Article

The Effect of Cyanoacrylate Fuming on Subsequent Protein Stain Enhancement of Fingermarks in Blood

Nicole Mutter [1]
Paul Deacon [2]
Kevin J. Farrugia [1]

Abstract: This study investigates the effect of cyanoacrylate (CA) fuming, at atmospheric and vacuum conditions, on subsequent protein stain (acid violet 17) enhancement of fingermarks in blood. Fingermark depletions in blood were deposited on three nonporous surfaces (e.g., plastic bag) and aged for a set period of time (up to 28 days) before enhancement with the water-ethanol-acetic acid and methanol formulations of acid violet 17 (AV17). All trials were carried out in duplicate. One depletion was pre-treated with CA fuming followed by the enhancement technique and the other depletion was treated with only the enhancement technique (control).

As expected, atmospheric CA fuming hindered the subsequent enhancement of blood with the AV17 water-ethanol-acetic acid formulation but not the methanol formulation. The same observations were also recorded under vacuum CA fuming conditions. Preliminary work with vacuum metal deposition did not hinder subsequent AV17 protein stain enhancement with either formulation.

Introduction

The use of cyanoacrylate fuming for the development of latent fingermarks was reported independently in Japan and the United Kingdom in the late 1970s [1]. The mechanism and adaption of this enhancement process has been the subject of

[1] School of Science, Engineering & Technology, Division of Science, Abertay University, Dundee, U.K.
[2] Dundee, Scotland, U.K.

Received April 17, 2018; accepted June 12, 2018

many research papers [2–7]. Because of the poor contrast of the white cyanoacrylate polymer with the background, a secondary process is generally required by means of powders or fluorescent stains [8]. More recently, the use of various one-step fluorescent processes has been investigated [9–16]. Such products have a number of advantages over two-step processes in terms of reduced processing times, space, and cost; however, there are also drawbacks, such as the degradation of fluorescence over time. The U.K. Home Office Centre for Applied Science and Technology (CAST) currently ranks a one-step process as a Category C process: "Processes at a developmental stage exhibiting potential as an effective fingermark recovery process. Optional processes for occasional operational use. Possible reasons for use: no other options available; all Category A options have been exhausted; niche applications" [17]. In contrast, the two-step cyanoacrylate process with fluorescent staining [e.g., basic yellow 40 (BY40) and basic red 14 (BR14)] is regarded as a Category A process: "Processes extensively evaluated by the Home Office and considered suitably effective to be incorporated into processing charts. Standard processes for routine operational use. They must be used in preference to other category processes where possible" [17]. Further and extensive research by the forensic community is required before such one-step processes improve their status.

Integrated Forensic Approach

If multiple types of evidence are present (e.g., fingermarks and body fluids), consideration must be given to which forensic technique is to be applied first and whether the application of one technique for detecting one type of evidence affects the other. Thus, an integrated forensic approach that provides a strategy to maximize evidence recovery and improve efficiency is an important consideration when multiple types of evidence are present. It has been reported that the use of CA fuming is not detrimental to subsequent STR profiling for DNA recovery; however, the use of a stain resulted in reduced DNA quantities [18]. Another study [19] reported that both two-step and one-step CA processes (Lumicyano and Polycyano) had similar impacts on DNA profiles; however, the degradation effect of Polycyano UV on DNA was greater. The noninhibitive effect of CA fuming on blood and saliva marks that underwent subsequent DNA typing was also reported [20]. Furthermore, CA fuming was not detrimental to the detection of other types of evidence by presumptive and confirmatory tests such as blood

[21], semen or sperm heads [22], and saliva [23], as well as the analytical detection of explosives [24]. There were minimal effects on the recovery of explosives by high-performance liquid chromatography from glass subjected to CA fuming; however, some losses on the percentage recoveries from plastic and aluminum foil were observed that were due to the probable trapping of the target compounds in the deposited CA polymer [24].

Sequential Treatment of Fingermarks in Blood

In the late 80s, McCarthy and Grieve [25] reported that the preprocessing of blood marks with CA fuming may be advantageous but this depended on the stain that was used (all contained methanol) and whether the surface was textured. Previous advice for the treatment of evidence suspected to have possible latent and blood marks suggested that the more effective latent techniques should be applied prior to blood enhancement techniques; however, the use of CA fuming is severely detrimental to the subsequent performance of blood techniques, resulting in a significant reduction in quality and contrast of the marks [26]. Current recommendations for sequencing such articles of evidence recommend the use of vacuum metal deposition (VMD)→fingerprint powders→acid dyes (water-ethanol-acetic acid protein stain formulation)→powder suspensions with the omission of CA fuming [17]. The use of VMD can improve the contrast of blood marks with the background and is not detrimental to the subsequent use of acid dyes because the fixative 5-sulfosalicylic acid dissolves the zinc coating that may have covered any blood marks that are present. Methanol-based acid dyes can be used as a corrective measure if blood has previously been CA fumed because the methanol is able to "soften" the CA polymer formed over the blood and facilitates the penetration of the protein stain. It is also possible that CA fuming is detrimental to other blood enhancement techniques such as luminol and leucocrystal violet (heme specific); however, although such techniques are useful for the enhancement of footwear marks, they are not, in general, suitable for the fine ridge detail recovery of fingermarks and are less sensitive than acid dyes [26, 27].

This study evaluated the effect of cyanoacrylate (atmospheric and vacuum) on AV17 enhancement of fingermarks in blood. The results were then compared to the effects by VMD enhancement.

Materials and Methods

The methodology for latent mark acquisition, depletion series, grading of marks, and chemical formulations is adapted from that described by CAST and the International Fingerprint Research Group [28, 29].

This project was approved and followed procedures in accordance with the ethical standards of the authors' institutional review board.

Collection and Preparation of Samples

White plastic bags, white ceramic tiles, and pure aluminum sheets were obtained locally, ensuring that each substrate was from the same source. To ensure replication of experiments and to remove unwanted contaminants, all surfaces were cleaned with warm, soapy water and then ethanol before drying and creating a grid system for the easier deposition of 10 depletions. The donor's finger was dabbed in lysed horse blood, the excess was removed by pressing twice for two seconds on a clean paper towel, and then the depletion series were created. The donating finger was cleaned in-between each depletion series to ensure no blood had accumulated in-between the ridges.

Two sets of all depletion series were prepared: one set was treated with only AV17 and the other set was CA fumed prior to AV17 treatment. Prior to enhancement, deposited fingermarks were aged for four different periods (1, 7, 14, and 28 days) to investigate whether fingermark age affects the overall quality of enhancement.

Atmospheric CA Fuming

An Air Science chamber (CA30S) with an approximate volume of 450 L and fitted with a heat source of about 100 °C in addition to a humidifier (set at 80%) were used for cyanoacrylate fuming items under consideration. A running time of 45 minutes ensured that most of the cyanoacrylate had evaporated (>99%).

Vacuum CA Fuming

Vacuum CA was performed in a 25 L chamber (Applied Vacuum Engineering, Bristol, U.K.). Cyanoacrylate (0.4 g) (CSI Equipment Ltd., U.K.) was placed into two new separate foil dishes (0.2 g x 2), which were placed above the items to be fumed. Vacuum fuming conditions consisted of a pressure of 5 Torr for 40 minutes [15].

VMD [17]

A VMD 360 (West Technology, Bristol, U.K.) was used as the vaporization chamber for VMD treatment using the conventional and successive vaporization of gold (Alfa Aesar, gold wire, Ø 0.25 mm, annealed, 99.999%) and zinc (Alfa Aesar, spheres, Ø 1–5 mm, 99.999%).

Acid Violet 17 Water-Ethanol-Acetic Acid Formulation (AV17 WEAA) [17]

The application of AV17 WEAA was applied via a three-step process including fixation, staining, and destaining. The fixative solution was prepared by dissolving 5-sulfosalicylic acid (23 g, Acros) in distilled water. The articles to be processed were then immersed in the fixative solution for at least 5 minutes. The staining solution was prepared by dissolving acid violet 17 (1 g, BVDA, CI 42650) in acetic acid (50 mL, Fisher), ethanol (250 mL, Fisher), and distilled water (700 mL). After fixation, the articles were immersed in the staining solution for at least 5 minutes before immersion in a destaining solution consisting of acetic acid (50 mL, Fisher), ethanol (250 mL, Fisher), and distilled water (700 mL).

Acid Violet 17 Methanol Formulation (AV17 MeOH) [17]

The processing procedure for AV17 MeOH is also a three-step process. The fixative solution consisted of methanol only and the immersion period was for at least 1 hour. The staining solution was prepared by dissolving acid violet 17 (2 g, BVDA, CI 42650) in methanol (900 mL, Sigma) and acetic acid (100 mL, Fisher) followed by the immersion of articles for at least 5 minutes. The destaining solution was performed by immersion in wash solution 1 (methanol, 900 mL, Sigma; acetic acid, 100 mL, Fisher) to remove the excess dye followed by immersion in wash solution 2 (methanol, 950 mL, Sigma; acetic acid, 50 mL, Fisher) for approximately 30 seconds.

Photography and Grading of Marks

All depletion series were photographed before and after each enhancement stage using a Nikon 5100 DSLR camera equipped with a 60 mm micro Nikon lens. Following enhancement, blood marks in the depletion series were graded on a scale of 0 to 4 as recommended by CAST [28].

Results and Discussion

The Relationship Between the Enhancement Method and Blood Fingermark Quality

Figure 1 documents the effect of CA fuming on subsequent blood enhancement techniques as compared to the enhancement of blood marks without prior CA fuming. AV17 WEAA yielded a significantly greater number of identifiable fingermarks on depletion series that were not pre-treated with cyanoacrylate. Blood depletion series treated with AV17 WEAA alone gave 67 identifiable fingermarks, about 8 times more than depletions pre-treated with CA fuming. This confirms the detrimental effect of CA fuming on the subsequent performance of blood techniques, resulting in a significant reduction in quality and contrast of the marks [26]. Nonetheless, a methanol formulation of AV17 can be employed as a corrective measure when CA fuming is used on blood marks. The methanol "softens" and penetrates the CA polymer and allows for the reagents to stain the blood proteins (Figure 2). The process of CA fuming did not have a detrimental effect on AV17 MeOH, and the same number of identifiable marks was detected regardless of whether CA fuming was used prior to AV17 MeOH. CAST recommends that the methanol formulation of the protein stains (acid black 1, acid violet 17, and acid yellow 7) should only be used as a corrective measure (Category D process) because it is less effective than the WEAA formulation, and methanol has toxic and flammable properties.

In general, such research would warrant the use of split depletions to account for any variability during deposition; however, such variability was minimized with the use of blood as a contaminant. Furthermore, other scoring systems, such as the University of Canberra (UC), allow the direct comparison of two halves of a split mark. Nonetheless, the CAST absolute scale provides "a means of assessing the overall performance of a technique across a multitude of different samples" [29].

Effect of Substrate and Aging on Enhancement

There was little to no difference in the number of identifiable marks across all aging periods and substrates. For blood marks, there was no significant change in the number of marks that were detected across the aging periods used in this study.

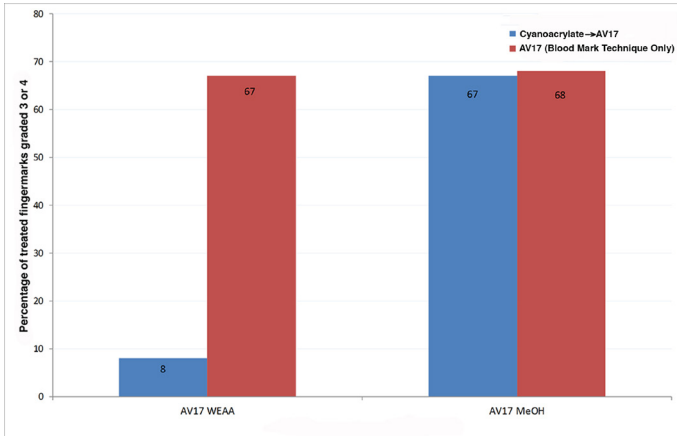
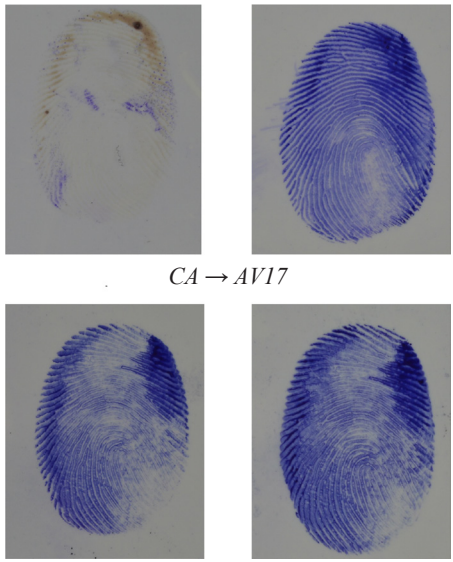


Figure 1

Comparison of the effect of blood mark techniques on fingermark quality with and without CA treatment across all aging periods and substrates (total number of fingermarks treated for each bar is 120).



CA → AV17

AV17

Figure 2

Effect of cyanoacrylate on blood mark enhancement; depletion 5; ceramic white tile; aging 14 days; CA followed by AV17 (above) and AV17 only (below): WEAA (images on left) and MeOH (images on right) formulations.

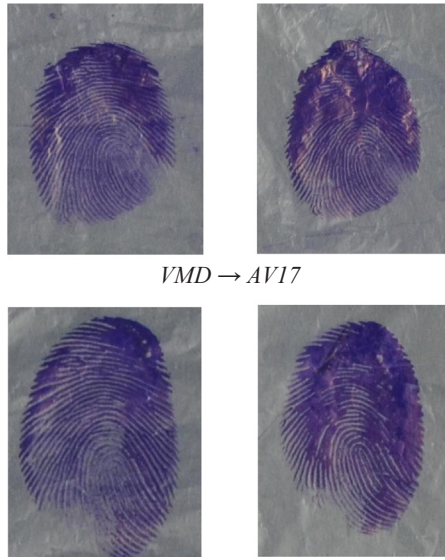
Vacuum CA and VMD

Further trials were carried out to assess the impact of vacuum CA on blood enhancement by the AV17 WEAA formulation and to compare to the atmospheric CA fuming. The vacuum CA fuming results in a thin, light, even coating observed as small, granular beads, which is different from the noodlelike structure formed under atmospheric (with humidity) conditions [15]. It was hypothesized that the thin CA layer that was created under vacuum conditions may not be detrimental to the WEAA formulation for protein stains. The results observed in this study showed that there was no difference in the detrimental effects of AV17 WEAA when comparing pre-treatment with atmospheric or vacuum CA. Vacuum CA fuming hindered the WEAA formulation of AV17, whereas the methanol formulation could be used again as a corrective measure, as observed with the atmospheric conditions.

VMD treatment of blood marks did not affect the WEAA formulation for protein stains as per the sequence recommended in the *Fingermark Visualisation Manual* [17] (Figure 3). A recent study [30] reported that exposure to vacuum conditions such as those found in VMD systems may result in a significant reduction in mass and lipid composition of a latent fingermark. Further work is necessary to understand the effects of such vacuum on blood marks and other body fluids.

Other Protein Stains and Subsequent Blood Tests

As expected, similar results were observed with preliminary trials using other protein stains recommended by the Home Office CAST [17] [i.e., acid black 1 (amido black) and acid yellow 7]. The use of CA fuming, protein stains (WEAA formulation), and VMD did not hinder the presumptive tests (KM–phenolphthalein) and Takayama tests. The methanol formulation affected the KM and Takayama tests, showing a reduction in the test efficacy: the blood reaction with KM was not instantaneous (ranging between 5 to 15 s on average), and very few crystals were observed for the Takayama reaction. Initial work with human blood also demonstrated that the techniques used in this study, including the methanol formulation, were not detrimental to immunochromatographic tests such as the RSID-Blood and Seratec-Blood test kits.



VMD → AV17

AV17

Figure 3

Effect of VMD on blood mark enhancement; depletion 5; white plastic bag; aging 7 days; AV17 only (above) and VMD followed by AV17 (below): WEAA (images on left) and MeOH (images on right).

Conclusions

This study investigated the effect of CA fuming (atmospheric and vacuum) on the subsequent enhancement of fingermarks in blood. CA fuming hindered the subsequent enhancement of blood marks by the water formulation of protein stains. A methanol formulation for protein stains can be used as a remedial process if CA fuming has been employed and blood marks are suspected to be present at a later stage. Nonetheless, the methanol formulation may damage some substrates and be detrimental to blood presumptive and confirmatory tests as compared to the WEAA formulation. It was hypothesized that the use of vacuum CA fuming may be a suitable option for when one is not sure whether both latent and blood marks are present because vacuum CA creates a thinner layer of polycyanoacrylate of different morphology across the substrate as compared to the atmospheric

CA fuming; however, there was no difference between both conditions. The use of VMD did not hinder any of the protein stain formulations.

For further information, please contact:

Kevin J. Farrugia
School of Science, Engineering and Technology
Division of Science
Abertay University
Dundee,U.K., DD1 1HG
Kevin.farrugia@abertay.ac.uk

References

1. Wood, L. W. The Discovery of Superglue Fuming. *Fingerprint Whorld* **1991**, *16* (64), 117–118.
2. Lewis, L. A.; Smithwick, R. W.; Devault, G. L.; Lewis, S. A.; Bolinger, B. Processes Involved in the Development of Latent Fingerprints Using the Cyanoacrylate Fuming Method. *J. For. Sci*. **2001**, *46* (2), 241–246.
3. Bandey, H. L.; Kent, T. *Superglue Treatment of Crime Scenes: A Trial of the Effectiveness of the Mason Vactron SUPERfume Process*; Home Office, Police Scientific Development Branch, Sandridge, U.K., 2003.
4. Wargacki, S. P.; Lewis, L. A.; Dadmun, M. D. Understanding the Chemistry of the Development of Latent Fingerprints by Superglue Fuming. *J. For. Sci*. **2007**, *52* (5), 1057–1062.
5. Wargacki, S. P.; Lewis, L. A.; Dadmun, M. D. Enhancing the Quality of Aged Latent Fingerprints Developed by Superglue Fuming: Loss and Replenishment of Initiator. *J. For. Sci*. **2008**, *53* (5), 1138–1144.
6. Algaier, D.; Baskaran, D.; Dadmun, M. The Influence of Temperature on the Polymerization of Ethyl Cyanoacrylate from the Vapor Phase. *React. Funct. Polym*. **2011**, *71* (8), 809–819.
7. Paine, M.; Bandey, H. L.; Bleay, S. M.; Willson, H. The Effect of Relative Humidity on the Effectiveness of the Cyanoacrylate Fuming Process for Fingermark Development and on the Microstructure of the Developed Marks. *For. Sci. Int*. **2011**, *212* (1–3), 130–142.
8. Bleay, S. M.; Sears, V. G.; Bandey, H. L.; Gibson, A. P.; Bowman, V. J.; Downham, R.; Fitzgerald, L.; Ciuksza, T.; Ramadani, J.; Selway, C. *Fingerprint Source Book*; Home Office Centre for Applied Science and Technology (CAST): Sandridge, U.K., 2012; pp 233–289.

9. Hahn, W.; Ramotowski, R. S. Evaluation of a Novel One-Step Fluorescent Cyanoacrylate Fuming Process for Latent Print Visualization. *J. For. Ident*. **2012**, *62* (3), 279– 298.
10. Prete, C.; Galmiche, L.; Quenum-Possy-Berry, F. -G.; Allain, C.; Thiburce, N.; Colard, T. Lumicyano: A New Fluorescent Cyanoacrylate for a One-step Luminescent Latent Fingermark Development. *For. Sci. Int*. **2013**, *233* (1–3), 104–112.
11. Farrugia, K. J.; Deacon, P.; Fraser, J. Evaluation of Lumicyano Cyanoacrylate Fuming Process for the Development of Latent Fingermarks on Plastic Carrier Bags by Means of a Pseudo Operational Comparative Trial. *Sci. Just*. **2014**, *54* (2), 126–132.
12. Farrugia, K. J.; Fraser, J.; Calder, N.; Deacon, P. Pseudo-Operational Trials of Lumicyano Solution and Lumicyano Powder for the Detection of Latent Fingermarks on Various Substrates. *J. For. Ident*. **2014**, *64* (6), 556–582.
13. Chadwick, S.; Xiao, L.; Maynard, P.; Lennard, C.; Spindler, X.; Roux, C. PolyCyano UV: An Investigation into a One-Step Luminescent Cyanoacrylate Fuming Process. *Aust. J. For. Sci*. **2014**, *46* (4), 471–484.
14. Groeneveld, G.; Kuijer, S.; de Puit, M. Preparation of Cyanoacrylate Derivatives and Comparison of Dual Action Cyanoacrylate Formulations. *Sci. Just*. **2014**, *54* (1), 42–48.
15. Farrugia, K. J.; Fraser, J.; Friel, L.; Adams, D.; Attard-Montalto, N.; Deacon, P. A Comparison Between Atmospheric/Humidity and Vacuum Cyanoacrylate Fuming of Latent Fingermarks. *For. Sci. Int*. **2015**, *257*, 54–70.
16. Khuu, A.; Chadwick, S.; Spindler, X.; Lam, R.; Moret, S.; Roux, C. Evaluation of One-Step Luminescent Cyanoacrylate Fuming. *For. Sci. Int*. **2016**, *263*, 126–131.
17. Bandey, H. L., Ed. *Fingermark Visualisation Manual*; Home Office Centre for Applied Science and Technology (CAST): Sandridge, U.K., 2014.
18. Bhoelai, B.; de Jong, B. J.; de Puit, M.; Sijen, T. Effect of Common Fingerprint Detection Techniques on Subsequent STR Profiling. *For. Sci. Int.: Genet. Suppl. Ser*. **2011**, *3* (1), e429–e430.
19. Khuu, A.; Chadwick, S.; Moret, S.; Spindler, X.; Gunn, P.; Roux, C. Impact of One-step Luminescent Cyanoacrylate Treatment on Subsequent DNA Analysis. *For. Sci. Int*. **2018**, *286*, 1–7.
20. Stein, C.; Kyeck, S. H.; Henssge, C. DNA Typing of Fingerprint Reagent Treated Biological Stains. *J. For. Sci*. **1996**, *41* (6), 1012–1017.

21. Duncan, G. T.; Seiden, H.; Vallee, L.; Ferraro, D. Effects of Superglue, Other Fingerprint Developing Agents and Luminol on Bloodstain Analysis. *J. Assoc. Off. Anal. Chem*. **1986**, *69* (4), 677–680.
22. Simmons, R.; Deacon, P.; Phillips, D. J.; Farrugia, K. The Effect of Mark Enhancement Techniques on the Subsequent Detection of Semen/Spermatozoa. *For. Sci. Int*. **2014**, *244*, 231–246.
23. McAllister, P.; Graham, E.; Deacon, P.; Farrugia, K. J. The Effect of Mark Enhancement Techniques on the Subsequent Detection of Saliva. *Sci. Just*. **2016**, *56* (5), 305–320.
24. King, S.; Benson, S.; Kelly, T.; Lennard, C. Determining the Effects of Routine Fingermark Detection Techniques on the Subsequent Recovery and Analysis of Explosive Residues on Various Substrates. *For. Sci. Int*. **2013**, *233* (1–3), 257–264.
25. McCarthy, M. M.; Grieve, D. L. Preprocessing with Cyanoacrylate Ester Fuming for Fingerprint Impressions in Blood. *J. For. Ident*. **1989**, *39* (1), 23–32.
26. Sears, V. G.; Butcher, C. P. G.; Fitzgerald, L. A. Enhancement of Fingerprints in Blood– Part 3: Reactive Techniques, Acid Yellow 7, and Process Sequences. *J. For. Ident*. **2005**, *55* (6), 741–763.
27. James, S. H.; Kish, P. E.; Sutton, T. P. *Principles of Bloodstain Pattern Analysis: Theory and Practice,* 1st ed.; CRC Press: Boca Raton, FL, 2005.
28. Sears, V. G.; Bleay, S. M.; Bandey, H. L.; Bowman, V. J. A Methodology for Finger Mark Research. *Sci. Just*. **2012**, *52* (3), 145–160.
29. International Fingerprint Research Group (IFRG). Guidelines for the Assessment of Fingermark Detection Techniques. *J. For. Ident*. **2014**, *64* (2), 174–200.
30. Bright, N. J.; Willson, T. R.; Driscoll, D. J.; Reddy, S. M.; Webb, R. P.; Bleay, S.; Ward, N. I.; Kirkby, K. J.; Bailey, M. J. Chemical Changes Exhibited by Latent Fingerprints after Exposure to Vacuum Conditions. *For. Sci. Int*. **2013**, *230* (1–3), 81–86.