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TECHNICAL NOTE

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Optimization and Initial Evaluation of 1,2-Indandione as a Reagent for Fingerprint Detection*

ABSTRACT: 1,2-Indandione has been used to develop fluorescent fingerprints on porous materials such as paper. The compound reacts with amino acid residues to produce highly fluorescent fingerprint ridges. An optimized formulation and treatment protocol for using the reagent is presented here.

The reagent is applied as a solution in HFE7100 containing acetic acid and ethyl acetate. Treated articles are heated at 100°C for 10 min at ambient humidity and stored in the dark before recording the fingerprints using fluorescence photography or digital imaging.

Photodecomposition of the fluorescent fingerprints has been observed. Storage in the dark reduces degradation, extending the lifetime of the fingerprints. Other chemical methods to stabilize the fingerprints proved unsuccessful.

Comparisons of the performance of 1,2-indandione with DFO in CFC113 performed on a limited range of substrates indicated that the reagent might be an effective method for the development of latent fingerprints despite the new reagent producing less intense fluorescence.

KEYWORDS: forensic science, latent fingerprints, 1,2-indandione, 1,8-diazafluoren-9-one, fluorescence

In 1997, researchers at the University of Pennsylvania discovered that 1,2-indandiones could react with amino acids to give fluorescent products (1). As amino acids are significant components of ecrine sweat, 1,2-indandiones are potential reagents for the development of latent fingerprints on porous surfaces such as paper (2,3).

Before any reagent is introduced into operational police use, it is essential to demonstrate that it will effectively develop latent fingerprints on articles encountered operationally. Optimum treatment and visualization conditions for the use of 1,2-indandione have therefore been determined by varying reaction conditions such as temperature and relative humidity. Carefully controlled comparisons using fraudulently passed bank checks and a range of commonly encountered paper exhibits have also been performed to assess whether the new reagent is likely to be as effective as existing reagents such as 1,8-diazafluoren-9-one (DFO) (4) and ninhydrin (5).

Post-treatment storage of articles is also important, particularly when a significant delay could occur between treatment with 1,2-indandione and the recording of developed fingerprints. This is particularly important when using indandione because the fluorescent intensity of the developed fingerprints decreases on exposure to daylight. Fingerprints developed with 1,2-indandione are easily recorded using the same fluorescence examination methods currently used for fingerprints developed with DFO.

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Experimental

Visualization of Developed Fingerprints

Fingerprints developed with 1,2-indandione are not usually visible to the naked eye and must therefore be recorded using fluorescence photography or digital imaging. To ensure that the correct excitation and emission bands were used for the visualization of fingerprints, the absorption and emission spectra of the fluorescent species produced by the reaction of 1,2-indandione and amino acid residues were measured using a Shimadzu RF-5301 fluorescence spectrophotometer. The excitation and emission maxima (λ_{max}) were found to be 552 and 559 nm, respectively (Fig. 1). Developed fingerprints were therefore excited with the 473 to 548-nm band of the high-intensity light source, in this case the Quaser 40 and viewing from 549 nm.

The fluorescent intensity of fingerprints developed with 1,2-indandione was assessed by making direct luminescence measurements using a Minolta LS100 spot meter. Six sequentially deposited fingerprints were cut in half and treated with the test solutions. After heating, the fluorescent intensity of the first, third, and sixth deposited fingerprints were measured and the average intensity calculated.

These average intensities were then normalized using a control procedure to eliminate errors caused by small variances in the power output of the Quaser 40. To do this, the power output of the

light source was recorded at the beginning of the experiment before any measurements of the fluorescent intensity were made, ensuring that the distance between the end of the light guide and the power meter remained constant (Fig. 2). This procedure was repeated before each set of data was collected. The figures obtained for the original power output of the light source (p_0) and later power outputs (p_n) were then used to relate the observed fluorescence of the developed fingerprints to the measured fluorescence at the original power output using the equation below:

$$I_{norm} \approx \frac{p_0}{p_n} \cdot I_{meas}$$

where I_{norm} is the normalized fluorescent intensity, and I_{meas} is the experimentally measured fluorescent intensity.

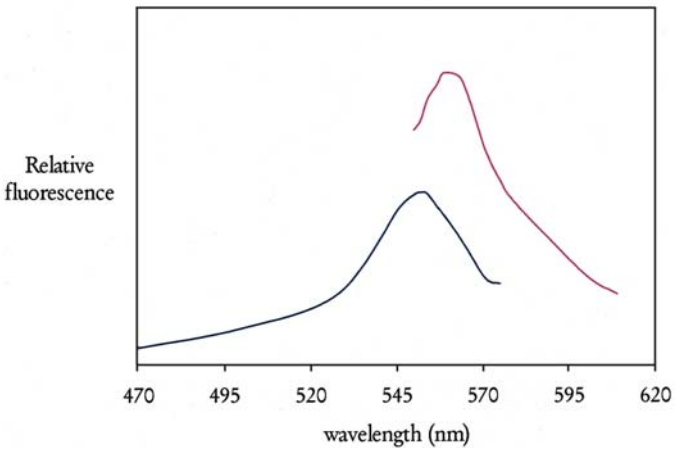

FIG. 1—*Absorption/emission spectrum of fluorescent product.*

Reagent Formulation and Post Treatments

It is desirable that all amino acid reagents used for the detection of latent fingerprints on porous surfaces are formulated in ozone friendly, nonflammable solvents so that fire and explosion risks and overall environmental impact are minimized. HFC4310mee and HFE7100 are therefore potential solvents for amino acid reagents, as both solvents have zero ozone–depleting potential (ODP) and are nonflammable. HFC4310mee, however, has already been shown to be an inferior solvent for 1,2-indandione, so only HFE7100 was used in this study. Aqueous formulations were not examined because amino acid residues in fingerprint ridges are water-soluble.

1,2-Indandione is considerably more soluble in solvents such as HFC4310mee and HFE7100 than DFO, another significant advantage of the reagent. It is also advisable to avoid the use of methanol and ethanol in reagent formulations, as it is known that 1,2-indandione formulations based either wholly or partly on alcohols are not stable over a period of weeks (6).

Zinc chloride treatment (2) was also examined to see if it significantly improved the fluorescence of deposited fingerprints developed with 1,2-indandione.

Heating Conditions

The test substrates were treated with 1,2-indandione by simply passing the paper through a shallow trough of the reagent formulation. The test strips were then heated in an oven, varying heating time, temperature, and relative humidity. Articles treated at ambient humidity were heated in a Heraeus oven, complying with the current PSDB specification for DFO ovens (5). All articles treated at raised humidity levels were heated using a Sanyo Gallenkamp fingerprint development chamber, a commercially available lami-

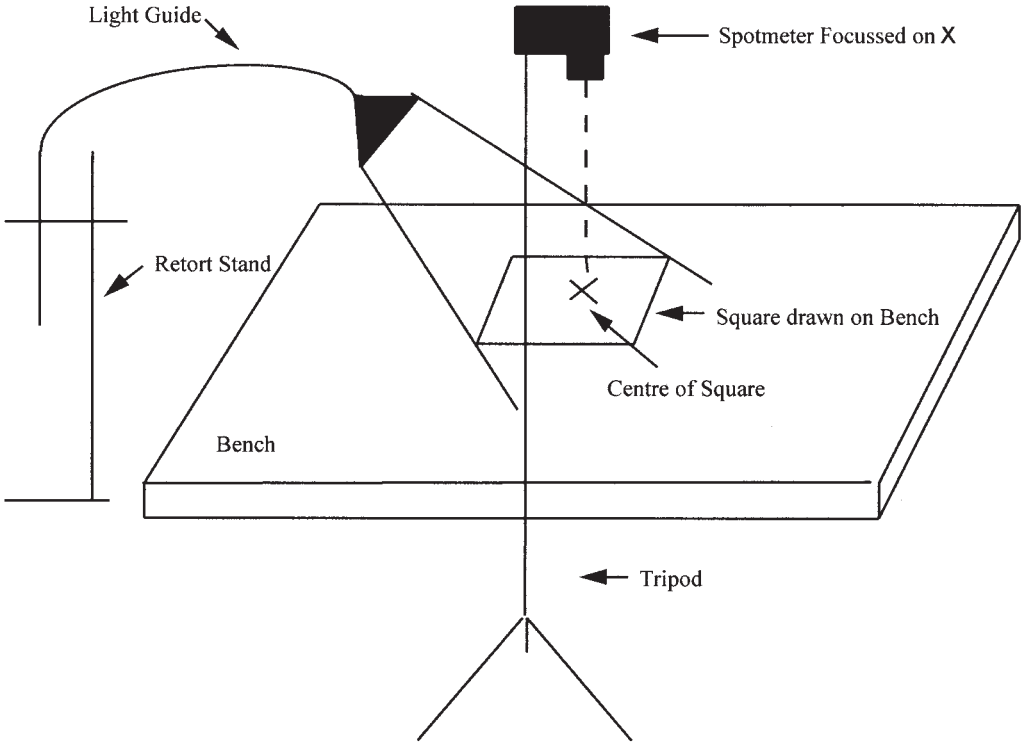

FIG. 2—*Apparatus for high-intensity light source power measurements.*

nar flow humidity oven specially adapted for use in the fingerprint laboratory (5).

Stability of Developed Fingerprints

The stability of developed fingerprints was assessed by measuring the fluorescence intensity. Measurements to assess the effects on fingerprint fluorescence of heating time, humidity, storage time, and exposure to light were made immediately after heating.

Initial Comparisons with DFO

Once the optimum heating conditions had been determined, comparisons were made of the relative effectiveness of 1,2-indandione (0.025% in HFE7100) and DFO (0.025% in CFC113) to determine if the new reagent exhibited increased sensitivity (4,5). Initial tests to compare 1,2-indandione with DFO were performed using deposited fingerprints that were divided in half and treated individually with different reagent formulations. These experiments, however, can be misleading, as experimentally deposited fingerprints often do not accurately model the behavior of latent fingerprints occurring on operational material. To overcome this problem, pseudo-operational trials using fraudulently passed bank checks were performed according to the protocol that has previously been used to evaluate ozone-friendly ninhydrin formulations (7).

Results and Discussion

Reagent Formulation and Post Treatments

The effects of the different components in the reagent formulation were examined: 1,2-indandione concentrations of 0.25, 0.50, 0.75, 1.00, 1.50, and 2.00 g/L were examined. The maximum fluorescence was obtained using a concentration of 0.25 g/L.

It has previously been shown that DFO at 0.25g/L develops very few fingerprints in the absence of acid and that the color of fingerprints developed using ninhydrin formulations depends to some extent on the acidity of the reagent formulation used. The ability of 1,2-indandione to develop latent fingerprints is also affected by the acidity of the reagent formulations. In general, increasing the acid concentration gives increased fluorescence of developed fingerprints. However, if the amount of acetic acid exceeds 10 mL/L, the background fluorescence increases to a level where the contrast between fingerprint ridge and background is adversely affected. This is particularly severe when 20 mL/L of acetic acid was used where both the fluorescence of the fingerprint and the background were high. The optimum formulation performance was achieved using 10 mL of acetic acid/L of carrier solvent (Fig. 3).

On the basis of these experiments, the optimized formulation below was used in all subsequent experiments.

1,2-indandione	0.25 g
ethyl acetate	90 mL
acetic acid	10 mL
HFE7100	900 mL

Heating Protocol

The optimal heating conditions were found to be 100°C for 10 min at ambient humidity, i.e., no additional humidification inside the oven chamber. The use of longer heating times or increased relative humidity inside the oven results in a significant decrease in the brightness of developed fingerprints (Figs. 4 and 5).

Stability of Developed Fingerprints

Fingerprints developed with 1,2-indandione and left in daylight degraded over 28 days to only 20% of the original fluorescent intensity (Fig. 6). Experimentally excluding light has extended the lifetime of developed fingerprints to at least six weeks. This is particularly important in busy fingerprint laboratories where there may be a significant delay between treatment and fingerprint recording. Articles treated with 1,2-indandione should therefore be stored in the dark to prevent degradation of developed fingerprints.

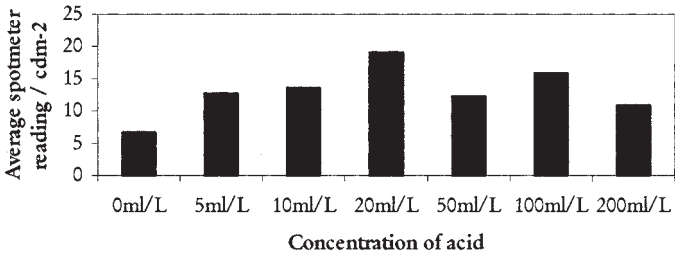

FIG. 3—*Variation of intensity of fluorescence with acid concentration.*

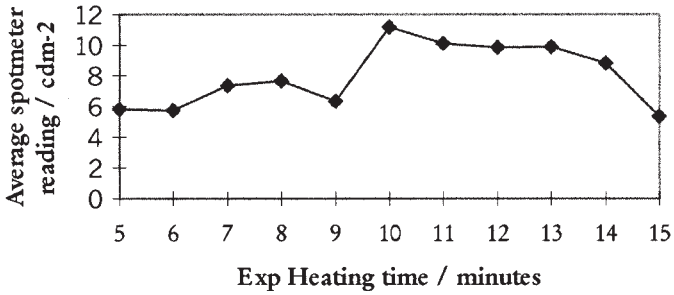

FIG. 4—*Variation of intensity of fluorescence with heating time at 100°C.*

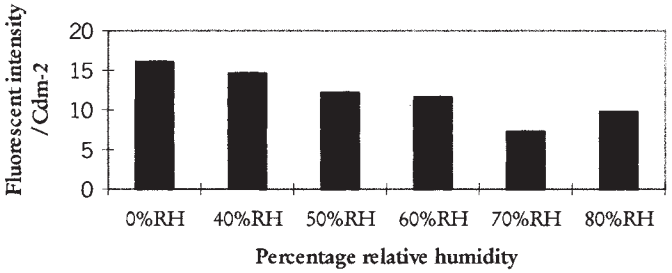

FIG. 5—*Variation of intensity of fluorescence with relative humidity.*

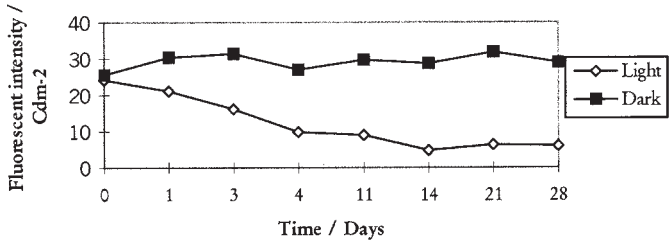

FIG. 6—*Effects of storage conditions on intensity of fluorescence.*

Regeneration of Degraded Developed Fingerprints

If fingerprints developed with 1,2-indandione have degraded, they may in certain circumstances be regenerated. If fingerprints that have degraded in daylight are retreated with the 1,2-indandione formulation and reheated, the fingerprints can be redeveloped. However, if the redeveloped fingerprint is allowed to degrade a second time, it is not possible to repeat the regeneration. Furthermore, degraded fingerprints cannot be regenerated simply by reheating: they must be retreated with fresh 1,2-indandione solution before reheating.

Similar degradation of the developed fingerprints has been observed when illuminating the fingerprint with the 473 to 548-nm excitation band of the Quaser for up to 3 h. However, when a freshly treated fingerprint that had been decomposed in this manner was left in the dark overnight, the fluorescent intensity of the fingerprint returned to its original intensity.

Fingerprints treated a week previously also decomposed when illuminated with the 473 to 548-nm excitation band of the Quaser. In this case, however, the fingerprints cannot be regenerated on storage in the dark. No firm conclusions have been made to account for these observations, although the degradation process could be due to initial photolysis of the fluorescent species. Regeneration of fluorescent fingerprints may be due to further reaction of amino acid residues with excess 1,2-indandione.

Several additional chemical processes were used to try to stabilize developed fingerprints against degradation. Zinc chloride treatment did not result in stabilization of fingerprints, although it did increase the fluorescent intensity (1,2). The use of trapping reagents such as N-phenylmaleimide (6,8,9) and 1,2-diphenylcyclopropenone to stabilize the developed fingerprints was also attempted. Unfortunately, neither of these reagents stabilized the fluorescence nor resulted in a color change in the developed fingerprints when used as a post treatment. Further experiments were performed to assess whether 1,2-diphenylcyclopropenone could stabilize developed fingerprints when dissolved in the 1,2-indandione formulation. Again, no stabilization of the fluorescent product was observed.

Initial Comparisons with DFO

The initial comparison with 0.025% DFO in CFC113 (5) was carried out using fraudulently passed bank checks. Checks were dipped once in the reagent solution and allowed to dry before being heated to 100°C. The results obtained on the day the checks were processed (Day 0) and after two weeks of storage (14 days) are shown in Fig. 7

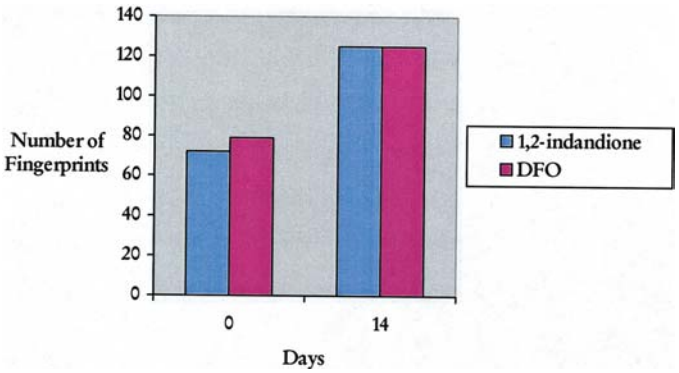

FIG. 7—*Effects of storage time on the total number of detectable fingerprints.*

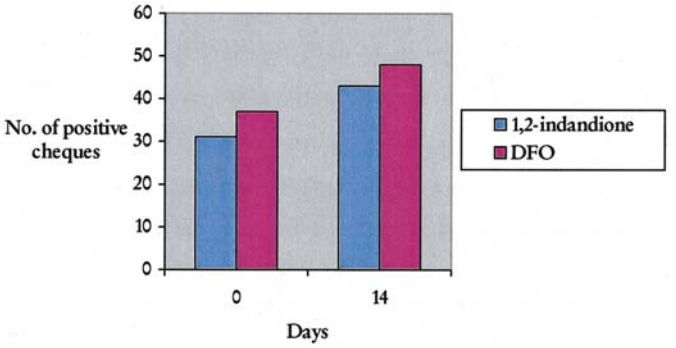

FIG. 8—*Effects of storage time on the total number of positive checks.*

TABLE 1—*Pseudo-operational trial of 1,2-indandione against 1,8-diazafluoren-9-one (DFO).*

Article	No. of Articles	Number of Developed Fingerprints	
		DFO	1,2-indandione
White envelopes	20	41	44
Brown envelopes	15	17	33
Photocopy paper	20	92	82
Newspaper	20	2	2
Receipts	20	5	7
Train tickets	19	10	6
TOTAL	114	167	174

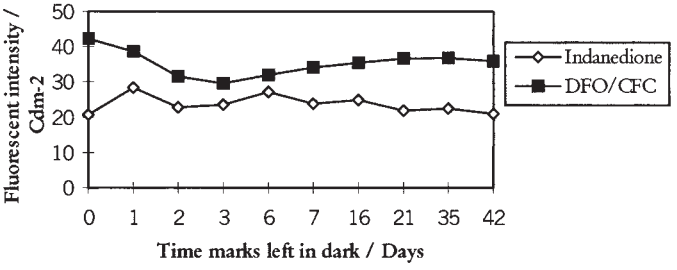

FIG. 9—*Comparison of the intensity of fluorescence of DFO and indandione-treated fingerprints over time.*

and Fig. 8. The trial demonstrated that 1,2-indandione was comparable to DFO in terms of the numbers of fingerprints developed on bank checks and in the number of positive cases.

To investigate the relative effectiveness of 1,2-indandione, further experiments were conducted using a range of pseudo-operational material such as used envelopes and train tickets (Table 1).

After treating 114 articles (envelopes, newspapers, train tickets, etc.) with each formulation, it is apparent that there are few practical differences in the effectiveness of 1,2-indandione and DFO in CFC113 in developing latent fingerprints.

The intensities of fingerprints developed with 1,2-indandione and DFO were also measured (Fig. 9). This shows that DFO produces fingerprints that initially exhibit stronger fluorescence than 1,2-indandione developed fingerprints. After storage in the dark for a week, the fluorescence intensity of the indandione-developed fingerprints had increased to almost equal that of the DFO-developed

fingerprints. Storing for longer periods did not result in any further improvement in fluorescence intensity.

Conclusion

1,2-Indandione may be a promising reagent for the development of latent fingerprints on paper surfaces despite the fact that the reagent produces fingerprints exhibiting less intense fluorescence than DFO. The development conditions are 100°C for 10 min at ambient humidity. After fingerprints have been developed, they should ideally be recorded immediately using fluorescence photography or digital imaging. If there is any delay, the articles should be stored in complete darkness until they can be examined under fluorescent conditions.

Although later trials[3] have demonstrated that there are other more effective methods for the development of latent fingerprints on porous surfaces, the work presented in this paper constitutes a useful starting point for evaluating formulations of other 1,2-indandione analogues for the detection of latent fingerprints.

Acknowledgments

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[3] Since completing the initial evaluation described in this paper, a large operational-scale operational trial comparing 1,2-indanedione to several formulations of 1,8-diazafluorenone (DFO) has been completed. These results have been published separately (10).

References

1. Ramotowski R, Cantu AA, Jouillé MM, Petrovskaia O. 1,2-Indanediones: a preliminary evaluation of a new class of amino acid visualising reagents. Fingerprint Whorld 1997;23(90):131–40.
2. Hauze DB, Petrovskaia O, Taylor B, Jouillé MM, Ramotowski R, Cantu AA. 1,2-indanediones: new reagents for visualising the amino acid components of latent prints. J Forensic Sci 1998;43(4):744–7.
3. Almog J, Springer E, Wiesner S, Frank A, Khodzhaev O, Lidor R, et al. Latent fingerprint visualisation by 1,2-indanedione and related compounds: preliminary results. J Forensic Sci 1999;44(1):114–8.
4. Hardwick S, Kent T, Sears V, Winfield P. Improvements to the formulation of DFO and the effects of heat on the reaction with latent fingerprints. Fingerprint Whorld 1993;19(73):65–9.
5. Kent T, editor. Manual of fingerprint development techniques. 2nd ed. Sandridge: Home Office, 1998.
6. Petrovskaia O. Design and synthesis of chromogenic and fluorogenic reagents for amino acid detection [dissertation]. Philadelphia, PA: University of Pennsylvania, 1999.
7. Hewlett DF, Sears VG, Suzuki S. Replacements for CFC113 in the ninhydrin process: part 2. J Forensic Ident 1997;47(3):300–6.
8. Grigg R, Malone JF, Mongkolaussavaratana T, Thianpatanagul S. X=Y-ZH compounds as potential 1,3-dipoles. Part 23: Mechanisms of the reaction of ninhydrin and phenalene trione with α-amino acids. X-ray crystal structure of protonated Ruhemann's Purple, a stable azomethine ylide. Tetrahedron 1989;45(12):3849–62.
9. Grigg R, Mongkolaussavaratana T, Pounds CA, Sivagnanam S. 1,8-diazafluorenone and related compounds. A new reagent for the detection of α-amino acids and latent fingerprints. Tetrahedron Letters 1990; 31(49):7215–8.
10. Merrick S, Gardner SJ, Sears VG, Hewlett DF. An operational trial of ozone-friendly DFO and 1,2-indandione formulations for latent fingerprint detection. J Forensic Ident 2002;52(5):595–605.

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