J Forensic Sci, March 2008, Vol. 53, No. 2
doi: 10.1111/j.1556-4029.2008.00671.x
Available online at: www.blackwell-synergy.com

TECHNICAL NOTE

Joseph Almog,[1] Ph.D.; Asne Klein,[1] M.Sc.; Inbal Davidi,[1] M.Sc.; Yaron Cohen,[2] M.Sc.; Myriam Azoury,[2] M.Sc.; and Michal Levin-Elad,[2] Ph.D.

Dual Fingerprint Reagents with Enhanced Sensitivity: 5-Methoxy- and 5-Methylthioninhydrin

ABSTRACT: "Dual fingerprint reagents" are chemical formulations which produce with latent fingerprints in a single step, impressions that are both colored and fluorescent. Pre-mixed solutions of the two commercially available ninhydrin analogues, 5-methoxyninhydrin (MN) and 5-methylthioninhydrin (MTN) with zinc or cadmium salts, are true dual reagents. They are much more sensitive than the parent dual reagent, ninhydrin/$ZnCl_2$. The main advantage of the new formulations is that they can be used at room temperature, with no need to cool the sample to liquid nitrogen temperature. At 0.05% concentration, which is 10-fold lower than the common ninhydrin working solution, MTN/$ZnCl_2$ is as sensitive as DFO in the fluorescence mode and considerably more sensitive in the color mode. MTN is also slightly cheaper than DFO.

KEYWORDS: forensic science, fingerprint reagents, dual fingerprint reagents, ninhydrin, 5-methoxyninhydrin, 5-methylthioninhydrin, DFO, genipin, cadmium chloride, zinc chloride

Recently, we have found that mixtures of ninhydrin with zinc or cadmium salts react with latent fingerprints to produce impressions that are both colored and fluorescent. When the ninhydrin concentration in the formulation is reduced from the commonly used 0.5–0.05%, fingerprints can be visualized only in the fluorescence mode. While the combined solution of ninhydrin and metal salt offers clear advantages over ninhydrin alone, or ninhydrin followed by metal salts, the process still suffered from a major disadvantage: to observe the fluorescence it was necessary to cool the exhibits to liquid nitrogen temperature (1). The purpose of this study was to find more "true" dual reagents that, in a single reaction, produce with latent fingerprints, fluorescent impressions with sufficiently intense color to be observed by naked eye. We hoped to find such dual reagents that do not require liquid nitrogen temperature for fluorescence emission. In view of the abundance of information on the reaction of ninhydrin and its analogues with amino acids and with latent fingerprints (2,3), we expected that not only ninhydrin but also some of its analogues might become dual fingerprint reagents by mixing them with zinc or cadmium salts. Moreover, since some of the analogues have exhibited enhanced sensitivity over that of ninhydrin, particularly in the fluorescence mode (4–7), it was expected that some of those compounds may become superior dual reagents as well. The first candidates we tried were the simple, commercially available, mono-substituted ninhydrins: 5-methoxyninhydrin (MN) (Fig. 1*a*) and 5-methylthioninhydrin (MTN) (Fig. 1*b*). The former was mentioned for the first time by Lennard et al. (5) and was used with remarkable success in resolving a blackmailing case shortly afterwards (8). The latter was prepared independently and almost simultaneously by Heffner and Joullie (9), and by Almog et al. (10). Both compounds have shown marked advantages over ninhydrin, as fluorogenic fingerprint reagents (5,8–10). They did not become widely used because of the introduction of the very efficient one-stage fluorogenic reagent, DFO, by Pounds and Grigg, in 1990 (11,12). We started by exploring pre-mixed solutions of MN and MTN with zinc and cadmium salts and compared their reactivity with that of the two-step process, ninhydrin analogue reaction followed by secondary treatment with metal salt, as well as with that of DFO, the currently leading fluorogenic reagent.

Ethanolic solutions containing the two ninhydrin analogues with zinc or cadmium chlorides, were prepared at various concentrations and applied to latent fingerprints on paper, which were deposited in a "depletion sequence" (1). The results were compared with those obtained with DFO under the same conditions, in the color and fluorescence modes. Results were also compared with those obtained with the ninhydrin–zinc chloride dual reagent, on which we reported earlier (1). For a more accurate, semi-quantitative comparison, similar experiments were also carried out on amino acid stains of known concentrations.

Experimental

Materials and Methods

5-Methylthioninhydrin was purchased from BVDA, Holland. MN was purchased from Aldrich. Both ninhydrin analogues and metal salts were reacted with amino acid stains and with latent fingerprints on paper. Development was carried out either by the common two-stage process: ninhydrin analogues followed by metal salt (the common procedure) or by the one-stage process: pre-mixed solutions of ninhydrin analogue and metal salt. We examined color wavelength and intensity, fluorescence wavelength and intensity, solution stability, and stability of the developed impressions.

[1]Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel.
[2]Latent Fingerprint Laboratory, Division of Identification and Forensic Science (DIFS), Israel Police, National H.Q., Jerusalem 91906, Israel.

Received 31 Jan. 2007; and in revised form 8 Nov. 2007; accepted 18 Nov. 2007.

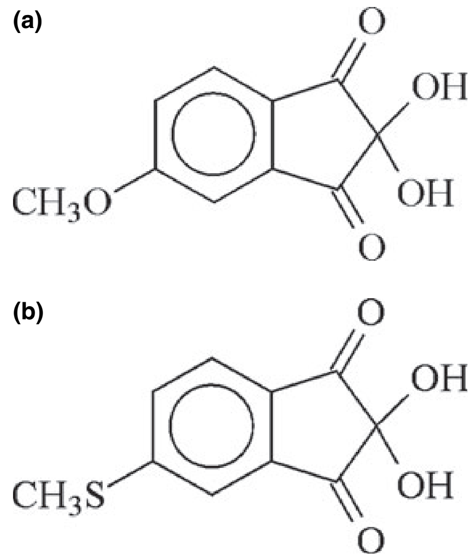

FIG. 1—*(a) 5-Methoxyninhydrin (MN). (b) 5-Methylthio-ninhydrin (MTN).*

Working Solutions

Initial working solutions contained 0.05% MN or MTN in ethyl alcohol containing also 1% acetic acid. The pre-mixed solution contained 0.2% $ZnCl_2$ or $CdCl_2$ in the same ninhydrin analogue solution. After the initial experiments in ethanolic solutions showed encouraging results, we also prepared non-ink-running solutions of MTN, in HFE 7100, according to the HOSDB Manual for ninhydrin (13). MTN working solutions contained 0.05% and 0.1% MTN in HFE 7100, the other ingredients being ethyl acetate (0.2%), acetic acid (0.5%), and ethyl alcohol (4.5%). Zinc chloride solution in HFE was prepared as suggested in the same manual. MTN "dual reagent" was prepared by adding 1 mL of the zinc chloride solution to 29 mL of the MTN solution. For comparison, DFO and ninhydrin solutions were also prepared following the HOSDB Manual (13).

Amino Acid Stains and "Depleted" Fingerprints

Two sets of reactions were carried out: with amino acid stains and with latent fingerprints. Drops of aqueous solutions of the amino acids alanine and glycine, at decreasing concentrations (from 5*EXP-3M to 5*EXP-5M) were pipetted on filter paper strips (each stain made of 5μL solution) and air dried. The paper strips bearing the stains were developed by pre-mixed solutions containing MTN and zinc chloride in HFE 7100, and by DFO.

Natural latent fingerprints of 10 individuals, varying in their "fingerprint donorship" from "good" to "poor," were collected on copier paper and brown wrapping paper in a depleted order. Each set of depleted prints was obtained by successive impressions of the same finger. A total of 36 sets of six depleted fingerprints were prepared by repeating the print deposition process. The latent prints were cut in halves along the middle, and each half was developed using the same above-mentioned protocols.

Development Conditions

In the two-stage process, the targets (amino acid stains or latent fingerprints) were dipped in the ninhydrin analogue solution, air dried, processed in a humidity oven under optimal working conditions (80°C and 65% RH) (13), and photographed. They were then dipped in a metal salt solution (0.2% in ethyl alcohol). In the one-stage process ("dual reagent"), the targets were developed by the pre-mixed solution and processed as before. The resulting colors and fluorescence were compared visually.

Amino acid stains, and latent fingerprints treated with ninhydrin analogues and with DFO, were air dried and processed in a humidity oven at 80°C and 65% RH (MTN and MN), and in a 100°C oven (DFO).

Light Absorption and Fluorescence Observation

Light absorption and emission wavelengths (λ_{max}) were measured in ethanolic solutions containing MTN, amino acids (alanine or glycine), and zinc chloride. The absorbance and fluorescence curves of the solutions were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Varian Australia Pty Ltd., 679 Springvale Rd., St. Helens, Australia), immediately after the reaction was completed.

Fluorescence study of fingerprints and amino acid stains processed by the MTN-metal salts formulations was carried out at room temperature, illuminating at 505 and 530 nm, using Polilight® PL 500 lamp (Rofin Australia Pty. Ltd., 6/42-44 Garden Boulevard, Dingley, Victoria, Australia) and observing through an orange filter (cutoff at 529 nm).

Results and Discussion

Mixtures of both ninhydrin analogues, MN and MTN, with zinc or cadmium salts were found to be true dual fingerprint reagents. By treating latent fingerprints with these formulations, colored impressions were obtained (MN: orange with zinc, pink with cadmium; MTN: pink with zinc, red with cadmium), showing remarkable fluorescence at room temperature. Lowering the temperature to −200°C was not necessary–it only marginally increased the fluorescence. Like in the previous study with unsubstituted ninhydrin (1), it seems that the metal ions, which are pre-mixed with the ninhydrin analogues, do not interfere with the reaction with amino acids and they react only with the initially formed Ruhemann's purple. The metal ions could potentially prevent the formation of Ruhemann's purple by reacting with the many intermediates that are formed in the ninhydrin reaction with amino acids, but this is not the case. It is, therefore, assumed that also other ninhydrin analogues can become "dual fingerprint reagents" by mixing them with salts of zinc or cadmium.

Formulations containing zinc or cadmium showed similar sensitivity, so we decided to carry on with zinc, since cadmium and its compounds are considered toxic and constitute an environmental hazard (14,15). MTN formulations gave slightly better results than MN formulations and hence, at a certain point, we focused on MTN/$ZnCl_2$ only. Spectrophotometric measurements showed that virtually identical results are obtained when the process is carried out in either one or two stages. The absorption curves of the MTN Ruhemann's purple and its metal complexes are identical with those reported previously for the two-stage process (10) (Fig. 2).

Both reagents were far more sensitive than the ninhydrin/metal salt, in the color as well as in the fluorescence modes. At a concentration of 0.05%, 10-fold lower than the normal ninhydrin concentration, MTN/$ZnCl_2$ developed pink fingermarks that could be readily visualized in white light, or as fluorescent images. Fingerprints developed with MTN/$ZnCl_2$ were comparable in

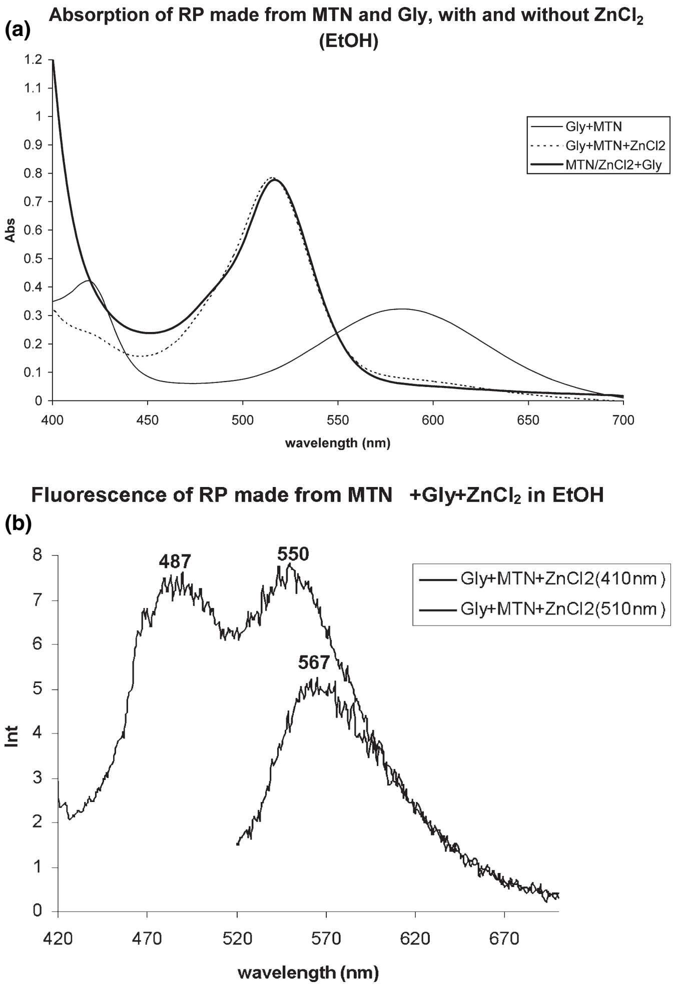

FIG. 2—*Absorption and fluorescence spectra of the product between MTN, glycine (Gly), and zinc chloride (EtOH solution). (a) Light absorption: zinc chloride was added either after the addition of the amino acid (two-step process) or before ("dual reagent"), and (b) fluorescence curves (excitation at 410 and 510 nm).*

fluorescence intensity to those developed with DFO; both fluoresced intensely under Polilight® illumination. The color of the former, however, was considerably stronger than that developed by DFO (Fig. 3). Interesting results were obtained on brown wrapping paper as a substrate. While neither of the dual reagents described above nor DFO developed any meaningful mark, genipin, the first described true dual reagent (16,17), produced good, identifiable fluorescent marks on this substrate (illumination at 590 nm and observation through a cut-off filter above 620 nm) (Fig. 4). This result is in accordance with earlier findings, that genipin (because of the longer excitation and emission wavelengths of its developed fingerprints, compared with ninhydrin analogues or DFO), is probably the only reagent that consistently develops good fingermarks on brown wrapping paper (17). Working solutions of MTN dual reagent, in dark bottles, were stable for at least 6 months at room temperature.

The metal ions, apparently, have a stabilizing effect on the developed prints: kept in a drawer at room temperature, the impressions remained unchanged after 6 months. This observation was not unexpected, as the original idea of adding metal salts to Ruhemann's purple had been suggested for stabilizing its color over time (18,19).

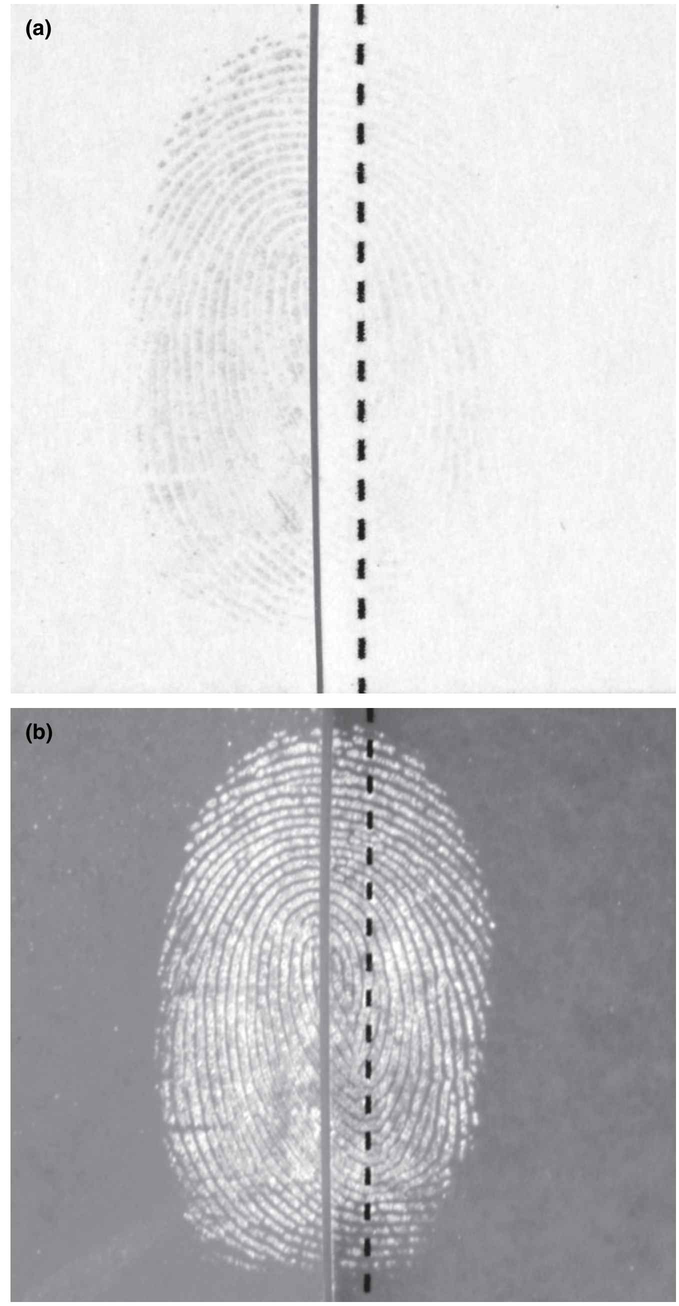

FIG. 3—*Comparison between DFO (right half) and MTN (left half) dual reagent. (a) Color mode (white light), and (b) fluorescence (excitation at 505 nm).*

The results obtained so far with dual reagents of the ninhydrin family and DFO can be summarized as follows:

$$\text{Color : MTN/ZnCl}_2 \approx \text{MN/ZnCl}_2 > \text{ninhydrin/ZnCl}_2 > \text{DFO}$$

Fluorescence (room temperature):

$$\text{MTN/ZnCl}_2 = \text{DFO} \geq \text{MN/ZnCl}_2 >> \text{ninhydrin/ZnCl}_2$$

These findings corroborate the earlier observations that were based on the two-stage process (10). The dual reagent, however, is advantageous since it is effective in one step, and lower concentrations of the reagents are used. In our opinion, the MTN/$ZnCl_2$

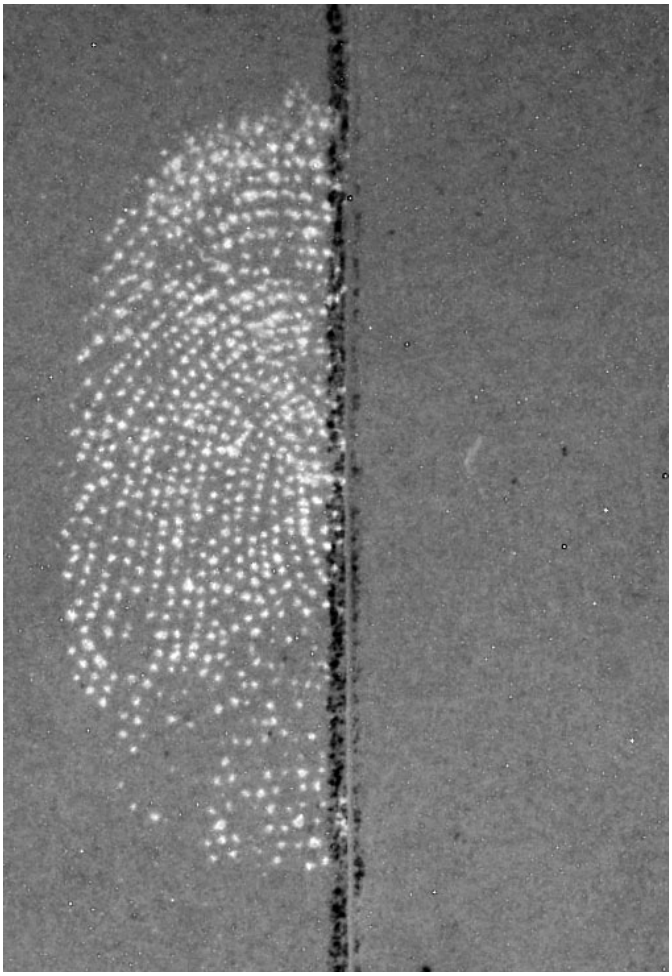

FIG. 4—*Comparison between genipin (left half) and MTN (right half) on brown wrapping paper (excitation at 590 nm).*

formulation has high potential of becoming a practical fingerprint reagent for paper surfaces. Cost comparison also supports this assumption; since all reagents are applied at 0.05% level, the cost of the working solution should be proportional to the cost of the reagents. Following are prices in US$ paid by the Israel Police Fingerprint Laboratory for 1 gram of each reagent (suppliers' names are in parentheses):

Ninhydrin 0.95 (Fluka); DFO 30 (Casali Institute, the Hebrew University, Jerusalem); MTN 24 (BVDA, Holland); MN 432 (Aldrich) (prices may vary with the amounts purchased and between different suppliers).

Just before completion of this manuscript, Stoilovic, Lennard, Wallace-Kunkel and Roux reported that 1,2-indanedione-zinc chloride is also a true dual fingerprint reagent (although they have not used this term), producing with latent fingerprints visible marks that fluoresce intensively upon illumination at 505 nm. (20).

Conclusions

1. Pre-mixed solutions of 5-methylthioninhydrin (MTN) and 5-methoxyninhydrin (MN) with zinc or cadmium salts are true dual fingerprint reagents: they develop latent fingerprints on paper as colored impressions that also fluoresce in the visible domain.
2. The new formulations are superior to the previously reported dual reagent, Nin/$ZnCl_2$ in all technical aspects. Their major advantages are:
   a. Fluorescence detection at room temperature.
   b. More intense color and much more intense fluorescence are obtained.
   c. Lower concentrations of the ninhydrin analogues are required.
3. MTN dual reagents are comparable to DFO in the fluorescence mode. They are superior to DFO in producing colored fingermarks.
4. On the basis of the preliminary results it seems that a pre-mixed solution of MTN with zinc chloride is a very efficient fingerprint reagent. A comprehensive study of the potential and advantages of MTN/metal salt reagent is underway.
5. Genipin continues to be the dual reagent of choice for fingermarks on brown wrapping paper.

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Additional information and reprint requests:
Joseph Almog, Ph.D.
Professor of Forensic Chemistry
Casali Institute of Applied Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904
Israel
E-mail: almog@vms.huji.ac.il