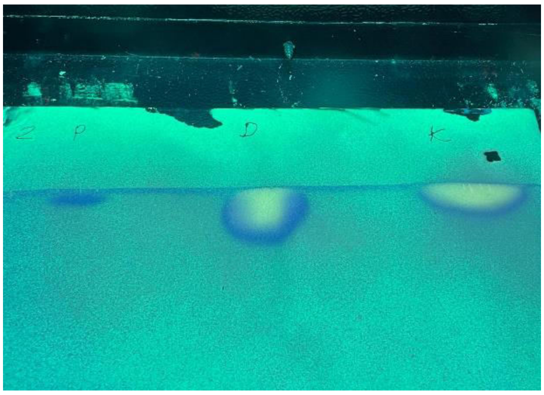

Fig 4.2.2.2 TLC plate treated with Eosin yellow, observed under short wave UV light.

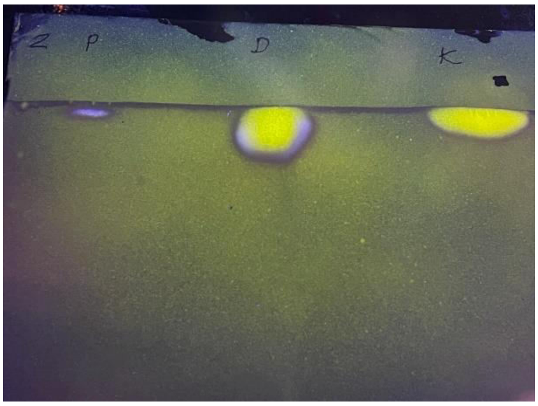

Fig 4.2.2.3 TLC plate treated with Eosin yellow, observed under low wave UV light.

4.2.3 FLUORESCIEN SODIUM

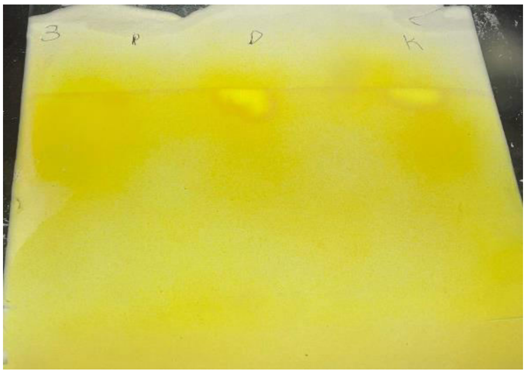

Fig 4.2.3.1 TLC plate treated with fluorescein sodium, observed under fluo tube.

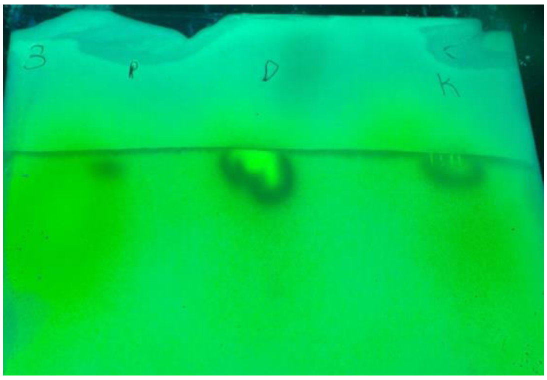

Fig 4.2.3.2 TLC plate treated with fluorescien sodium, observed under short wave UV light.

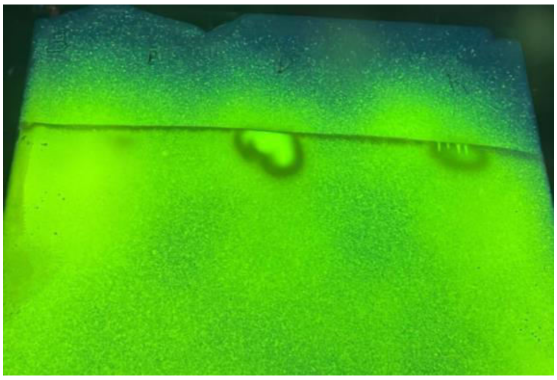

Fig 4.2.3.3 TLC plate treated with fluorescien sodium, observed under low wave UV light.

4.2.4 ROSE BENGAL

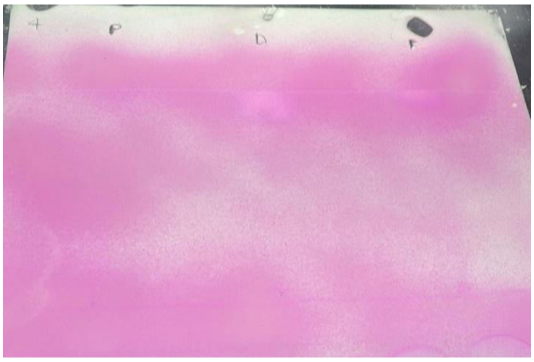

Fig 4.2.4.1 TLC plate treated with Rose bengal, observed under fluo tube.

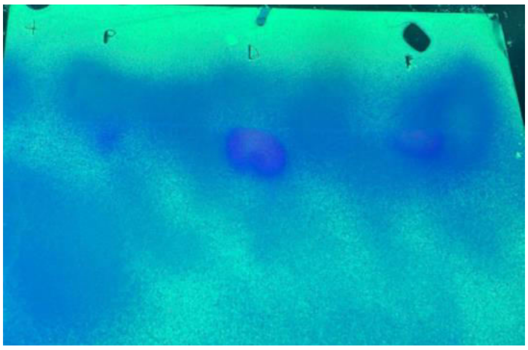

Fig 4.2.4.2 TLC plate treated with Rose bengal, observed under short wave UV light.

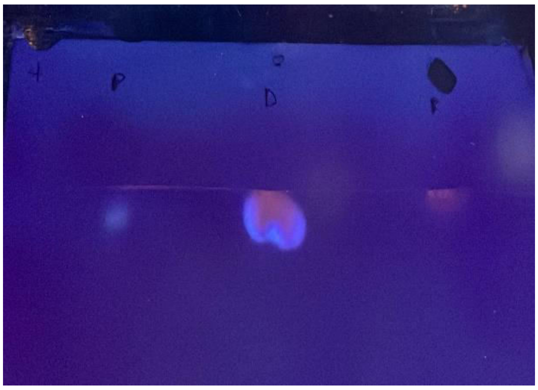

Fig 4.2.4.3 TLC plate treated with Rose bengal, observed under low wave UV light.

4.2.5 COUMARIN

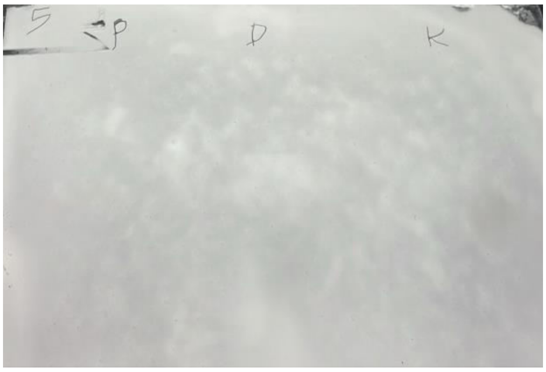

Fig 4.2.5.1 TLC plate treated with coumarin, observed under fluo tube.

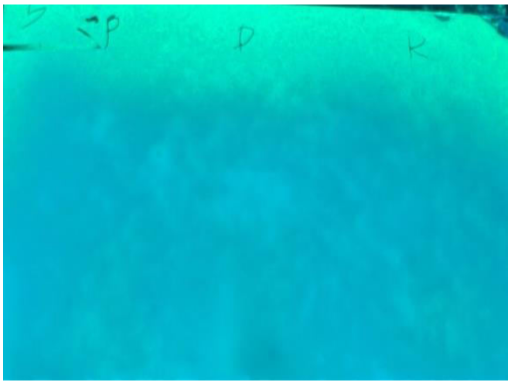

Fig 4.2.5.2 TLC plate treated with coumarin, observed under short wave UV light.

Fig 4.2.5.3 TLC plate treated with coumarin, observed under low wave UV light.

4.3 HYPOTHESIS-BASED INTERPRETATION

The analysis was conducted based on two hypotheses. The first hypothesis suggested that

Rhodamine B remains the most widely used fluorescent dye in forensic TLC for detecting accelerants. The results of this study do not refute this hypothesis, as Rhodamine B is indeed widely used in forensic TLC. However, the study's findings do suggest that this may not be the optimal choice, given the availability of alternative dyes with improved safety profiles and comparable or superior performance.

The second hypothesis suggested that there are alternative fluorescent dyes that can be used in TLC for detecting accelerants, which are safer and equally or more effective than Rhodamine B. The results of this study strongly support this hypothesis. Eosin Yellow, in particular, was found to outperform Rhodamine B in terms of sensitivity, specificity, and safety. The biodegradability and lower toxicity of Eosin Yellow make it a more attractive option for forensic TLC applications.

In conclusion, this study confirms that Rhodamine B is widely used in forensic TLC for detecting accelerants, but also reveals that alternative dyes like Eosin Yellow offer improved safety profiles and superior performance. The findings strongly support the existence of safer and more effective alternatives to Rhodamine B, with Eosin Yellow emerging as a prime candidate for future forensic TLC applications.

4.4 SUMMARY

The comparative study of fluorescent dyes for TLC detection of arson accelerants demonstrated that Eosin Yellow is a more effective and safer alternative to Rhodamine B. It provides superior fluorescence intensity, better compatibility with accelerants, lower toxicity, and greater environmental safety. Additionally, its cost-effectiveness makes it a practical choice for forensic applications.

Based on these findings, forensic laboratories should consider adopting Eosin Yellow as a replacement for Rhodamine B in arson investigations. Future studies can further explore its application across a broader range of accelerants and forensic conditions to solidify its effectiveness in real-case scenarios.

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 INTRODUCTION

Forensic science continually seeks safer and more sustainable analytical techniques. Concerns over the chemical hazards of Rhodamine B have driven the search for alternatives. Reviews by Stevanovic et al. (2024) and Shaki et al. (2010) highlight the advantages of HPTLC and the role of functional groups in fluorescent dyes, emphasizing the need for innovative materials in forensic applications.

The results of this study reveal that Eosin Yellow outperforms Rhodamine B and other tested dyes in terms of detection sensitivity, cost-effectiveness, and environmental safety. This finding resonates with Stevanovic et al.'s (2024) emphasis on the importance of efficient and practical analytical tools, as well as Shaki et al.'s (2010) insights into the design and optimization of fluorescent dyes. By identifying Eosin Yellow as a safer and more effective alternative, this study contributes to the growing emphasis on sustainability and safety in forensic science, paving the way for greener and more responsible forensic practices.

5.2 MAJOR FINDINGS

- Eosin Yellow emerged as the most effective alternative to Rhodamine B for detecting arson accelerants.
- It demonstrated superior detection capabilities, excelling in sensitivity, clarity of detection, and cost-effectiveness.
- The study emphasizes the importance of environmental and safety considerations in selecting dyes for forensic applications.
- Other tested dyes (Fluorescein Sodium, Rose Bengal, and Coumarin) showed promising results but were less effective than Eosin Yellow in detection clarity and sensitivity.

5.3 DISCUSSIONS RELATED TO HYPOTHESIS

The study's findings confirmed the two primary hypotheses, shedding light on the effectiveness and limitations of Rhodamine B and alternative fluorescent dyes in forensic Thin Layer Chromatography (TLC) for detecting accelerants.

With respect to the first hypothesis, the results showed that Rhodamine B is indeed widely used in forensic TLC due to its effectiveness, but its chemical hazards and environmental risks were evident, supporting the need for safer alternatives. However, specific details on the extent of these hazards could not be obtained due to the limitations of the current study. Details on the environmental impact of Rhodamine B and its alternatives are also not obtainable using the current methodologies. Third-party assessments developed specifically for this cause must be implemented to get satisfactory results.

Onto the second hypothesis, it is also proved that alternative fluorescent dyes like Eosin Yellow can be used in TLC for detecting accelerants, which are safer and equally or more effective than Rhodamine B. However, it must be taken into consideration that not all dyes will have the same level of effectiveness and this observation may vary depending on the specific application and conditions. These findings may occur in different contexts, be it in forensic or research applications.

5.4 LIMITATIONS

- The analysis was conducted on a limited number of accelerant samples, restricting the generalizability of the findings to diverse arson cases.
- Some alternative dyes may not be as readily available as Rhodamine B, posing challenges for widespread adoption.
- The study used standard TLC techniques, and more advanced analytical methods could provide deeper insights into dye performance.

5.5 SUGGESTIONS FOR FURTHER STUDY

- Expand sample analysis by testing a wider range of accelerants and environmental conditions to validate the effectiveness of Eosin Yellow and other alternatives.

- Utilize advanced analytical techniques (e.g., chromatographic and spectroscopic methods) for deeper insights into dye performance.
- Conduct long-term environmental impact studies to assess the ecological implications of using alternative dyes in forensic applications.

5.6 IMPLICATIONS

The study underscores the importance of environmental safety in forensic applications, highlighting the potential benefits of adopting safer dyes like Eosin Yellow. By reducing the use of hazardous chemicals like Rhodamine B, forensic investigations can improve their safety and sustainability without compromising detection efficiency.

Furthermore, this shift aligns with global efforts to minimize environmental pollution and promote green chemistry, emphasizing the need for regulatory bodies to encourage the use of safer alternatives in forensic laboratories

5.7 CONCLUSIONS

The present study demonstrates the potential risks associated with the continued use of Rhodamine B in forensic TLC, highlighting the need for safer alternatives. However, it is possible to identify more environmentally friendly and safer dyes like Eosin Yellow, which offers additional benefits in terms of biodegradability and cost-effectiveness. There is a noticeable difference in the performance of Eosin Yellow compared to Rhodamine B, particularly in terms of safety and sustainability. However, the study's findings cannot be completely generalized for a wider range of accelerants and conditions, as further research is needed to validate these results. The technology currently used for TLC analysis is not enough for a more detailed analysis on the chemical properties of the dyes. The present study would be further relevant if there was a more comprehensive analysis of the chemical hazards associated with Rhodamine B, and technologically advanced methods were developed to conduct detailed analysis and further help in identifying safer alternatives to provide more accurate and reliable results.

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