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"A Systematic Review of HCR, PDR, and ARR-Based Approaches for Enhancing Bloody Fingerprint Evidence"

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Abstract

With the development of new reagents, sophisticated materials, and cutting-edge processes during the past ten years, the field of blood fingerprint enhancement has made considerable advancements. Blood fingerprint enhancement is an important part of forensic science, even though it is not as visually appealing as latent fingerprint development. It provides important evidence for identity verification, forensic investigation, and crime scene reconstruction. For improving blood fingerprints, conventional chemical reagents that target heme, protein, and amino acids are frequently utilised. These reagents have been modified and optimized to improve sensitivity, selectivity, and contrast. However, false positives remain a significant concern, and partial blood fingerprint enhancement is still challenging. Emerging enhancement techniques based on advanced materials, new equipment, or methods have also shown great potential. For instance, magnetic nanoparticles, fluorescent dyes, and micro-structured surfaces have been used to enhance blood fingerprints with high sensitivity and specificity. However, the compatibility of blood enhancement techniques with DNA analysis remains a crucial issue.Other critical issues in forensic science related to blood fingerprint enhancement include fingerprint age determination, the development of standard operating procedures, and the validation of enhancement techniques. Additionally, the use of blood enhancement techniques in different environmental conditions and substrates requires further investigation. To address these issues, researchers in the field must collaborate to establish a standardized protocol for blood fingerprint enhancement, develop techniques with low false-positive rates and high sensitivity and specificity, and investigate the compatibility of enhancement techniques with DNA analysis. In conclusion, blood fingerprint enhancement is a vital area of forensic science, and recent advances in conventional chemical reagents and emerging techniques have provided promising avenues for further research. However, there are still many critical issues that need to be addressed before blood fingerprint enhancement can reach its full potential in forensic science.

Keywords: Fingerprint enhancement; reconstruction; nanoparticles; Amino acids; validation; emerging techniques

1. Introduction

Fingerprints have maintained their unparalleled importance in forensic science since Henry Faulds first discovered their evidential value over a century ago [1]. With the unique ridge details, lifetime invariability, and detectability at crime scenes, fingerprints remain the gold standard for identification [2]. Despite the advent of modern technologies, such as DNA analysis, the individuality of fingerprints and their ability to link a suspect to a crime scene has never been shaken [3]. In the realm of forensic investigation, there are two primary forms of fingerprints: latent and patent [4]. While patent fingerprints are visible to the naked eye, most prints found at crime scenes are latent or invisible. This has led to a greater focus on the development of latent fingerprints by forensic investigators and researchers in chemistry, materials science, and optical science [5]. In recent decades, there has been a surge of interest in the combination of nanotechnology and fluorescence imaging for the detection of latent fingerprints [6]. This innovative approach has resulted in higher sensitivity, higher contrast, and higher selectivity in latent fingerprint detection. As a result, forensic scientists can now extract more information from latent prints than ever before, aiding in the identification and prosecution of criminals [7]. Contrary to popular belief, blood fingerprints can sometimes be patent marks, meaning that specific treatment is not required to visualize them [8]. However, this doesn't mean that development methods for blood fingerprints are given less attention. In fact, despite their potential visibility, it is still necessary to use specific methods to enhance their visibility in cases where the bloodstain is blurry due to low amounts or longer aging times, or when the background color is similar to that of the bloodstain.

It is a well-known fact that blood can often contaminate fingerprints found at crime scenes, especially in cases of violent crimes [9] However, with the help of advancements in technology and materials, there have been significant developments in

enhancing blood fingerprints over the past decade [10]. This review highlights the evolution of traditional chemical development techniques such as heme-catalytic reagents (HCR), protein-dyed reagents (PDR), and amino-reacted reagents (ARR) for blood fingerprint development, which have shown promising results in recent studies.

2. Heme-catalytic reagent (HCR) based method

There are various compounds such as benzidine, aniline, lumino, leuco crystal violet (LCV), and other alternative reagents have been used to develop blood fingerprints. However, despite the effectiveness of some HCR-based methods, they are still considered presumptive tests rather than confirmatory ones [11]. Recent research in HCR has focused on improving the accuracy of blood fingerprint development by reducing false positive and negative reactions, as well as exploring new fixing agents that are non-toxic [12]. Innovative approaches to fixing blood fingerprints while using HCRs include using alginate to prevent vertical diffusion of blood fingerprints prior to luminol treatment and utilizing 5-sulfosalicylic acid (SSA) as a stabilizer for the protein in blood fingerprints before LCV enhancement [13]. Additionally, researchers are currently exploring new compounds and techniques to improve the accuracy and reliability of blood fingerprint development, which could have important implications for forensic investigations [14].

2.1 Benzidine

In 1904, Oskar Adler and Rudolf Adler made a breakthrough discovery regarding benzidine compounds, when they noticed that these compounds reacted with blood to produce blue substances [15].

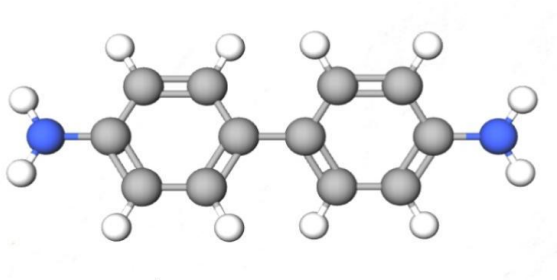

Figure 1: Molecular Structure of Benzidine

The development of bloodstains increasingly used benzidine. Nonetheless, safer options were sought out due to its significant risk of carcinogenicity [16]. Holland proposed tetramethylbenzidine (TMB) as a less dangerous substitute in 1974 [17]. Other body fluids are unaffected by conventional HCRs, which only react with blood [18]; nevertheless, they may interact with vegetable peroxidases and trigger cross-reactions. Hussain and Pounds offered diaminobenzidine (DAB) as a highly specific replacement in 1989 [19, 20] to overcome this problem. DAB doesn't react incorrectly with iron or rust and has a pH high enough to prevent cross-reactions with vegetable peroxidase.

2.2 Aniline compounds

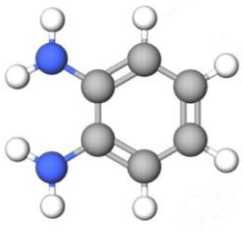

Figure 2: Molecular Structure of o-phenylenediamine (OPD).

Aniline compounds, such as o-phenylenediamine (OPD) and p-phenylenediamine (PPD), have proven to be dependable and less harmful alternatives to 3,30-diaminobenzidine (DAB) for blood fingerprint enhancement.

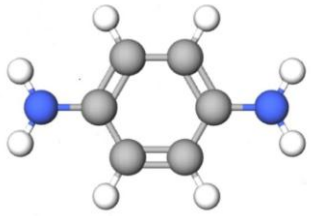

Figure 3: Molecular Structure of p-phenylenediamine (PPD).

OPD has been shown by Caldwell and Kim to significantly improve blood fingerprints on paper surfaces at pH 5.4 and glass surfaces at pH 7.4 [21]. Although OPD was developed in 2002, there has been little progress in the sector until Oliver found that OPD solutions at pH 5.4, 6.4, and 7.4 demonstrated equal efficacy in improving blood fingerprints, with pH 5.4 on ceramic substrates producing somewhat better results in 2018. They also found that OPD could enhance both recent blood markings and latent marks that were up to 90 days old [22].

2.3 Luminol

The use of luminol has been a popular method for decades in detecting hidden bloodstains due to its high sensitivity, simple preparation, and low cost [23]. While Albrecht is generally credited as the pioneer who reported the chemiluminescent reaction of luminol in 1928, the chemical's interference immunity has been a longstanding concern [24]. Luminol's chemiluminescence can be affected or blocked by a wide range of substances, including some antioxidants or iron-containing reagents, horseradish, and household bleach [25]. Another limitation is that luminol does not contain a fixing agent, which can cause ridge details to become diffuse with repeated applications on non-porous surfaces [26]. Additionally, recent research by Akemann et al. in 2018 suggests that luminol may produce a false negative result when detecting bloodstains on some surfaces exposed to heat, fire, soot, or water. Despite its popularity, the limitations of luminol call for alternative methods or improved technologies in forensic investigations [27].

2.4 Leuco crystal violet (LCV)

For years, leuco crystal violet (LCV) has been a popular developer for latent fingermarks in blood due to its quick and relatively simple process [28]. LCV is colorless, but when combined with hydrogen peroxide and blood, it is oxidized and turns purple, making it ideal for enhancing blood fingermarks on light-colored surfaces [29]. However, the use of LCV and other peroxidase reagents is not currently recommended in the forensic community due to their carcinogenic properties [30].Despite these concerns, recent studies have shown that LCV has biocompatibility and can be improved with better fixatives and luminescence properties. Fox et al. (2014) reported that messenger RNA profiling may be affected by the use of LCV for blood treatment, which could impact subsequent genetic analysis for body fluid identification [31]. Therefore, it is crucial to investigate the impact of bloodstain age and lengthen the time between enhancement and genetic analysis in real cases [32]. Although LCV remains a useful tool in forensic investigations, caution must be taken to ensure its safe and effective use.

Moreover, studies have shown that employing different chemicals might reduce bubbling while processing blood fingerprinting with LCV [33]. McCarthy (2014) discovered that 5-sulfosalicylic acid (SSA) can enhance LCV's capacity to produce and detect luminescence [34] and serve as a fixative for proteins in bloodstains. A method to decrease LCV bubbling was developed in a manner similar to this by Seo and Yu (2019) by first treating blood samples with an ethanol-based aminotriazole (AT) solution [35]. With these state-of-the-art methods, blood fingerprint particular and idiosyncrasies can be better retained during the forensic investigation process.

2.5 Other methods of HCR

The hemochrome reaction (HCR) approach uses a number of other techniques in addition to those already described, including the use of leucomalachite green (LMG), fluorescein, and 2,2-azino-di-[3ethylbenzthiazolinesulfonate] diammonium salt (ABTS). When subjected to a redox reaction, ABTS yields a pale green tint and is a safer substitute for DAB. For some surfaces, the brilliant green colour of oxidised ABTS can be advantageous, although its production costs can be expensive. Due to their poor visibility or restricted applicability, LMG and fluorescein are less frequently used in blood fingerprint enhancement [36].

3. Protein-dyed reagent (PDR) based methods

The interaction between the proteins in blood and the dye reagents is influenced by various factors such as pH, resulting in different charges that allow positive and negative bonding to occur [37]. In addition to electrostatic forces, physical interactions such as hydrogen bonding and Van der Waals forces may also play a role in the affinity of acid dyes to protein molecules [38]. Among the commonly used protein dye reagents are Amido black, Coomassie brilliant blue, Hungarian red, Acid Yellow 7, and other biological dyes. Interestingly, the names of these acidic dyes often reflect their distinct colors, with some dyes like Hungarian red and Acid Yellow 7 being fluorescently excited while others such as Amido black and Coomassie brilliant blue are fluorescence-free [39].

3.1 Amido black (AB)

This unique protein dye has a fascinating history of aliases, including Buffalo black NBR, naphthol blue-black, pontacyl blue-black SX, and naphthalene black 10. Its usefulness in forensics was recognized by the British Police Scientific Development Branch, who recommended it for fingerprint enhancement in blood in 2010, with two different formulations based on methanol or water [40]. Over time, forensic investigators have carefully examined its compatibility with DNA analysis and sought to optimize its formulation for maximum effectiveness [41]. Despite some concerns about its potential impact on DNA recovery and analysis, recent studies have shown that the use of aqueous AB can actually enable the recovery of two distinct DNA profiles from touch DNA and blood samples, respectively, increasing its value for identifying suspects and victims alike. As a result, this versatile and valuable protein dye continues to be a vital tool for forensic scientists and investigators around the world.

3.2 Coomassie brilliant blue

Coomassie Brilliant Blue, also known as Acid Blue 83, is a well-known protein staining reagent that produces a bright blue color upon reacting with proteins [42]. However, its uses are not limited to protein staining, as it has also been found to be effective in enhancing the weak fingerprints of blood diluted up to 1 in 125 folds on porous and non-porous surfaces, as demonstrated by Mattson and Bilous in 2014 [43]. Despite its usefulness in enhancing fingerprints, there have been concerns regarding its biotoxicity on touch DNA, as pointed out by Tsai et al. in 2016. In their analysis, they found Coomassie Brilliant Blue to be unsuitable for subsequent STR typing due to observable stochastic effects on DNA quantity and quality [44].

3.3 Hungarian red

The history of Hungarian red can be traced back to the 1990s when the Dutch CHEMZIS workgroup discovered a protein-dyed reagent that was being used by the Miskolc police in Hungary. However, the workgroup was unaware of the ingredients in the solution and later found out that it was acid fuchsin [45], which gave the reagent its distinctive red color. Hungarian red was initially believed to be most effective on smooth and non-porous surfaces rather than porous or semi-porous ones [46]. Despite this, Corcoran's study demonstrated that Hungarian red was equally effective in enhancing blood fingerprint ridge patterns on contaminated glass and metal substrates [47]. However, recent research conducted by Petretei in 2019 revealed that Hungarian red has a low detecting sensitivity when applied to diluted bloodstains that have been diluted with water [48]. Even at a dilution ratio as low as 1:1, Hungarian red can only visualize ridge outlines, while AB exhibits better detection sensitivity in diluted bloodstains [49].

3.4 Acid yellow 7 (AY7)

With its exceptional fluorescence properties, AY7 stands out as a highly sensitive nonfluorescent PDR in comparison to other reagents [28]. In the field of forensic science, AY7 has been proven to be an invaluable tool in the detection and visualization of blood fingerprints on a variety of fabrics, including black cotton, polyester, and nylon/lycra [7]. Researchers have conducted extensive verification experiments that demonstrate AY7's ability to produce clear fingerprint details of Level 3 on non-porous surfaces under alternate light sources, making it the top choice among numerous blood reagents [50]. A study conducted by Mattson and Bilous further confirms the exceptional developing sensitivity of AY7, as it was able to effectively develop latent fingerprints on blood tiles diluted 1:125 [43].

3.5 Other methods of PDR

Benzoxanthene yellow and Crowle's Double Stain are two common techniques used to identify blood fingerprints on nonporous surfaces, but they both have their drawbacks. Although benzoxanthene yellow is effective, its availability is currently limited. On the other hand, prolonged exposure to Crowle's Double Stain can reduce STR amplification efficiency [51]. In contrast, a recent study by Chingthongkham et al. (2020) discovered a new approach using natural Lac dye (Laccifer lacca) that has promising results for detecting blood fingerprints on non-porous substrates. Lac dye could be a potential replacement for AB and other chemicals because it is less expensive and safer. The technique is effective in identifying and enhancing blood fingerprints on various types of non-porous surfaces, and it provides comparable color contrast and detail level to AB [52]. However, the results were relatively poor when it was applied to porous surfaces. Therefore, the use of Lac dye on nonporous surfaces shows potential for the detection and enhancement of blood fingerprints [53].

4. Amino-reacted reagent(ARR) based method

Hemoglobin, a vital protein in red blood cells responsible for oxygen transport, is composed of a complex arrangement of amino acids [54]. Techniques for enhancing bloodstains in forensic investigations often involve the use of reagents that react with specific chemical groups on proteins and peptides, including amino acids in plasma that have dissociated from proteins [55]. These enhancement methods based on amino acid reactive reagents (ARR) differ significantly from those based on protein digestion reagents (PDR) in their mechanisms of action [56]. Among the most commonly used ARR for staining bloodstains are ninhydrin and its analogues.

4.1 Ninhydrin

Ninhydrin has a long history in the field of forensic research, having been created for the first time in 1910 by Ruhemann, a professor of chemistry at Cambridge University [57].

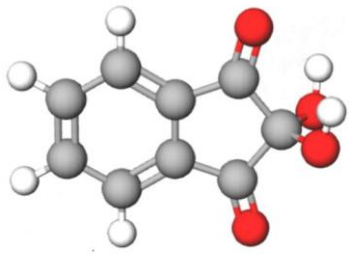

Figure : Molecular Structure of Ninhydrin.

However, it was not until 1954 that ninhydrin was discovered to be a powerful reagent for fingerprint enhancement [58]. Ninhydrin ability to develop sweat fingerprints on porous surfaces is well-known, but its effectiveness in developing blood

fingerprints has also been noted [59]. Over the years, the developing sensitivity of ninhydrin has been constantly improved, making it a reliable technique for detecting even small amounts of amino acids in fingerprints.

In 2014, Yang and Lian took NIN development to the next level by creating a new series of fingerprint developing membranes (FDM) that utilize ninhydrin as a developer. This solid-medium NIN membrane was found to be even more effective than traditional methods, with a detection limit of 0.1mg/L amino acid for both sweat and blood fingerprints deposited on porous and non-porous materials [60]. This breakthrough in fingerprint enhancement using ninhydrin technology has revolutionized the field of forensic science and has paved the way for further innovations in the future.

4.2 1,2-Indanedione (IND)

The potential of 1,2-Indanedione (IND) as a superior substitute for the conventional fingerprint detection approach employing ninhydrin (NIN) on porous surfaces has been established by cutting-edge research in the field of fingerprint detection [61]. Indanedione is a viable alternative for forensic investigators since its reaction product fluoresces strongly when excited by green light [62]. The amino acids in the fingerprint residues can be fully utilised by 1,2-Indanedione-ZnCl2 (IND-Zn) by lowering the electrostatic interactions or hydrogen bonds between the amino acids and the substrate, according to recent study on the reactivity and processing sequence of this compound. An evaluation of the impact of blood fingerprint detection technologies on DNA typing after treatment with either indanedione or ninhydrin on porous surfaces found no appreciable effects [63]. However, further study has shown that blood concentration influences the ability of a polyvinylpyrrolidone and 1,2-indanedione (PVP-IND) mixture to recognise fingerprints. Although PVP-IND was found to perform better than the aqueous solution of AB for fingerprints in diluted blood, it was found that the AB formula performed better for fingerprints in undiluted blood due to the diffusion of friction ridges, which may be attributed to the reaction of PVP with amino acids in the blood [64].

4.3 1,8 Diazafluoren-9-one (DFO)

Grigg created 1,8 diazafluoren-9-one (DFO), a highly luminous ninhydrin derivative, for the first time in 1990 [65]. It is a useful tool in forensic investigations because it combines with amino acids to produce a distinctive magenta colour. In tests using blood fingerprints and shoe impressions, Pereira discovered that ninhydrin and DFO performed remarkably well on porous surfaces [66]. DFO has been proven to have negligible negative effects on DNA recovery in addition to its forensic applications, as indicated by Laurin's systematic testing of numerous reagents/products and DNA analysis carried out in 2015 [67]. Overall, DFO is a useful tool in forensic investigations because of its distinctive fluorescence and compatibility with DNA recovery.

5. Conclusion

The fingerprints remain a crucial tool in forensic science for identifying suspects and linking them to crime scenes. This systematic review provides a comprehensive overview of the effectiveness of HCR, PDR, and ARR in enhancing bloody fingerprint evidence. The review highlights the potential of these techniques in improving the visualization and detection of bloody fingerprints, which can aid in criminal investigations. The development of blood fingerprints through Heme-catalytic reagents (HCRs) has been a significant breakthrough in forensic science. Although several compounds, including benzidine, aniline, luminol, and leuco crystal violet (LCV), have been used as alternative reagents, HCR-based methods remain presumptive tests rather than confirmatory ones. To improve the accuracy and reliability of blood fingerprint development, recent research has focused on reducing false positive and negative reactions and exploring new fixing agents that are non-toxic. Protein-dyed reagent (PDR) interact with the proteins in blood through various physical and electrostatic forces, resulting in distinct colors that can aid in visualizing latent prints. In this review we have discussed about protein dye reagents Amido black, Coomassie brilliant blue, Hungarian red, Acid Yellow 7 have distinct properties and varying levels of sensitivity. Natural lac dye shown promising results and used as a potential substitute for AB and other chemicals due to its lower cost and safer properties. Despite the concerns about biotoxicity and potential impact on DNA recovery and analysis, protein-dyed reagents continue to be an important tool for forensic scientists and investigators worldwide. The amino acid reactive reagents (ARR), such as ninhydrin, 1,2-Indanedione (IND), and 1,8 Diazafluoren-9-one (DFO), react with specific chemical groups on proteins and peptides, including amino acids, which are the building blocks of hemoglobin. The effectiveness of these reagents

in detecting even small amounts of amino acids has led to significant breakthroughs in fingerprint enhancement, making them a reliable tool for forensic investigators. The compatibility of these reagents with DNA recovery, further expanding their usefulness in forensic investigations.

Overall, this review paper highlights the potential of HCR, PDR, and ARR-based approaches for enhancing bloody fingerprint evidence and improving the accuracy of forensic investigations. With continued research and development, these techniques have the potential to become valuable tools for forensic investigators in solving complex criminal cases.

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