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Chemical enhancement of footwear impressions in urine on the surface of tiles

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Abstract Enhancement of footwear impressions in urine on the surface of tiles by using p-dimethylaminocinnamaldehyde (DMAC), which react with urea, and ninhydrin, 1,8-diazafluoren-9-one (DFO), 1,2-indanedione/zinc (1,2-IND/Zn), which react with amino acid, was studied. As a result of comparing the application methods of reagents, the ninhydrin and the 1,2-IND/Zn were suitable for application with spray method, which is spray directly on footwear impression, DFO and DMAC were suitable for application with dry contact method, which is applying heat with press to DMAC impregnated paper on footwear impression. In addition, DMAC applied with dry contact method showed best contrast and enhancement result in both white and black colored tiles by comparing of the sensitivity by different dilution ratio of urine and the aging time of footwear impressions in urine. And the result of applied with DMAC (with dry contact method) on the floor tiles collected at various places in a building's men’s and women’s bathrooms, it can be successfully enhanced that footwear impressions in urine. So it is believed that the method can be used to recover footwear impressions in urine from real crime scenes.

Key words: p-dimethylaminocinnamaldehyde, footwear impressions in urine, enhancement, dry contact method

1. Introduction

A footwear impression refers to the impression left on a surface from the downward pressure by the weight of a person wearing footwear. When footwear are worn, their soles bear down in a unique pattern, and as a result, each footwear leaves its own unique impression, which is used as an important link between the suspect and the crime scene.[1-2] There are two types of footwear impressions — two dimensional (2D) and three dimensional (3D) footwear impressions — depending on the characteristics of the surface that the footwear come in contact with.[1-4] Of these, 2D footwear impressions are created when footwear come in contact with a hard surface, and if there is high contrast between the color of dust (dirt) that was on the soles of the footwear and the color of the surface, such a difference in color is visually identifiable.[1,5,6] However, if the contrast between the two substances is poor, the impressions are left as latent footwear impressions that are not readily visible, and in such cases, they are treated by various

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methods, including electrostatic dust print lifting, gelatin lifting, and cyanoacrylate fuming, to make them visible.[1,7-10]

A crime may be committed in an indoor bathroom, and in such incidents, footwear impressions may be used as important clues in solving the case. However, because indoor bathrooms in modern buildings do not have much dust (dirt) on the surface, 2D footwear impressions on dust are rarely found. In such cases, footwear impressions in urine created by the urine smeared on the soles of footwear can be found; however, because urine is a substance with no color or a light yellow color, footwear impressions in urine are rarely visually detectable even if they are present. Therefore, visualization of footwear impressions in urine by chemical enhancement is a very important task for criminal investigation.

Urine contains various components, including urea and trace levels of amino acids and proteins;[11] hence, enhancement using reagents that produce a color change by reacting with these components can enable the visualization of footwear impressions in urine that were previously undetectable by visual inspection. Ninhydrin, DFO, and 1,2-IND/Zn solutions are known reagents used to produce latent fingerprints by making amino acids left on porous surfaces, such as papers, visible, whereas DMAC is a reagent known to produce latent fingerprints by making urea and amino acids left on porous surfaces visible.[12-22] Farrugia *et al.* used acid black 1, acid violet 17, and acid yellow 7, which are used for the visualization of proteins, in addition to ninhydrin, DFO, 1,2-IND/Zn, and DMAC, to enhance footwear impressions in urine left on fabrics, which have porous surfaces; thereafter, the sensitivity of these reagents were compared. The results showed that the best enhancement effect was achieved when DFO was used; however, all the 7 reagents listed above showed poor enhancement effect on dark fabrics or fabrics with patterns.[23,24]

Footwear impressions in urine may be left on non-porous surfaces such as bathroom tiles. However, there are no reported study results on techniques for enhancing footwear impressions in urine left on non-porous surfaces. Therefore, in this study, we investigated techniques for enhancing footwear impressions in urine left on a tile, a non-porous surface.

2. Materials and Methods

2.1. Materials

White and black tiles from Central Deco (Korea) were used. The powders used in this study were from the following sources: ninhydrin and 1,2-IND powders from Sirchie (USA); DFO powder from BVDA (Netherlands); DMAC powder from Sigma-Aldrich (USA); and 5-sulfosalicylic acid powder from Daejung (Korea). Kimtech Science Wiper from Yuhan Kimberly (Korea) was the tissue used in this study.

The urine sample was first morning urine from a 25-year-old female, and was stored in a refrigerator (4 ºC) for up to two days before use.

Photographs were taken using an AF-S DX NIKKOR 18-55 mm standard lens and a D5500 camera (Nikon, Japan). Polilight Flare Plus 2 from Rofin (Australia) was used as the 505 nm green light source, whereas CombLite from Altlight (Korea) was used as the white light source. An orange-colored filter from Altlight (Korea) was used as the barrier filter.

2.2. Preparation of working solutions and dry contact paper

A ninhydrin working solution was prepared by dissolving 6 g of ninhydrin in 50 mL of ethanol and then adding 950 mL of petroleum ether. Ninhydrin dry contact paper was prepared by submersing A4 copy paper in a concentrated solution prepared by dissolving 6 g of ninhydrin in 100 mL of ethanol, and then removing the paper and drying it.

A DFO working solution was prepared by dissolving 0.5 g of DFO in a mixture containing 100 mL of methanol, 100 mL of ethyl acetate, and 20 mL of glacial acetic acid, and then adding 780 mL of petroleum ether. DFO dry contact paper was prepared by submersing A4 copy paper in a concentrated solution prepared by dissolving 1 g of DFO in a mixture containing 200 mL of methanol, 200 mL of ethyl acetate, and 40 mL of glacial acetic acid, and then removing the paper and drying it.

A 1,2-IND/Zn working solution was prepared by dissolving 0.8 g of 1,2-IND in a mixture containing 90 mL of ethyl acetate, 10 mL of glacial acetic acid, and 80 mL of zinc chloride, and then adding 820 mL of petroleum ether. A zinc chloride solution was prepared by dissolving 0.4 g of zinc chloride in a mixture containing 10 mL of ethanol and 1 mL of ethyl acetate, and then adding 190 mL of petroleum ether. 1,2-IND/Zn dry contact paper was prepared by submersing A4 copy paper in a concentrated solution prepared by dissolving 1 g of 1,2-IND in a mixture containing 90 mL of ethyl acetate, 10 mL of glacial acetic acid, and 20 mL of zinc chloride stock, and then removing the paper and drying it.

A DMAC working solution was prepared by mixing a solution with 0.25 g of DMAC dissolved in 50 mL of ethanol and a solution with 1 g of 5-sulfosalicylic acid dissolved in 50 mL of ethanol. DMAC dry contact paper was prepared by submersing A4 copy paper in a concentrated solution prepared by dissolving 0.25 g of DMAC in 100 mL of ethanol, and then removing the paper and drying it.

All the reagents were used within 15 days of preparation, whereas the dry contact papers were used within one week from preparation.

2.3. Deposition of footwear impressions in urine

Footwear impressions were deposited by standing on tiles for 15 s while wearing footwear with the soles smeared with urine solution using tissues. The tiles with footwear impressions were dried for 1 h at room temperature before being used in the experiment.

2.4. Treatment and photographing of footwear impressions

For treatment via the spray method, a sprayer was used to evenly spray the working solution on the footwear impressions (total of 7 times from a height of approximately 30 cm). The sprayed solution was left to dry for 5 min at room temperature. For treatment via the tissue method, a tissue was placed on the sample and an eyedropper was used to soak the tissue with the working solution. For treatment via the dry contact method, the sample was covered with a dry contact paper and pressed for 90 s using an iron heated to 100–110 ^{o}C.

Table 1. The grading system to determine the degree of enhancement of footwear impressions in urine

Grading	Definition
0	No footwear impression detail.
1	Partial footwear impression detail present.
2	Partial footwear impression detail present only can observe their site.
3	Full footwear impression detail present but probably cannot be used for identification.
4	Full footwear impression detail present; identifiable footwear impression.

Footwear impressions enhanced by ninhydrin were photographed under white light using an aperture value of F/8, ISO 800, and a shutter speed of 1/25 s (white tile) or 1/10 s (black tile).

Footwear impressions enhanced by DFO, 1,2-IND/Zn, and DMAC were photographed with an orange-colored barrier filter loaded in front of the camera and under white light using the same conditions, except with a shutter speed set to 1/5 s and under irradiation with a 505 nm green light source.

2.5. Assessment of footwear impressions

Footwear impressions were graded in accordance with the criteria shown in *Table* 1 by 7 students who had completed at least 1 year of forensics education. The mean score was derived from the individual assessment scores submitted by the students, and was used to assess the intensity of the footwear impressions.

3. Results and Discussion

3.1. Optimal methods for using reagents

To determine the most appropriate method for treating footwear impressions in urine with ninhydrin, DFO, 1,2-IND/Zn, and DMAC, footwear impressions in urine were enhanced using three different methods (spray, tissue, and dry contact methods), the results

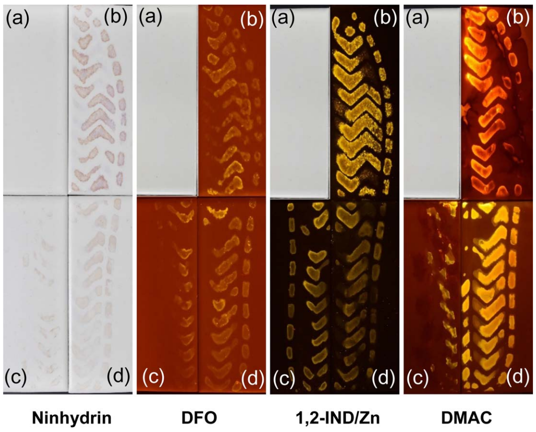

Fig. 1. Performance comparison of the spray, tissue, and dry contact method on the enhancement of footwear impressions in urine on white tiles. (a) Control (b) Spray method (c) Tissue method (d) Dry contact method

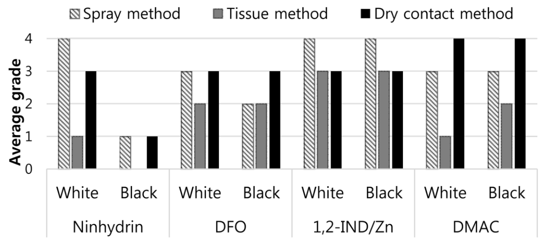

Fig. 2. Variation of the average grading when the footwear impressions in urine on white and black tiles were enhanced by spray, tissue and dry contact method.

of which are shown in *Fig.* 1. Moreover, *Fig.* 2 shows the result of footwear impressions that assessed *Fig.* 1 according to the criteria shown in *Table* 1. Based on the results of treating different samples with each reagent, the best enhancement effects were found when ninhydrin and 1,2-IND/Zn were used with the spray method, and when DFO and DMAC were used with the dry contact method. Moreover, when DMAC was used with the spray or tissue method, the footwear impressions became smudged, which indicated that this method should not be used.

3.2. Comparison of sensitivity of enhancement reagents

Footwear impressions in urine were produced using urine diluted ~0–100-fold with deionized water. These footwear impressions were enhanced using

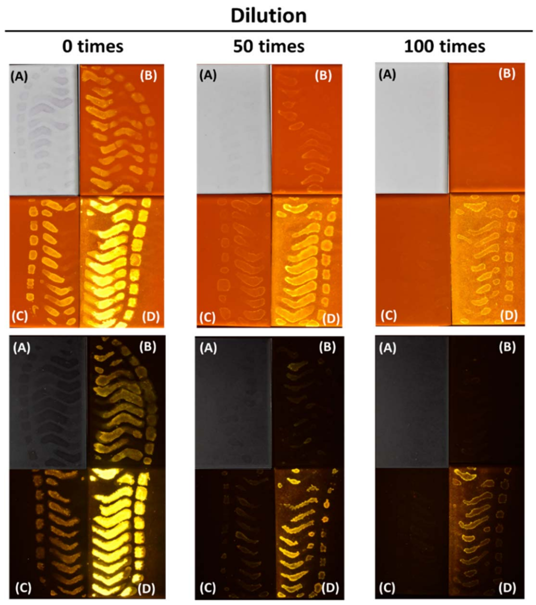

Fig. 3. The enhancement results of footwear impressions in diluted urine deposited on the surface of white (top) and black (bottom) tiles. (A) Ninhydrin, (B) DFO, (C) 1,2-IND/Zn, (D) DMAC

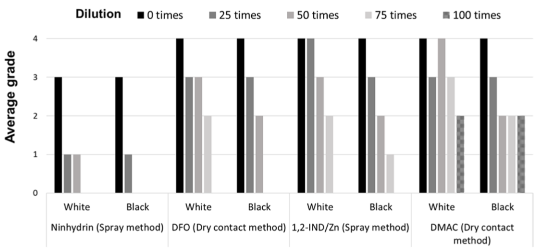

Fig. 4. Comparison of sensitivity of urine sensitive reagents for the enhancement of footwear impression in urine.

ninhydrin (spray method), DFO (dry contact method), 1,2-IND/Zn (spray method), and DMAC (dry contact method) to compare the enhancement effects, the results of which are shown in *Fig.* 3 and *Fig.* 4. When light irradiated the footwear impressions in diluted urine not treated with any reagent, photoluminescence of the impressions was not observed. However, when light irradiated footwear impressions in urine treated with a reagent, photoluminescence was observed, and regardless of the tile color or dilution factor of urine, DMAC (dry contact method) showed the best enhancement effect. The amount of urea present in urine is known to be approximately

10 times higher than that of amino acids.[11] Therefore, it is believed that the enhancement sensitivity increases more upon treatment with DMAC, which reacts with urea, than upon treatment with ninhydrin, DFO, or 1,2-IND/Zn, which react with amino acids. Farrugia et al. reported that when footwear impressions in urine on fibers were enhanced with 1,2-IND/Zn, ninhydrin, DFO, and DMAC, DFO showed a better enhancement effect than DMAC;[23] this is contradictory to DMAC showing a better enhancement effect than DFO in this study. It is believed that such results may be due to DFO being a reagent for porous surfaces, whereas the tiles used in this study have non-porous surfaces.[25]

3.3. Enhancement of footwear impression over time

When footwear impressions in urine are left as such, the components of urine diffuse into the air over time. Consequently, the amount of reactants in the urine decreases, causing a decrease in the intensity of the reaction between the urine and reagents.[14] To compare the efficacy of reagents over time, footwear impressions in urine were produced with urine diluted ~0–100-fold with deionized water. These footwear impressions were stored indoors for 1, 7, 14, and 21 days at an average temperature of 18 ºC and relative humidity of 29 %, after which, the impressions were enhanced with ninhydrin (spray method), DFO (dry contact method), 1,2-IND/Zn (spray method), and DMAC (dry contact method) to compare the enhancement effects. The assessment results of the intensity of enhancement are shown in *Fig.* 5. As shown in the figure, enhancement with DMAC (dry contact method) showed the least change in score over time, which reconfirmed that this method has the best enhancement effect on footwear impressions.

3.4. Enhancement of footwear impressions in urine left on actual bathroom tile

Considering the experimental results, it was determined that treatment with DMAC (dry contact method) was the best method for enhancing footwear impressions in urine left on tiles. To confirm whether the method of treatment with DMAC (dry contact method) could be used in an actual crime scene, 7 tiles (45 cm × 45 cm each) from the floor near the entrance (n = 2), basin (n = 2), and toilet (n = 3) in men's and women's indoor bathrooms in a single

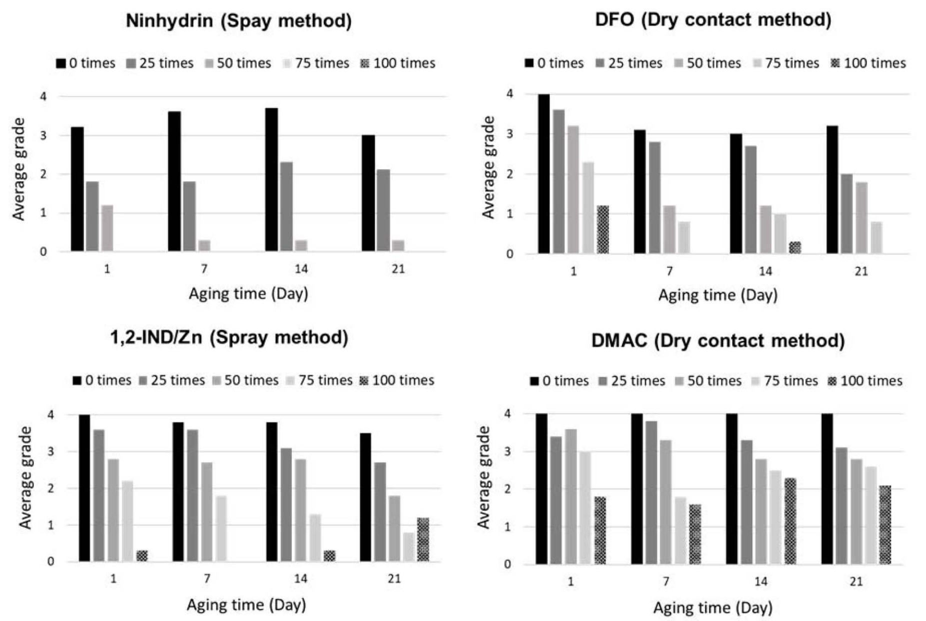

Fig. 5. Changes in the grading of the footwear impressions in urine over time.

Table 2. The number of footwear impressions in urine recovered from the floor tiles in men's and women' bathroom

	Number of footwear impressions in urine (average grade)	
	Men's bathroom	Women's bathroom
Entrance floor	2 (0.5)	0
Basin floor	4 (0.2)	0
Toilet floor	5 (0.2)	2 (0.8)
Urinal floor	7 (2.5)	Not tested
Squat toilet floor	Not tested	5 (1.8)

university building were selected. In addition, 3 additional tiles near urinals in a men's bathroom and 3 additional tiles near squat toilets were selected. The selected tiles were treated with DMAC (dry contact method). To maintain the condition for leaving the footwear impressions on the tiles as close as possible, all the footwear impressions were collected 6 h after the bathroom floor was cleaned. When only an ultraviolet light source was used to inspect the bathroom floor prior to treatment with the reagent, no footwear impressions were observed. However, when footwear impressions in urine were collected after treating with DMAC (dry contact method), as shown in *Table* 2, 18 footwear impressions in the men's bathroom and 7 footwear impressions in the women's bathroom were found, even though only a few people used the bathrooms due to the footwear impression collection period being the school vacation time. It is believed that the reason why more footwear impressions were collected in the men's bathroom than in the women's bathroom was due to the fact that men are more likely to spill urine on the bathroom floor than women.

4. Conclusions

When using ninhydrin, 1,8-diazafluoren-9-one (DFO), 1,2-indanedione/zinc (1,2-IND/Zn), or p-dimethylaminocinnamaldehyde (DMAC) to enhance footwear impressions in urine left on white or black tiles, the best enhancement effect was obtained using the method of spraying ninhydrin or 1,2-IND/Zn directly on the samples, and with the dry contact method of covering the sample with paper soaked in DFO or DMAC and ironing the paper. Moreover, the best detection sensitivity for footwear impressions in urine left on white and black tiles was achieved with treatment via the dry contact method using DMAC. An observation of the changes in sensitivity over 21 days after the footwear impressions were left showed that treatment via the dry contact method using DMAC showed the best sensitivity. Furthermore, compared to the control (using only ultraviolet light), enhancement by treatment with DMAC showed better results with respect to the intensity of photoluminescence and the sensitivity for urine. In addition, when the method of applying DMAC via the dry contact method was used on tiles in actual men's and women's bathrooms, footwear impressions were successfully detected, which indicated that this method could be used to enhance and detect footwear impressions in urine on tiles in the bathroom floor of an actual crime scene.

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