Printed in the Republic of Korea
ANALYTICAL SCIENCE & TECHNOLOGY
Vol. 38 No. 5, 271-280, 2025
https://doi.org/10.5806/AST.2025.38.5.271

Use of benzalkonium chloride and cetyltrimethylammonium bromide for post-treatment of ninhydrin developed fingermarks on A4 papers

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(Received May 15, 2025; Revised July 18, 2025; Accepted August 28, 2025)

Abstract: Previous studies have shown that the cationic surfactants benzalkonium chloride (BAC) and cetyltrimethylammonium bromide (CTAB) enhance the visualization of ninhydrin-amino acid compounds (Ruhemann's purple). However, applying BAC or CTAB directly to fingermarks composed of amino acids, after development with amino acid sensitive reagents has not been investigated. Therefore, the authors studied whether the development of fingermarks left on paper with amino acid sensitive reagents and the subsequent post-treatment with cationic surfactants would result in further enhancement. First, the purple compound obtained by treating the amino acids contained in a spot with ninhydrin turned blue when post-treated with BAC or CTAB, indicating that BAC or CTAB has the potential to darken the color of ninhydrin-developed fingermarks and make it more visible. But when fingermarks on paper were developed with ninhydrin and then post-treated with BAC or CTAB, the color of the fingermarks shifted from purple to blue, but the overall sensitivity was similar to fingermarks without BAC or CTAB. Furthermore, no significant effect was observed when fingermarks developed with 1,8 - diazafluoren - 9 - one or 1,2 - indanedione/zinc instead of ninhydrin were treated with BAC or CTAB. However, further testing on colored papers showed that adding BAC or CTAB to ninhydrin-developed fingermarks may provide better contrast on red-based papers.

Key words: benzalkonium chloride, cetyltrimethylammonium bromide, cationic surfactant, fingermark, ninhydrin

1. Introduction

Latent fingermarks can be used as important evidence in criminal investigations. However, they are not readily visible to the naked eye and require visualization. On porous surfaces, this visualization can be achieved by using amino acid sensitive reagents such as ninhydrin and its analogs.[1,2] These analogs such as 1,2 – indanedione/zinc (IND) and 1,8 – diazafluoren – 9 – one (DFO) produce better contrast and higher sensitivity under fluorescence compared to ninhydrin. Thus, they are preferred for developing fingermarks.[1,3]

However, IND and DFO are relatively more expensive and require a light source for visualization. In contrast, ninhydrin is cheaper and produces purple-colored fingermarks that can be viewed directly

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under white light (without the use of any specialized light sources). This makes it especially useful in cases where fluorescence producing reagents or techniques may not be feasible,[1-7] and thus it may be preferred in forensic laboratories with a lower budget. However, ninhydrin still has limitations such as insufficient contrast on certain backgrounds and lower sensitivity compared to amino acid sensitive reagents that produce fluorescent fingermarks.[1,3,8,9] Thus, there is still a need to improve the overall performance of ninhydrin.

Benzalkonium chloride (BAC) and cetyltrimethylammonium bromide (CTAB) are cationic surfactants that have shown positive effects on the reactions involving amino acid sensitive reagents based on previous studies.[10-15] In the study by Chadwick *et al.* (2017), when fingermarks were deposited using hands covered with non – alcoholic sanitizers (that included BAC) or an aqueous BAC solution and then developed with ninhydrin or IND, the ridge quality of ninhydrin developed fingermarks, and the fluorescence intensity and quality of IND developed fingermarks improved.[10] These effects were especially evident in the case of poor fingermark donors (i.e. donors who produce a low amount of sweat).[10] Kinetic and spectrophotometric studies on CTAB showed that it improved the rate of reaction when added to ninhydrin and amino acids.[11-15] The reaction between ninhydrin and amino acids was catalyzed within the stern layer (the hydrophilic head group layer) of the CTAB micelles.[11-15] Thus, based on the results of the previous studies, it is expected that BAC and CTAB may have similar positive effects due to the formation of micelles when applied directly to fingermarks after development with amino acid sensitive reagents on paper. However, to the best of the authors' knowledge, no such study has been published.

Therefore, to check whether BAC (or CTAB) post-treatment of fingermarks developed with amino acid sensitive reagents showed any positive effects, optimal BAC and CTAB reagents were formulated for direct application to fingermarks developed with amino acid sensitive reagents on A4 paper. The performance of amino acid sensitive reagents with and without using BAC or CTAB were compared by testing on A4 as well as colored paper. Any advantages that this novel post-treatment method may show compared to development with amino acid sensitive reagents without post-treatment were investigated.

2. Experimental

2.1. Materials and equipment

The chemicals used in this study were BAC, CTAB, serine, glycine, and asparagine from Daejung (Korea); ninhydrin, alanine, leucine, threonine, histidine, and valine from Samchun (Korea); lysine from Sigma-Aldrich (USA); DFO and IND from Sirchie (USA). 505-nm light source (Polilight-Flare Plus 2) from Rofin (Australia); an orange barrier filter from Altlight (Korea); a heat press machine (Dreame81) from Designdream (Korea); Nikon D5300 DSLR Camera from Nikon (Japan) and a 60 mm lens from Laowa (China) were also used.

2.2. Preparation of reagents

Petroleum ether (PE) based DFO was prepared by dissolving DFO (500 mg) in a mixture of methanol (100 mL), ethyl acetate (100 mL) and acetic acid (20 mL) and then adding PE (780 mL). PE based IND was also prepared. Working solution of IND was prepared by adding IND (0.8 g) to ethyl acetate (90 mL), glacial acetic acid (10 mL) and zinc chloride stock solution (80 mL), followed by PE (820 mL). The zinc chloride stock solution was prepared by first dissolving zinc chloride (0.4 g) in absolute ethanol (10 mL) and ethyl acetate (1 mL) followed by adding PE (190 mL).[16] A modified PE based ninhydrin formulation, referred to as nin(PE) henceforth, was prepared by dissolving ninhydrin (6 g) in ethanol (90 mL)[16] and then adding acetic acid (10 mL). The solution was then diluted with PE (900 mL).[16]

Artificial sweat was prepared by adding serine (0.98 g), glycine (0.588 g), alanine (0.294 g), lysine (0.39 g), leucine (0.098 g), threonine (0.146 g), asparagine (0.146 g), histidine (0.146 g), valine (0.098 g), sodium chloride (6.6 g), magnesium chloride (0.008 g), calcium chloride (0.032 g) and zinc chloride (0.004 g) to

deionized water (1 L).[17]

2.3. Fingermark deposition

Natural fingermarks are fingermarks that are composed of skin residues naturally present on the surface of the fingers and deposited without immediate hand-washing, producing excess sweat or increasing the sebaceous content by touching areas such as the face, neck etc.[18,19] Thus, natural fingermarks were deposited by two good donors (who produce a high amount of sweat), two average donors (who produce an average amount of sweat) and two poor donors (who produce a low amount of sweat). The donors were asked not to wash their hands for 30 minutes before fingermark deposition and to deposit each fingermark with light pressure and brief contact time. The donors were asked to rub their hands together for approximately 5 seconds before depositing each fingermark.[20]

2.4. Treatment and visualization of fingermarks

Nin(PE) developed fingermarks were heated at 160 °C for 10 s using a dry heat press and visualized under white light.[8]

2.5. Fingermark assessment

The fingermarks were assessed by 5 assessors who were forensic science graduate students trained in fingermark development and assessment for more than 1 year.[21]

3. Results

3.1. Effect of BAC or CTAB

When fingermarks were deposited with hands covered with hand sanitizers (containing BAC) or an aqueous (aq.) solution of BAC and then developed with amino acid sensitive reagents, the overall ridge development, quality and fluorescence intensity of the fingermarks showed improvement.[10] But the effect of applying aq. BAC (or CTAB) directly to fingermarks after development with amino acid sensitive reagents is not known.

However, before testing the effect of aq. BAC (or CTAB) on fingermarks developed with amino acid sensitive reagents, the effect of aq. BAC (or CTAB) was tested on spots of artificial sweat containing amino acids, to exclude the influence of impurities such as oils and other contaminants that may be present in a fingermark. While preparing the spots, the volume of each of the reagents spotted was 10 μL. Ethanol-based BAC and CTAB were also considered since a volatile organic solvent such as ethanol is preferred compared to water while using amino acid sensitive reagents as it is less likely to cause blurring of fingermark ridges[22] and will allow the paper to dry faster.

3.1.1. Control spot tests

Control spot tests were conducted to exclude any potential interactions between BAC (or CTAB) and the individual reagents, as well as to rule out the influence of blank solvents on the reactions. No reaction was observed when 10 μL of 0.1 % w/v aqueous or ethanol-based BAC (or CTAB) was added to a 10 μL spot of artificial sweat. Similarly, no reaction occurred when the same amount of BAC (or CTAB) solution was applied to a 10 μL spot of a PE-based amino acid-sensitive reagent (ninhydrin, DFO, or IND). On adding 10 μL of a blank solvent (deionized water or ethanol) to the reaction between a 10 μL spot of artificial sweat and 10 μL of a PE based amino acid sensitive reagent (ninhydrin, DFO or IND) after heating, no significant result was obtained. Heating was carried out with a dry heat press at 160 °C for 10 s in case of ninhydrin and IND spots, and 180 °C for 10 s in case of DFO spots. Therefore, any effects observed in subsequent experiments can be attributed to the influence of BAC or CTAB on the reactions, rather than to the effects of the blank solvents or any direct interaction between BAC (or CTAB) and the individual reagents.

3.1.2. Effect of BAC or CTAB on amino acid - DFO (or IND) complex

DFO and IND are preferred over ninhydrin as they produce fluorescent fingermarks which have higher contrast and better sensitivity.[1,3] Thus, before testing

the effect of BAC or CTAB on the reaction between ninhydrin and amino acids, the effect of BAC or CTAB was tested on the reaction between DFO (or IND) and amino acids as any improvement in the performance of DFO or IND due to BAC or CTAB would prove to be more advantageous. For this purpose, PE based DFO (or IND) was added to spots of artificial sweat on paper. DFO spots were heated at 180 °C for 10 s using a dry heat press and visualized through an orange filter under light of 505 nm wavelength.[8,23] IND spots were heated at 160 °C for 10 s using a dry heat press and visualized through an orange filter under light of 505 nm wavelength.[8,23]

The addition of 0.1 % w/v BAC or CTAB (aqueous or ethanol-based formulations) to these spots did not produce any significant effects (such as a color change or increase in fluorescence intensity) that could be useful for fingermark development on paper. Therefore, the effect of BAC or CTAB on the reaction between DFO (or IND) and amino acids was not considered for further investigation.

3.1.3. Effect of BAC or CTAB on amino acid - ninhydrin complex

1) Aqueous BAC or CTAB

Since BAC or CTAB did not show significant effects on the reaction between DFO (or IND) and amino acids, the effect of these cationic surfactants were then tested on the reaction between ninhydrin and amino acids. On adding aq. BAC to a spot of artificial sweat that was developed with nin(PE) and heated, a color change from purple to a strong blue color was observed. Adding aq. CTAB to a spot of artificial sweat developed with nin(PE) after heating the spot also produced a faint blue color. This shows that BAC or CTAB can be used to change the color of the amino acid – ninhydrin reaction product formed to improve contrast with the background. Therefore, using this sequence of application, the effect of BAC or CTAB was considered for further investigation.

2) Ethanol-based BAC or CTAB

Ethanol is less likely to cause blurring of fingermarks ridges.[22] Additionally, the use of water in the post-treatment reagent will cause damage to the paper[24] while ethanol will evaporate quickly leading to faster drying of paper. Thus, ethanol-based BAC and CTAB were tested to see if a similar blue color change was produced. For this purpose, artificial sweat was spotted on A4 paper followed by addition of nin(PE) and then heated giving a purple color. However, on addition of 0.1 % w/v ethanol-based BAC or CTAB to the nin(PE) developed spot, color change from purple to blue was not observed.

3.1.4. Effect of $CaCl_2$

In Section 3.1.3. (B.), no color change was observed on using 0.1 % w/v ethanol-based BAC or CTAB. This could be due to the absence of micelles as it was seen that micelle formation is required for the cationic surfactants to have an effect on the reaction between ninhydrin and amino acids[11-15] and thus, micelles may likely be required for BAC or CTAB to give a color change as well. Since the CMC (critical micelle concentration - the concentration of the surfactant required to form micelles) will be higher if the amount of ethanol increases,[25-27] 0.1 % w/v concentration of BAC or CTAB may not be a sufficient concentration to form micelles in the presence of a higher volume of ethanol. Thus, in order to form micelles to obtain a color change in 100 % ethanol, increasing the concentration of BAC or CTAB (to attain the CMC) or using a salt ($CaCl_2$) in the formulation to reduce the CMC - lower the concentration required to form micelles,[28] may be required.

For this purpose, 8 sets of 3 natural fingermarks were deposited by a good donor on A4 paper, giving a total of 24 fingermarks. Average and poor donors were not considered as the aim was not to check the effectiveness of the post-treatment method, rather to check the percentage of BAC or CTAB required to get the color change in 100 % ethanol. Thus, the fingermarks of a good donor were considered to be sufficient as it can provide proper visualization of the color change. Each fingermark was deposited using fresh sweat. These fingermarks were developed with nin(PE), heated and then divided to form split fingermarks. Each of the 8 sets was treated with 0.1 %, 0.5 %, 1 % and 2 % w/v ethanol-based BAC

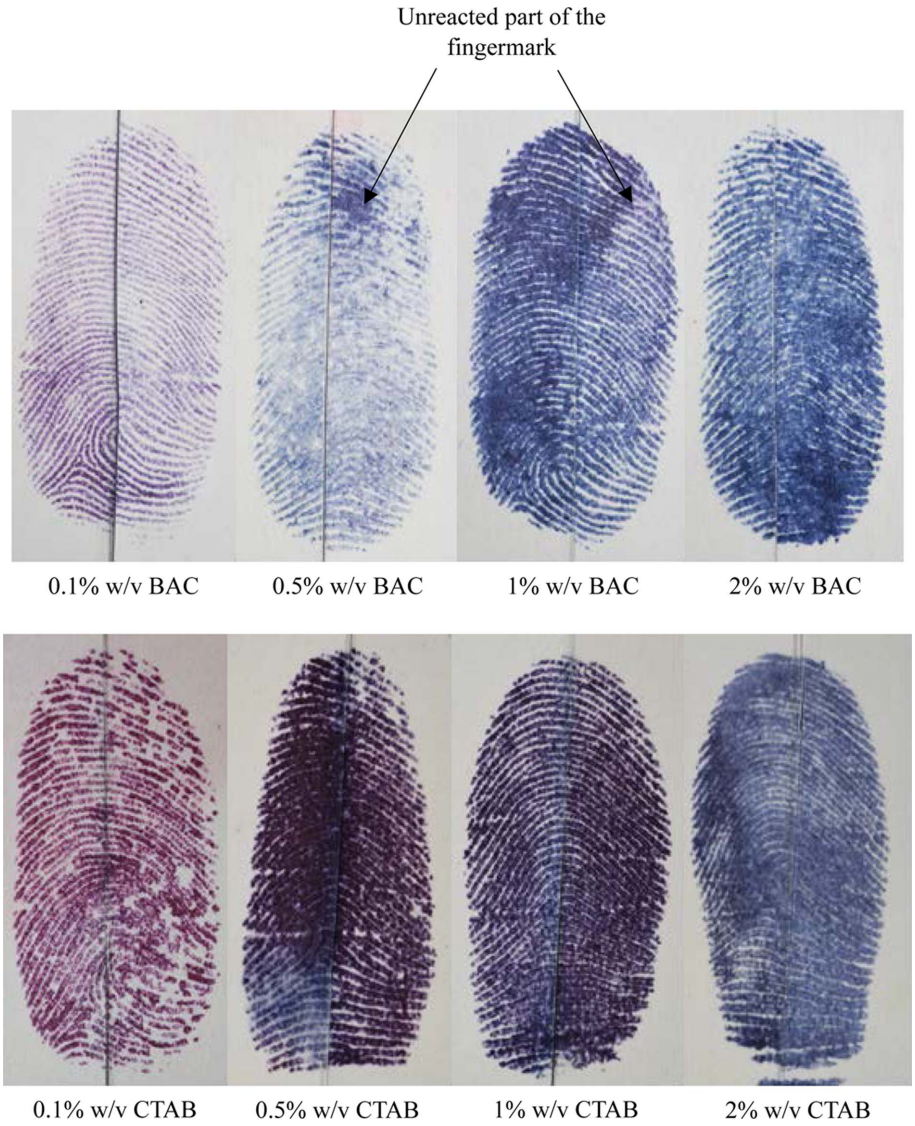

Fig. 1. Blue color change obtained with 2 % w/v ethanol-based BAC and CTAB post-treatment. Contrast of the images enhanced to observe the unreacted part.

and CTAB formulations, respectively. The left half of the fingermark was treated with a BAC (or CTAB) formulation containing 0.005 M $CaCl_2$ and the right half was treated with a formulation not containing $CaCl_2$, at the same BAC (or CTAB) concentration.

Among the BAC concentrations tested, although blue color was obtained using 0.5 % and 1 % w/v ethanol-based BAC as well as 2 % w/v ethanol-based BAC with $CaCl_2$, it appeared to have a greater extent of unreacted parts (as shown in *Fig.* 1) in some fingermarks compared to 2 % w/v ethanol-based BAC without $CaCl_2$. In case of CTAB post-treatment, color change to blue was obtained only on using 2 % w/v ethanol-based CTAB. The blue color change indicates the formation of micelles.

To determine whether $CaCl_2$ had any effect on the color intensity over a period of time, 3 sets of 3 natural fingermarks were deposited by a good donor, giving a total of 9 natural fingermarks. 2 sets were post-treated with 2 % w/v ethanol-based BAC and CTAB

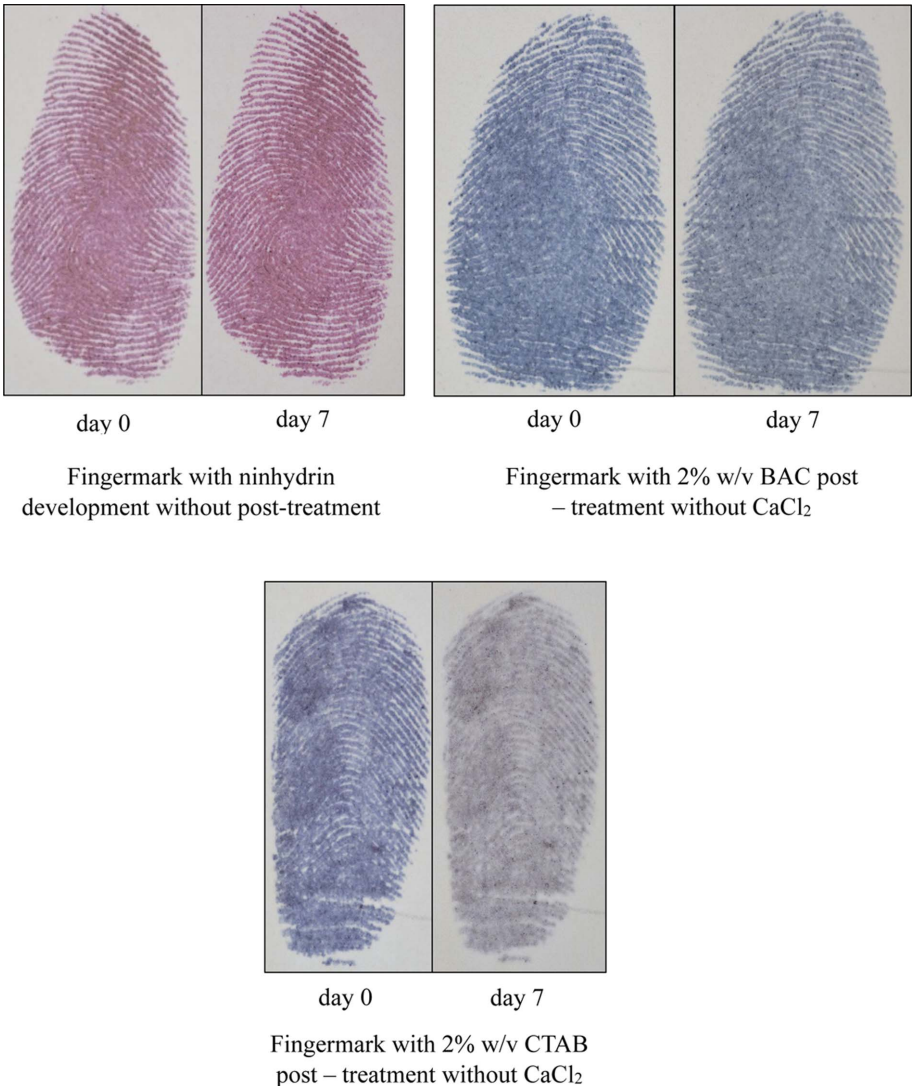

Fig. 2. Fading of fingermarks observed on day 7 after post-treatment with 2 % w/v Ethanol-based BAC and 2 % w/v Ethanol-based CTAB compared to ninhydrin development without post-treatment.

respectively, each formulation containing 0.005 M $CaCl_2$. The third set was not subjected to post-treatment. On observing the fingermarks after 7 days, the color of the fingermarks with ethanol-based BAC (or CTAB) post-treatment faded compared to the fingermarks developed with nin (PE) without post-treatment. This fading was observed even in case of fingermarks with BAC (or CTAB) post-treatment without $CaCl_2$ (as shown in *Fig.* 2) and it was very prominent in the case of CTAB post-treatment for all the fingermarks observed. Therefore, nin(PE) development without post-treatment showed the best results while CTAB post-treatment was not as effective as BAC post-treatment or nin(PE) development without post-treatment.

Addition of $CaCl_2$ did not produce satisfactory results and further research is required to understand why. Nevertheless, it was not included in the final formulation as color change was obtained just by increasing the concentration of BAC or CTAB. Thus,

Table 1. Modified University of Canberra (UC) comparative scale used for fingermark assessment

Grade	Definition
+1	BAC (or CTAB) post-treatment shows an increase in enhancement compared to nin(PE) development without post-treatment.
0	No significant difference between BAC (or CTAB) post-treatment and nin(PE) development without post-treatment.
-1	BAC (or CTAB) post-treatment shows a decrease in enhancement compared to nin(PE) development without post-treatment.
N	Developed fingermark but no/incomplete color change to blue.
00	No/poor visibility of the fingermark (i.e., no result from either technique).

Abbreviations: BAC: benzalkonium chloride; CTAB: cetyltrimethylammonium bromide; nin(PE): petroleum ether-based ninhydrin

2 % w/v BAC in 100 % ethanol and 2 % w/v CTAB in 100 % ethanol were selected as the optimal formulations for further testing of fingermarks.

3.2. Ethanol-based BAC or CTAB post-treatment vs. nin(PE) development without post-treatment on A4 paper

To check whether BAC (or CTAB) post-treatment had any significant advantages, the performance of ethanol-based BAC (or CTAB) post-treatment was compared with nin(PE) development without post-treatment. Thus, the following experiment was designed based on the International Fingerprint Research Group (IFRG) guidelines for Phase I experiments.[18]

For this purpose, natural fingermarks were collected from 6 individuals (a mix of good, average, and poor fingermark donors) to provide variable fingermarks. The donors were asked to deposit seven repeats of their thumb, index, and middle finger on A4 paper giving a total of 21 fingermarks. This was considered as one set. 4 sets were collected from each of the 6 donors. Thus, 84 fingermarks were collected from each donor. A time interval of 20 minutes was maintained between the collection of each set,[20] during which the donors could carry out their regular activities.[19] The fingermarks were developed using nin(PE) and then split into three portions. One portion was not subjected to further treatment while the other two portions were treated with BAC and CTAB, respectively.

The scale proposed by the University of Canberra (UC)[18] was considered for fingermark assessment as it is used for comparing the performance of reagents. The post-treatment method did not show any additional minutiae or ridge line formation, mainly showing a difference in contrast due to the change in color. Thus, it was considered to be the appropriate scale for comparing the performance of the post-treatment method in relation to just using ninhydrin (without post-treatment). However, the BAC (or CTAB) post-treatment method did not show a significant superior or inferior performance when observed and compared to nin(PE) using the UC scale. Thus, only a single positive grade showing superior performance (+1) and a single negative grade showing inferior performance (-1) were considered to be sufficient.[29] After post-treatment, it was also observed that some of the fingermarks did not show the color change or were not visible enough for analysis (irrespective of the treatment used). Thus, additional grades (N) and (00) were added to denote such fingermarks.[29] Therefore, the scale[18] proposed by the University of Canberra (UC) was modified to include these grades as shown in *Table* 1. This modified scale was used for the assessment of fingermarks by 5 assessors.

Fig. 3 shows the graphical representation of the comparison of ethanol-based BAC (or CTAB) post-treatment with nin(PE) development without post-treatment in split fingermarks based on the modified UC comparative scale. As shown in *Fig.* 3, considering only the fingermarks that received grades +1, 0 and -1, the percentage of nin(PE) developed fingermarks that showed superior performance (grade +1) with ethanol-based BAC (or CTAB) post-treatment was

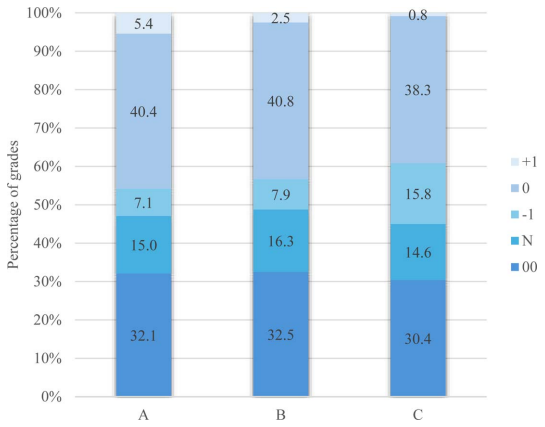

Fig. 3. Distribution of the percentage of grades received on comparing Ethanol-based BAC or CTAB post-treatment with nin(PE) development without post-treatment. A. Comparison between nin(PE) development without post-treatment and BAC or CTAB post-treatment within the same split fingermark. B. Comparison before and after BAC or CTAB post-treatment within the same fingermark. C. Comparison between nin(PE) deve-lopment without post-treatment and BAC or CTAB post-treatment across several repeats

BAC post-treatment CTAB post-treatment

Fig. 4. Comparison of ninhydrin-developed fingermarks on crimson paper before (left halves) and after (right halves) 2 % w/v ethanol-based BAC/CTAB post-treatment.

the lowest while the percentage of fingermarks that showed inferior performance (grade -1) on using ethanol-based BAC (or CTAB) post-treatment was higher. The highest percentage of fingermarks showed similar enhancement (grade 0) between ethanol-based BAC (or CTAB) post-treatment and nin(PE) development without post-treatment.

Overall, ethanol-based BAC (or CTAB) post-treatment showed similar clarity of ridges, contrast, ridge development and intensity across several repeats when compared to nin(PE) development without post-treatment. Thus, ethanol-based BAC or CTAB post-treatment did not appear to present any particular advantage over nin(PE) development without post-treatment. However, the authors have not found the reason yet.

3.3. Effect of ethanol-based BAC/CTAB post-treatment on Nin(PE) developed colored paper

To assess whether post-treatment with BAC or CTAB could enhance the color contrast of ninhydrin-developed fingermarks, natural fingermarks were deposited on eight types of colored paper: crimson, red, yellow, green, light blue, dark blue, purple, and pink. Each fingermark was split; one half was treated with Nin(PE) alone, while the other half was treated with Nin(PE) followed by immersion in 2 % w/v ethanol-based BAC or CTAB solution. As a result, the contrast enhancement effect of the 2 % w/v ethanol-based BAC or CTAB post-treatment was observed only on red-colored papers (red and crimson). No significant contrast improvement was noted on the other colored papers. *Fig.* 4 illustrates the enhanced contrast observed on crimson paper.

4. Discussion

Overall, BAC (or CTAB) post-treatment exhibited performance comparable to that of nin(PE) development alone. However, in some fingermarks, no color change was observed following post-treatment, and fading was noted after seven days—phenomena that were not observed in fingermarks developed solely with nin(PE). The underlying causes of these effects remain unclear and are beyond the scope of the present study. Previous studies have shown that the reaction between ninhydrin and amino acids takes place in the stern layer of micelles.[11-15] The blue color indicates the formation of these micelles. Thus, the blue color could be due to a complex formed between these micelles and Ruhemann's purple. In Chadwick's

earlier study, water was used as the solvent;[10] however, water is not suitable for amino acid-sensitive reagents, as it may damage the paper[24] and cause diffusion of amino acids,[30] leading to blurred ridge detail. Therefore, ethanol, an organic solvent, was employed in this study, as it is more appropriate for preserving the clarity of fingermark ridges. On white paper substrates, BAC (or CTAB) post-treatment did not provide any significant advantage over nin(PE) development alone and showed several limitations. Accordingly, the use of nin(PE) without post-treatment remains the recommended approach for developing fingermarks on A4 paper. However, BAC (or CTAB) post-treatment showed better contrast due to the blue color change when used on ninhydrin-developed fingermarks on red-based papers. Further research is necessary to identify the factors that influence the occurrence or absence of the blue color change.

5. Conclusion

On adding benzalkonium chloride (BAC) or cetyltrimethylammonium bromide (CTAB) after heating artificial sweat spots developed with petroleum ether-based ninhydrin, referred to as nin(PE), a color change was obtained from purple to blue on A4 paper. Thus, this application sequence was used in further experiments. Different formulations were tested (including the use of $CaCl_2$ to reduce the critical micelle concentration of BAC or CTAB) to get the color change in 100 % ethanol. But $CaCl_2$ did not give satisfactory results and was not included in the final formulation. Thus, 2 % w/v BAC in 100 % ethanol and 2 % w/v CTAB in 100 % ethanol were the optimal reagent formulations selected for further fingermark testing.

Although blue color was obtained, BAC (or CTAB) post-treatment showed similar performance when compared to nin(PE) development without post-treatment on A4 paper. Additionally, some of the fingermarks did not show the color change after post-treatment, and the color of the fingermarks faded after 7 days, which was not observed in fingermarks with nin(PE) development without post-treatment. Since post-treatment with BAC or CTAB did not show any significant advantages compared with nin(PE) development without post-treatment, and due to the current limitations of the BAC (or CTAB) post-treatment method, nin(PE) development without post-treatment is still recommended for fingermarks on A4 paper. The addition of BAC or CTAB also did not produce any significant effect on the reaction between 1,8-diazafluoren-9-one (or 1,2-indanedione/zinc) and amino acids. However, when further tests were conducted on colored papers, BAC (or CTAB) post-treatment showed better contrast when applied to ninhydrin-developed fingermarks on red-based papers. Thus, this post-treatment method can enhance the visualization of ninhydrin – developed fingermarks on red-based papers.

Acknowledgements

This research was supported by the Soonchunhyang University Research Fund.

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