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TECHNICAL NOTE

CRIMINALISTICS

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Latent Fingermark Development on a Range of Porous Substrates Using Ninhydrin Analogs—A Comparison with Ninhydrin and 1,8-Diazofluoren

ABSTRACT: Three relatively new reagents for developing latent fingermarks on porous substrates, 1,2-indandione (IND), 5-methylthioninhydrin (5-MTN), and lawsone, are compared with the more widely used ninhydrin and 1,8-diazofluoren (DFO). Developed latent fingermark visualization on 10 different substrates comprising colored papers, cardboard, and cellophane rather than conventional printer and writing/notepad paper is assessed using latent fingermark deposits from 48 donors. Results show improved fluorescent fingermark visualization using IND compared with DFO on a range of colored cardboards and thick white paper, thus extending the range of substrates known to yield improved visualization with IND. Adding zinc chloride to IND failed to yield any further improvement in fluorescent fingermark visualization. 5-MTN (with and without zinc chloride posttreatment) showed no improvement in visualization compared with ninhydrin and DFO although visible fingermarks were developed. Lawsone produced fluorescent visible fingermarks only with white substrates, which were inferior to those produced with DFO.

KEYWORDS: forensic science, latent fingermark, fingermark reagents, 5-methylthioninhydrin, 1,2-indandione, lawsone, ninhydrin, 1,8-diazofluoren

The development of latent fingermarks deposited on porous surfaces such as paper and cardboard is now mature with a range of chemical treatments readily available (1). The most widely used chemical reagents on such surfaces, as specified by the U.K. Home Office, are ninhydrin and 1,8-diazofluoren (DFO) (2) and research continues into extending the usefulness of these reagents to different substrates (3). In recent years, alternative reagents to ninhydrin and DFO have been reported to produce enhanced development of latent fingermarks. Most notable among these are a range of ninhydrin analogs, structurally similar to ninhydrin, that include 1,2-indandione (IND) (4,5), 5-methylthioninhydrin (5-MTN) (6), and, more recently, 2-hydroxyl-1,4-naphthoquinone (lawsone) (7). A review of ninhydrin analogs may be found in Lee and Gaensslen (1) and, more recently, in Champod et al. (8). No doubt, in recent years, some of the driving force for investigating the potential of alternative amino acid–specific reagents has been the 1999 U.S. Department of Justice Review of forensic science needs (9), which was cited by Crane et al. as motivation for their own work, albeit on infrared spectroscopic imaging of latent fingermarks (10).

IND was first suggested as a latent fingermark development reagent by Ramotowski et al. in 1997 (11), and since then, several other workers have compared its effectiveness against ninhydrin and DFO (4,5,12–20). Unlike ninhydrin, IND was found not to produce intense color development, although developed fingermarks fluoresced strongly when excited with green light (500–550 nm) (4). The addition of zinc salts (principally zinc chloride) was reported as either enhancing the intensity of the fluorescence (11,17,19–21) or having little effect on the fluorescence (16,18). These inconsistencies, together with problems of photodecomposition (12), solubility, and determination of an ideal formulation (4,5) have, to some extent, prevented IND from entering the mainstream of latent fingermark development reagents on porous surfaces. Its chemical structure is shown in Fig. 1*a*.

5-MTN was first reported by Heffner and Joullie (22) and Almog et al. (23) and, with the addition of zinc chloride, showed improved fluorescence compared with DFO. However, the widespread introduction of DFO in the early 1990s has been suggested as the reason why 5-MTN has not found greater use in latent fingermark development (6). More recently, Almog et al. (6) investigated premixed solutions of 5-MTN (and 5-methoxyninhydrin) with either zinc or cadmium chloride to produce developed latent fingermarks that are both colored and fluorescent. They found that the fluorescence of 5-MTN-Zn was comparable to that of DFO, but the 5-MTN-Zn-developed prints were more colored and visible in natural daylight. 5-MTN's chemical structure is shown in Fig. 1*b*.

Lawsone is a relatively new addition to latent fingermark development and was reported initially by Jelly et al. (7) and, more recently, by Lafratta et al. (24). It is derived from the leaves of *Lawsonia inermis* and is thought to produce the staining qualities of henna (7). In addition to its reported purple-brown color development of latent fingermarks, it is also reported to exhibit strong fluorescence emission at 650 nm when excited at 590 nm (7,24). Its chemical structure is shown in Fig. 1*c*.

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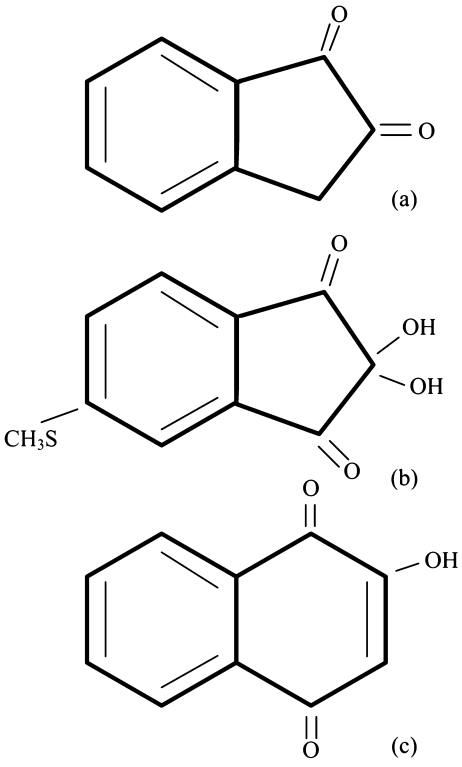

FIG. 1—*Chemical structure of (a) 1,2-indandione (IND), (b) 5-methylthioninhydrin (5-MTN), and (c) 2-hydroxyl-1,4-naphthoquinone (lawsone).*

To date, the effectiveness of ninhydrin analogs when compared with ninhydrin and DFO on a wide range of porous substrates has not been evaluated fully. Experimentation has been, generally, limited to white or colored business grade papers (5,12,18–20), wallpaper and untreated wood (5), brown wrapping paper (6), white filter paper (7), and thermal paper (20).

In this technical note, we compare the effectiveness of 5-MTN (with and without posttreatment with zinc chloride), IND (with and without the addition of zinc chloride), and lawsone with ninhydrin and DFO on a range of common colored paper, cardboard, and cellophane substrates, the color and absorbency of which were felt likely to make developed fingermark visualization with ninhydrin or DFO difficult. A comparison against ninhydrin and DFO and previous workers' results is given, and substrates where these reagents appear to offer improved fingermark visualization are identified.

Experimental Details

Materials and Method

For these experiments, latent fingermarks were taken from 24 men and 24 women donors of varying ages as shown in Table 1. Each donor deposited latent fingermarks on each of 10 different substrate materials. These substrates are listed in Table 2 and were chosen as they are known to be difficult to contrast developed latent fingermarks because of their color and absorbency. While more usually treated by cyanoacrylate fuming (1,2), various colored cellophanes were included (Table 2) as it has been reported that cellophane (the clear film produced from cellulosic fiber) can be treated as a porous surface (25). Each donor deposited latent fingermarks on sufficient samples of each substrate to enable separate comparisons to be made for each of the three reagents under test (IND, 5-MTN, and lawsone) with ninhydrin and DFO as shown in Table 3. All deposits were "first impressions," that is, a depletion series was not employed. Each donor was assigned a different number to enable comparisons for the same donor with different reagents on different substrates to be made. All fingermark deposits were treated <24 h after deposition and may therefore be considered as "fresh." The deposited fingermarks were traced using a black pen or a white-colored pencil, depending on the color of the substrate. The traced fingermark was then cut in half in order that the comparisons between the reagent under test and ninhydrin or DFO could be made. Fingermarks were deposited by pressing a finger onto a substrate for 1–2 sec with a light pressure sufficient to ensure contact between the finger and substrate. While no attempt was made to regulate the amount of pressure applied by individuals, this procedure was intended to produce reasonably uniform deposition. No artificial stimulation of sweat was employed such as placing the hand in a plastic bag (26) or wearing a latex glove prior to deposition (27). Donors did not wash their hands within the 20-min period preceding fingermark deposition.

TABLE 1—*Age range of donors.*

	Number of donors	
Age range	Male	Female
18–29	6	6
30–39	6	6
40–49	6	6
50–59	6	6

Assessment Scale for Developed Fingermarks

Fingermarks from each donor (Table 1) for each comparison (Table 3) on each substrate (Table 2) were assessed using a system devised by McLaren et al. (28). Samples for the reagent under test that showed a significant improvement over the comparison reagent

TABLE 2—*Substrates used in these experiments.*

Number	Substrate	Comments
1	White thick paper shopping bag	
2	Black thick paper shopping bag	
3	Red thick paper shopping bag	With yellow and white printed patterns
4	Thin card food wrap lid	Shiny green with white print
5	Brown cardboard	
6	White cardboard	
7	Dark purple cardboard	
8	Black cardboard	
9	Black cardboard	Shiny
10	Cellophane	Red, yellow, green, blue, and purple

TABLE 3—*Comparisons made with reagents under test.*

Test	Reagent Under Test	Comparison with
1	IND	DFO
2	IND-Zn	IND
3	IND	Ninhydrin
4	5-MTN	Ninhydrin
5	5-MTN-Zn	DFO
6	Lawsone	DFO

DFO, 1,8-diazofluoren; IND, 1,2-indandione; 5-MTN, 5-methylthioninhydrin.

were allocated a score of +2. For example, for comparison 1 in Table 3, if the reagent under test (IND) showed significant improvement in fingermark development over the comparison reagent (DFO), then this particular fingermark for that donor on that substrate was allocated a score of +2. Fingermarks showing a minor improvement were allocated a score of +1 and no improvement a score of 0. Fingermarks showing a decrease in quality were allocated a score of −1 or −2 as appropriate. In a similar fashion to McLaren et al. (28), clarity of ridge detail and contrast against the background were the primary factors considered for score allocation.

1,2-Indandione

As stated previously, the ideal IND formulation has been subject to debate (5), and in these experiments, the formulation proposed by Bicknell and Ramotowski (4) was used. This consisted of adding 1.0 g of IND (BVDA, Haarlem, Holland) to 30 mL of dichloromethane, followed by 60 mL of ethyl acetate and then 10 mL of glacial acetic acid while stirring. Finally, the volume was brought up to 1 L with 900 mL of methyl nonafluoroisobutyl ether (HFE-7100; Tetra Scene of Crime, Basildon, U.K.). For experiments requiring the addition of zinc chloride, the solution was prepared using the current U.K. Home Office formulation (U.K. Home Office, personal communication, 2010) of 0.125 g of IND, 45 mL of ethyl acetate, 5 mL of acetic acid, 0.25 mL of zinc chloride stock solution, and 500 mL of HFE-7100. The zinc chloride stock solution consisted of 0.2 g of zinc chloride in 5 mL of absolute ethanol. The working solution was poured into a tray and the substrates dipped for 5 sec, air dried, and then heated in an oven at 100°C with no raised humidity for 15 min (4).

5-Methylthioninhydrin

5-MTN (BVDA) was prepared according to the formulation given by BVDA (26). 5-MTN (0.34 g) was dissolved in a mixture of acetic acid (1 mL), ethanol (2.5 mL), and ethyl acetate (14.5 mL). The mixture was stirred for 15 min. Then, 10 mL of methyl *tert*-butyl ether and 72 mL of HFE-7100 were added to the solution. For experiments requiring posttreatment with zinc chloride, the stock solution was prepared as stated previously and applied to the 5-MTN-treated substrate by spraying (27). The working 5-MTN solution was poured into a tray, substrates dipped for 5 sec, air dried, and then heated in an oven at 80°C with 70% relative humidity for 5 min.

Lawsone

The formulation used here was based on Jelly et al.'s formulation (7). Lawsone (0.2 g) (Sigma-Aldrich, Gillingham, U.K.) was added to 40 mL of ethyl acetate and stirred. HFE-7100 (160 mL) was then added to the mixture and stirred. The working solution was poured into a tray, substrates dipped for 5 sec, air dried, and then heated in an oven at 80°C with 70% relative humidity for 5 min.

The lawsone did not dissolve completely in the solvent and attempts to find a solvent that did completely dissolve the lawsone were unsuccessful. This problem was also reported by Jelly et al. (7). Alternative sources of lawsone (Body Art Shop, Manchester, U.K. and Elixir, Carlisle, U.K.) showed no improvement in solubility.

Working solutions of both ninhydrin and DFO were prepared according to current formulations given by the U.K. Home Office (2).

Results and Discussion

1,2-Indandione

IND was found to produce a strong fluorescence when viewed with a Quaser OG549 long pass filter (549 nm band edge) and excited with a bandwidth setting of 473–548 nm on a Quaser 2000/30 (Foster and Freeman, Evesham, U.K.) as has been observed by other workers (4,5). The format OGXXX is a manufacturer reference in which XXX represents the long pass filter cut-on in nanometers. It was also noted that an excitation bandwidth setting of 468–526 nm and OG529 viewing filter and excitation bandwidth setting of 400–519 nm and OG593 viewing filter produced sufficient contrast to enable an IND-developed latent fingermark to be visualized (4,5). Compared with DFO, IND was found to give improved fingermark definition on cardboards (substrates 5–9) and on the white shopping bag (substrate 1) but not the colored shopping bags (substrates 2 and 3), thin card (substrate 4), or cellophane (substrate 10). The red-colored shopping bag (substrate 3) was not ideal because of low contrast between the substrate and developed fingermark. Figure 2 shows an example of a part DFO- and part IND-developed latent fingermark on the white shopping bag (substrate 1). These results are consistent with other workers reporting of improved visualization of IND-developed latent fingermarks over DFO, albeit for different substrates. Comparison 1 in Table 4 (IND with DFO) shows the average score for each age and gender group of donor (Table 1) for each substrate (Table 2). For substrates where IND gave improved fingermark definition, the average improvement was minor (slightly <+1). There was no apparent difference in improvement score between the different age or gender groups. Bicknell and Ramotowski (4) and Wallace-Kunkel et al. (5) have commented at length on the effect that both ambient relative humidity and moisture content of the substrate have on the effectiveness of IND. Bicknell and Ramotowski (4) reported that critical moisture level times occurred immediately following the dipping of a substrate in IND and the subsequent drying. Clearly, paper, cardboard, or shiny (less absorbent) surfaces are likely to have variable moisture contents and consequently result in variations in quality with respect to developed fingermarks.

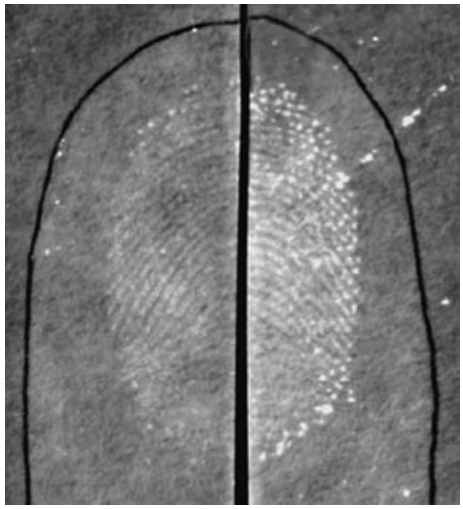

FIG. 2—*White shopping bag (substrate 1) showing (left) 1,8-diazofluoren (DFO) and (right) 1,2-indandione (IND) developed latent fingerprint. Images recorded in the luminescence mode with, for (left), excitation at 503–587 nm and observation using an OG593 long pass filter and, for (right), excitation at 473–548 nm and observation using an OG549 long pass filter.*

TABLE 4—*Average improvement score for each age and gender group for each comparison and each substrate. For a given comparison and substrate, the eight matrix elements represent the age and gender distribution shown in Table 1.*

	Substrate																			
Comparison	1		2		3		4		5		6		7		8		9		10	
IND with DFO	+0.8	+1	0	0	0	0	0	0	+0.7	+0.5	+0.5	+0.8	+0.8	+0.7	+0.7	+1.2	+0.7	+0.7	0	0
	+0.8	+0.8	0	0	0	0	0	0	+0.7	+0.8	+0.8	+0.8	+0.8	+0.8	+0.8	+0.7	+0.8	+0.8	0	0
	+0.7	+0.8	0	0	0	0	0	0	+0.5	+0.7	+0.8	+0.7	+0.8	+0.7	+1.2	+0.8	+0.8	+0.7	0	0
	+0.7	+1	0	0	−0.2	0	0	0	+0.5	+0.5	+0.7	+0.7	+0.7	+0.7	+0.5	+0.7	+0.7	+0.8	0	0
IND-Zn with IND	0	0	0	0	0	+0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	−0.2
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+0.2	0	0	0	0
	0	0	0	−0.2	0	0	0	0	−0.2	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+0.2	0
IND with Ninhydrin	−1.8	−1.8	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−1.8	−1.8	−2	−1.8	−2	−2	−2	−2	−1.8
	−1.7	−2	−1.8	−2	−1.8	−1.8	−1.8	−1.8	−1.7	−1.8	−2	−2	−2	−1.8	−1.7	−2	−2	−1.8	−1.8	−2
	−2	−1.8	−2	−1.8	−2	−1.8	−2	−2	−1.8	−2	−1.8	−2	−2	−1.8	−2	−1.8	−1.8	−1.8	−1.8	−1.8
	−1.8	−2	−1.8	−2	−1.8	−2	−1.7	−2	−2	−1.8	−2	−1.8	−1.8	−2	−1.8	−2	−2	−1.8	−1.8	−2
5-MTN with Ninhydrin	0	0	−0.2	0	0	−0.2	0	0	0	0	−0.2	0	0	0	0	0	0	0	−1.7	−1.8
	0	0	0	0	0	+0.2	0	+0.2	0	0	0	+0.2	−0.3	+0.2	0	+0.2	0	−0.2	−2	−1.8
	0	0	0	0	0	0	+0.2	0	0	0	0	0	0	0	+0.2	0	+0.2	0	−1.8	−1.7
	0	0	+0.2	0	−0.2	0	0	0	0	0	0	0	0	0	0	−0.2	0	0	−1.8	1−.7
5-MTN-Zn with DFO	−0.2	0	0	+0.2	0	0	0	0	0	+0.3	−0.3	0	0	0	+0.3	0	−0.2	0	0	0
	0	−0.2	0	0	0	−0.3	0	−0.2	−0.2	0	0	+0.2	0	−0.2	0	0	0	0	0	+0.3
	+0.2	0	+0.3	0	0	0	+0.3	0	0	−0.2	0	0	+0.3	0	0	−0.3	+0.2	−0.2	0	−0.2
	0	0	0	0	+0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	−0.3	0
Lawsone with DFO	−1.8	−1.7	−2	−2	−2	−2	−2	−2	−2	−2	−1.8	−1.7	−2	−2	−2	−2	−2	−2	−2	−2
	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−2	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−2
	−2	−2	−2	−2	−2	−2	−2	−2	−2	−2	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−2
	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−2	−1.8	−2	−2	−2	−2	−2	−2	−2	−2	−2

DFO, 1,8-diazofluoren; IND, 1,2-indandione; 5-MTN, 5-methylthioninhydrin.

FIG. 3—*White shopping bag (substrate 1) showing (left) 1,2-indandione (IND) and (right) IND-Zn developed latent fingerprint. Images recorded in the luminescence mode with, for both (left) and (right), excitation at 473–548 nm and observation using an OG549 long pass filter.*

The addition of zinc chloride to IND (as described previously) was found not to produce any additional visualization; Fig. 3 showing a part IND- and a part IND-Zn-developed latent fingermark on substrate 1. The IND-Zn-developed fingermarks were best viewed with an excitation bandwidth setting of 473–548 nm and OG549 viewing filter (20). It was noted that both IND and DFO produced a faint pink-colored fingermark visible under natural daylight, but the addition of the zinc chloride to the IND concealed it. This natural daylight visualization was found to be inferior to that obtained from ninhydrin. The concealment of the faint pink color following IND-Zn treatment is interesting as previous studies have indicated that the addition of zinc does not change the color of the developed fingermarks and may, in fact, enhance the color (4). This observation may result from the U.K. Home Office formulation for IND-Zn used here differing from that used in previous studies (4) and also in having a lower IND concentration. The lack of effectiveness of IND in some previous studies conducted by the U.K. Home Office (compared with DFO) has been attributed to the lower IND concentrations used (5). Clearly, this observation is worthy of further study.

Comparisons 2 and 3 in Table 4 show the average score for each age and gender group for both of these comparisons. Comparison 2 confirms the lack of additional visualization with IND-Zn, while comparison 3 shows the inferiority of natural daylight visualization of IND in comparison with ninhydrin, the average score being slightly < -2.

5-Methylthioninhydrin

Both ninhydrin and 5-MTN produced visible purple fingermarks with the same degree of visualization, and 5-MTN was very similar to ninhydrin in terms of development time, developed color, and range of useful substrates. Both reagents worked best on light-colored substrates, and Fig. 4 shows an example of a part ninhydrin- and part 5-MTN-developed latent fingermark on a yellow-colored part of substrate 3. The black thick paper shopping bag (substrate 2) gave the poorest contrast for both ninhydrin and 5-MTN. Not unexpectedly, neither reagent worked well with red-colored backgrounds. Ninhydrin was observed to give better visualization on all of the colored cellophane (substrate 10), which was visible at oblique viewing

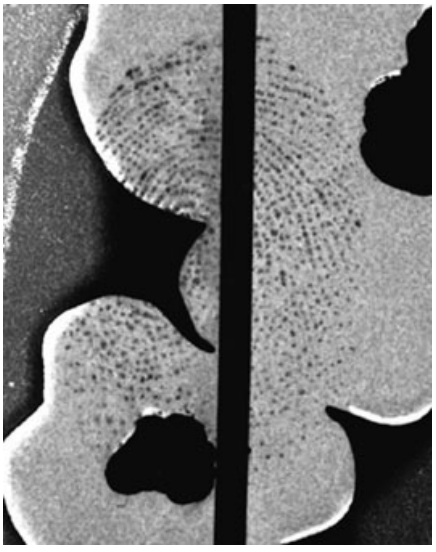

FIG. 4—*Yellow colored part of substrate 3 showing (left) ninhydrin and (right) 5-methylthioninhydrin (5-MTN) developed latent fingerprint. Images recorded in absorption mode with, for both (left) and (right), illumination at 430–510 nm and observation using an OG549 long pass filter.*

angles. The relative humidity recommended for developing latent fingermarks with 5-MTN of 80% (http://www.bvda.com/EN/prdctinf/en_mtn_1.html, accessed November 13, 2010) was found to encourage the reagent to produce poorly defined fingermark patterns. A lower relative humidity of 70% produced better defined fingermarks.

Spraying 5-MTN-treated substrates with zinc chloride did not give any improvement in visualization when compared with DFO. Optimum fluorescence occurred for both 5-MTN-Zn and DFO with an excitation bandwidth setting of 503–587 nm and OG593 viewing filter. Both reagents worked best on cardboard (substrates 5–9); Fig. 5 showing an example of a part DFO- and part 5-MTN-Zn-developed latent fingermark on brown cardboard (substrate 5). In accordance with U.K. Home Office recommendations (2), zinc chloride was not added to DFO.

Comparisons 4 and 5 in Table 4 show the average score for each age and gender group for both of these comparisons with the majority of scores being 0, other than the colored cellophanes (substrate 10) for comparison 4 where the average score was slightly < -2.

Lawsone

It has been reported that lawsone will readily stain latent fingermarks (7,24). With an excitation bandwidth setting of 400–519 nm and OG593 viewing filter, lawsone-developed fingermarks were observed to fluoresce albeit very weakly and much less than latent fingermarks developed with DFO. In these experiments, fluorescent lawsone-developed latent fingermarks could be observed only for the white substrates (substrate 1 and substrate 6); Fig. 6 showing an example of a part DFO- and part lawsone-developed latent fingermark on substrate 6. Comparison 6 in Table 4 confirms the negative score for the lawsone/DFO comparison with only substrates 1 and 6 giving scores slightly < -2. There was no apparent difference in score between the different age or gender groups. Other workers have also only observed lawsone fluorescence on white substrates (7,24), but with different excitation and viewing wavelengths (7). Clearly, more work needs to be carried out to gain a better understanding of the variable nature of lawsone latent fingermark development. Jelly et al. (7) did not state how many different people donated fingermarks in their study, but it would seem reasonable to suppose that of the 48 donors here, the success rate would have been higher. Given the limited work on lawsone to date, it cannot be seen as a viable reagent for latent fingermark development at this time.

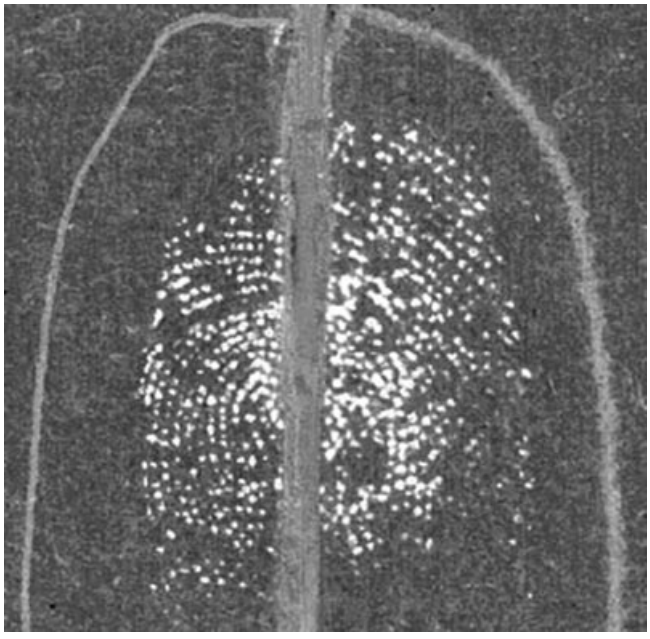

FIG. 5—*Brown cardboard (substrate 5) showing (left) 1,8-diazofluoren (DFO) and (right) 5-MTN-Zn developed latent fingerprint. Images recorded in the luminescence mode with, for both (left) and (right), excitation at 503–587 nm and observation using an OG593 long pass filter. 5-MTN, 5-methylthioninhydrin.*

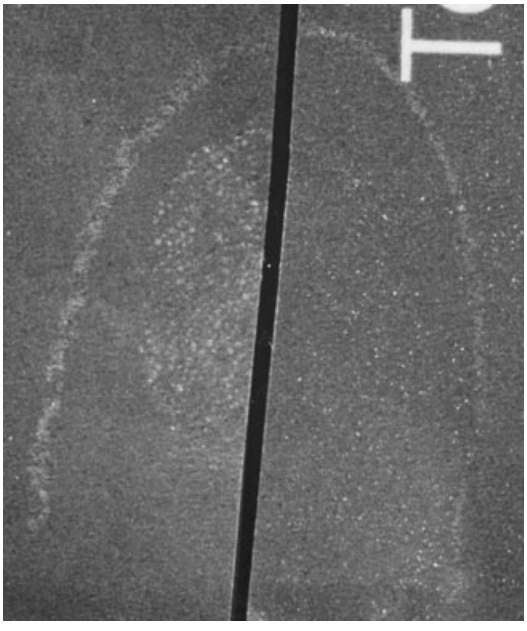

FIG. 6—*White cardboard (substrate 6) showing (left) 1,8-diazofluoren (DFO) and (right) lawsone developed latent fingerprint. Images recorded in the luminescence mode with, for (left), excitation at 503–587 nm and observation using an OG593 long pass filter and, for (right), excitation at 400–519 nm and observation using an OG593 long pass filter.*

According to Jelly et al. (7), lawsone-treated substrates heated for 1 h at 140–170°C produced a more uniform fingermark development. Here, where fingermark development was observed, a heating time of >5 min was found not to improve the visualization.

Conclusion

A comparison of three, relatively new, reagents to develop latent fingermarks on a range of substrates comprising colored papers, cardboard, and cellophane rather than conventional printer and writing/notepad paper has shown varying improvement over the more commonly used treatments of ninhydrin and DFO. The most successful of the three, IND, gave improved visualization of developed latent fingermarks on a range of colored cardboards and thick white paper and has thus extended the range of substrates that yield improved visualization with IND. The success of IND is interesting, given its reported sensitivity to relative humidity and substrate moisture content. This, and the reported variation in optimum formulation, may well imply that the success of IND will be dependant on local conditions unless carefully controlled.

5-MTN is less well reported that IND but, again, the lack of any improvement in visualization reported here (both with and without posttreatment with zinc chloride) may imply its benefit for latent fingermark development lies in making it a ''dual fingermark reagent'' to replace both ninhydrin and DFO as suggested recently by Almog et al. (6).

Lawsone clearly requires more research as, at present, there is insufficient data to demonstrate its potential.

It would also be useful to examine the effect that aging fingermark deposits (say, for 1 month and for 6 months) had on visualization, especially with a similar number of donors to these experiments. Part of any future work for all three reagents (IND, 5-MTN, and lawsone) could usefully include where each one might sit in a program of sequential treatment.

Acknowledgments

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