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Fingerprint Reagents with Dual Action: Color and Fluorescence

ABSTRACT: We define "dual fingerprint reagents" as chemical formulations that produce with latent fingerprints in one stage impressions that are both colored and fluorescent. Solutions containing ninhydrin and group IIb metal salts appear to be true dual reagents. Application of these formulations to latent fingerprints on paper is as efficient as the two-step process beginning with ninhydrin and followed by treatment with metal salt. In the color mode, fingerprint detectability with the two ninhydrin–metal salt reagents (one with zinc chloride and the other with cadmium chloride) is comparable with that of ninhydrin itself, in spite of the difference in color. The sensitivity is significantly higher in the fluorescence mode. To view the latent impressions the exhibits are treated with ninhydrin–metal salt reagents and observed under white light illumination and under fluorescence conditions. Cooling to liquid nitrogen temperature enhances the fluorescence considerably. In the shorter wavelength domain, ninhydrin–metal salt reagents exhibit higher sensitivity than the recently reported dual reagent, genipin. The latter is advantageous, however, in the longer wavelength domain, on paper items with strong self-fluorescence, such as brown wrapping paper or paper printed with fluorescent ink. Upon reduction of the ninhydrin concentration 10-fold, ninhydrin–metal salt formulations become purely fluorogenic reagents; no color is noticed but the fluorescence is as intense as with concentrated solutions. Working at lower concentrations is an advantage from ecological and economical viewpoints.

KEYWORDS: forensic science, fingerprint reagent, amino acid reagent, ninhydrin, fluorogenic, colorimetric, cadmium chloride, zinc chloride

It was noticed long ago that analytical examinations that are based on the formation of fluorescent products from nonfluorescing reactants (fluorogenic reactions) exhibit a much higher sensitivity—up to four orders of magnitude—than reactions that are based on color formation (1). Modern research into fluorescence techniques for latent fingerprint visualization started perhaps in 1976 with H. Ohki's report on the potential of fluorescamine as a fluorogenic reagent (2). Several other fluorogenic fingerprint reagents have been explored since (3–5). Menzel and Herod in 1982 found that treatment of weak ninhydrin-developed fingerprints with zinc chloride changed the color of the prints from purple to orange, and the prints also exhibited visible fluorescence upon excitation with an argon-ion laser (6). This two-stage process has been thoroughly studied by Lennard and coworkers; other metal salts have been tested (7,8); the structures of the fluorescent products with zinc and cadmium salts have been determined (9,10) (Fig. 1); and some ninhydrin analogues have shown even stronger fluorescence than the parent compound under similar conditions (11–15). All these amino acid reagents produced colored fingerprint impressions in the first stage that turned fluorescent by the secondary treatment with metal salt. We define "dual fingerprint reagents" as chemical formulations that produce with latent fingerprints in one stage impressions that are both colored and fluorescent. DFO, undoubtedly the most significant fluorogenic fingerprint reagent to date, can also be considered a dual reagent, but the pink color it produces with latent prints is very faint and insufficient for the detection of most latent prints. DFO impressions are actually detected only by their fluorescence (16). Similar behavior is exhibited by a more recent reagent, 1,2-indanedione, which also produces with latent fingerprints colorless to faint pink impressions that fluoresce in the visible domain (17,18). Genipin, a true dual reagent that was recently reported by this group, develops latent fingerprints on paper as dark blue impressions that fluoresce above 600 nm (19,20).

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 the naked eye. We started by exploring premixed solutions of ninhydrin and group IIb (now named Group 12) metal salts and compared their reactivity with that of the two-step process and with that of genipin.

Materials and Methods

Ninhydrin and metal salts were reacted with amino acid stains and with latent fingerprints on paper. The development was performed in a two-stage process, ninhydrin followed by metal salt (the common procedure), and in one stage (premixed solutions of ninhydrin and metal salt). We examined color wavelength and intensity, fluorescence wavelength and intensity, solution stability, and stability of the developed impressions.

Working Solutions

Ninhydrin working solution contained 0.5% ninhydrin in ethyl alcohol and 1% acetic acid. The premixed solution contained 0.2% $ZnCl_2$ or $CdCl_2$ in the same ninhydrin solution. Genipin solution for comparison was prepared according to the conditions suggested in a recent paper (0.17% in ethyl alcohol) (20).

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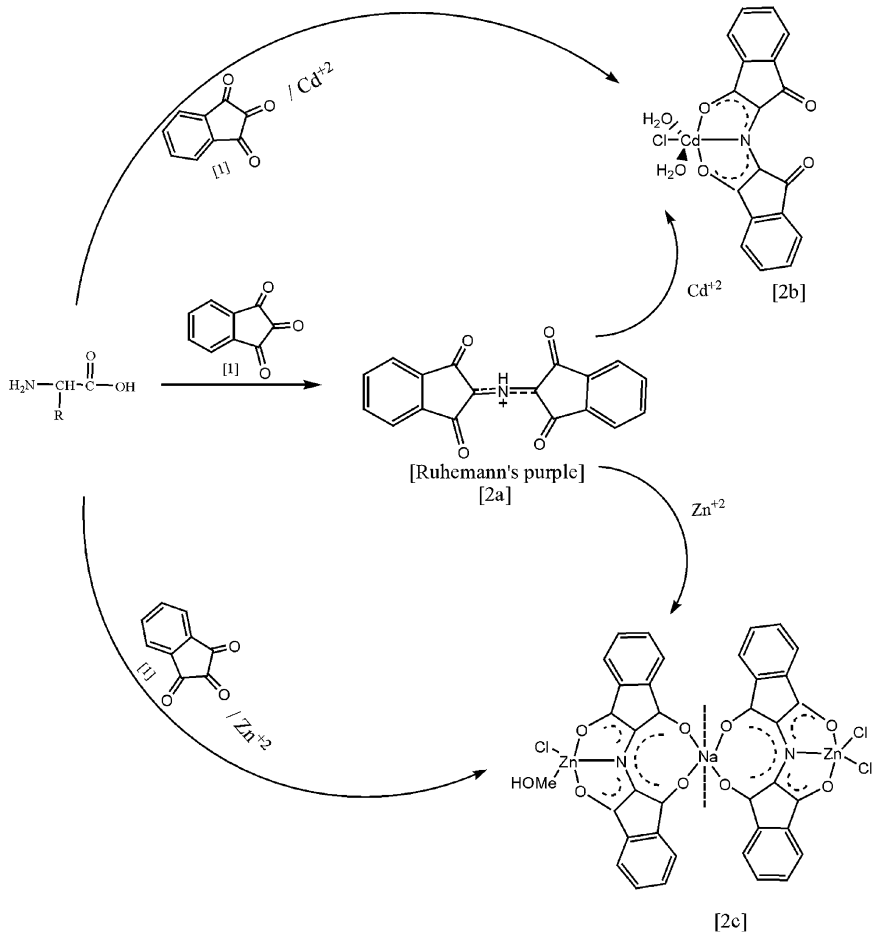

FIG. 1—*Course of the reaction between ninhydrin, amino acid, and metal salt to form fluorescent complexes in one or two stages.*

Amino Acid Stains and Depleted Fingerprints

Two sets of experiments were carried out: reaction with amino acid stains and with latent fingerprints. Drops of the amino acid threonine solutions in 1:1 ethanol:water at decreasing concentrations (from 0.05 to 0.0005 M) were pipetted on copier paper and filter paper strips (each stain made of 5 μL solution) and dried in air. The paper strips bearing the stains were developed by the following reagents: ninhydrin, ninhydrin followed by metal salts (two-stage process), premixed solution containing ninhydrin with metal salts (one-stage process), and genipin, until no noticeable reaction, color, or fluorescence could be observed.

Natural latent fingerprints of nine individuals of various levels of "fingerprint donorship" were collected on copier 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 ninhydrin solution, dried in air, processed in a humidity oven under the optimal working conditions (80°C and 65% RH) (21), and photographed. They were then sprayed with metal salt solution (0.2% in ethyl alcohol). In the one-stage process, the targets were developed by the premixed solution and processed as before. The resulting colors and fluorescence were compared visually.

Amino acid stains and latent fingerprints treated with ninhydrin and genipin were dried in air and processed in a humidity oven at 80°C and 65% RH.

Fluorescence Observation

Fluorescence study of the ninhydrin–metal salts experiments was performed by cooling the sample in liquid nitrogen, illuminating at 505 and 530 nm using a Polilight® PL 500 lamp (Rofin Australia Pty. Ltd., Dingley, Victoria, Australia) and observing through an orange filter (cutoff at 529 nm).

In the genipin experiments, targets at room temperature were illuminated at 590 nm and observed through a red filter (cutoff at 620 nm). A further series of experiments aimed at reducing the concentration of the ninhydrin in the working solution were conducted under similar conditions.

Solution Measurements

For solution measurements of the one-stage reaction, ninhydrin in ethanolic solution and threonine in ethanol:water (1:1) were mixed at a 2:1 molar ratio, metal salt was added to a final concentration of 0.2%, and the solution was heated to 80°C and cooled to room temperature. For the two-stage reaction, solid Ruhemann's purple was dissolved in ethanol and metal salt was added to a final concentration of 0.2%. The absorbance curves of the solutions

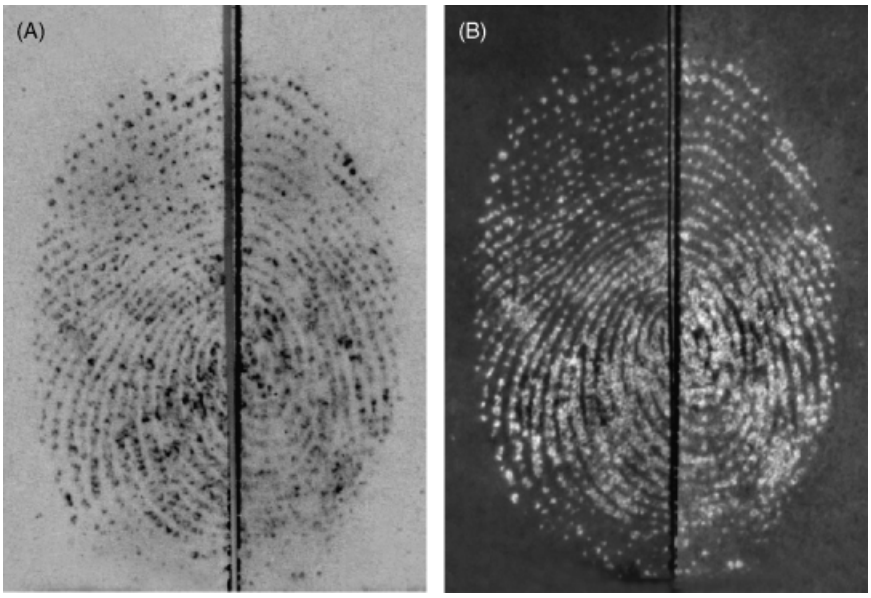

FIG. 2—*Half prints developed on a copier paper with ninhydrin and $ZnCl_2$, in the one-stage process (left side) and the two-stage process (right side). (A) Recorded under white light and (B) recorded under fluorescence conditions.*

were recorded with a Cary Eclipse Fluorescence Spectrophotometer (Varian Australia Pty. Ltd., St. Helens, Australia) immediately after the reaction was completed.

Results

Amino acid stains and latent fingerprints behaved similarly in all our experiments and the following observations apply to both of them.

1. In the one-stage process, latent fingerprints develop with a quality similar to the two-stage process in the color and fluorescence modes (Fig. 2).
2. In the color mode, the impressions that are formed by the addition of metal salt (one stage or two stages) are at least as clear as the impressions of Ruhemann's purple without the metallic addition. So, in spite of the color change from purple to orange (zinc) or red (cadmium), there is no decline in impression quality by adding metal salt. Orange prints darken when viewed with a blue filter or illuminated with blue light while red prints darken with blue–green viewing or illumination.
3. In the fluorescence mode, the impressions produced by ninhydrin–cadmium (the two versions) produce a stronger fluorescence than the ninhydrin–zinc combination (Fig. 3). In the lower wavelengths, below 550 nm, both ninhydrin–metal combinations produce stronger fluorescence than genipin, but genipin produces stronger fluorescence upon illumination above 590 nm, its optimal domain.

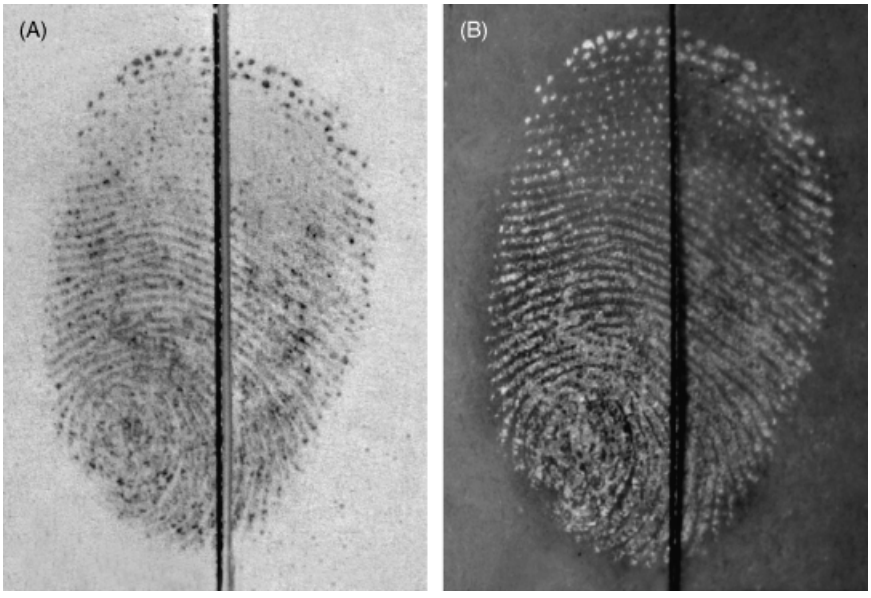

FIG. 3—*Half prints developed on a copier paper with ninhydrin–cadmium (left side) and ninhydrin–zinc (right side). (A) Recorded under white light and (B) recorded under fluorescence conditions.*

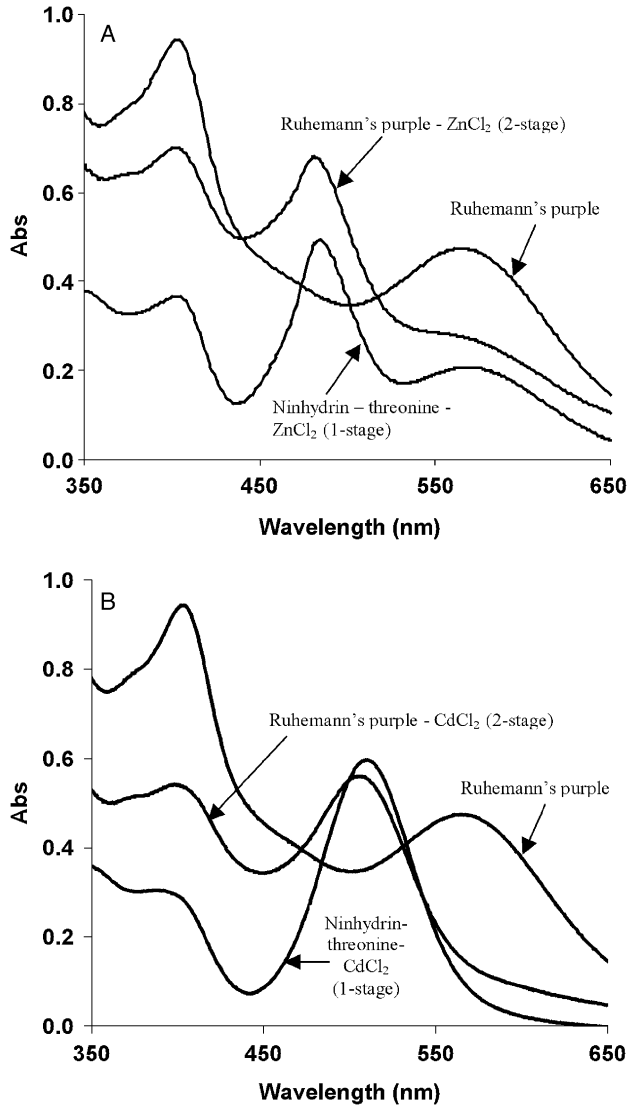

FIG. 4—*One-stage and two-stage reactions between ninhydrin, threonine, and metal salt; comparison of the absorption curves in solution. (A) Zinc complex and (B) cadmium complex.*

4. In solution, the products of the two processes exhibit very similar absorption curves (Fig. 4).
5. The impressions that are formed by ninhydrin–metal salt combination are stable for at least 1 year at room temperature.
6. In both processes it is possible to reduce the ninhydrin concentration 10-fold (to 0.05%) with no ill effect on the quality of the fluorescent images (although no color is observed; Fig. 5).

Discussion

The reaction of an ideal reagent is expected to produce with latent fingerprints impressions that are both colored and fluorescent. Color observation does not require specific instrumentation, and can be performed and observed in a moderately equipped laboratory or even in the field. While fluorescence imaging requires more equipment, the results generally demonstrate higher sensitivity. The use of ninhydrin, followed by treatment with metal salts is a two-step process and hence cannot be considered a true dual reagent. It produces distinct color—Ruhemann's purple—in the first stage, which is changed by the action of metal salt into

FIG. 5—*Print developed with ninhydrin (0.05%) and $ZnCl_2$ recorded under fluorescence conditions.*

orange (zinc) or red (cadmium). The colored complexes thus produced also fluoresce in the visible domain, and the fluorescence can be significantly enhanced by reducing the temperature (6–9). The assumption that premixed formulations of ninhydrin with metal salts will behave similarly and provide both effects, or any visible effect in one shot, is not obvious. 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.

Indications that such combinations might work appeared in the literature long ago. In 1967, Krauss in Germany observed that colors of the whole spectrum were formed upon the reaction of ninhydrin mixtures with various metal salts on amino acids and that they were more stable than plain Ruhemann's purple (22). Cadmium–ninhydrin mixtures have been recommended for following the decomposition of dairy products (23,24) and biochemical assays (25). A similar combination is routinely used for fingerprint visualization in at least one European country, Croatia, but only in the color mode (V. Salamunovic, personal communication). Metal–ninhydrin formulations have been compared with ninhydrin for fingerprint development, again, only in the color mode (26).

Although we have not fully characterized the products that are obtained by the one-stage process, based on the similarity of their spectrophotometric curves (Chart 1), we assume structures similar to the products obtained by the one-stage and two-stage processes. Thus, we assume structures [2b] and [2c], suggested earlier for the two-stage products with zinc and cadmium (9,10), to be also the structures of the complexes that are formed in the one-stage reactions. We assume a relatively slow formation of Ruhemann's purple [1a] first, in the reaction between ninhydrin and amino acids, followed by a fast reaction with the metallic salt to form the fluorescent complex [2b or 2c]. If the formation of Ruhemann's purple was quicker than the metallic complex formation, one would expect to observe the purple color forming, before it turns into the fluorescent complex, which is not the case.

The observation that 10-fold dilution of the ninhydrin solution provides good fluorescent impressions indicates that in fingerprint laboratories, ninhydrin can be used at much smaller amounts without compromising on the performance. As mentioned before, the lower concentration ninhydrin–zinc formulation is no longer a "dual" but purely a fluorogenic reagent. The potential advantages are economical, but also environmental. Indications that continuous use of ninhydrin might be hazardous have appeared in the literature lately (27,28). From a forensic point of view, using the dilute solution has an additional advantage: the fluorescent prints thus obtained are barely visible in white light and after the chemical treatment, the exhibits appear untouched.

Conclusions

1. Premixed solutions of ninhydrin with zinc or cadmium salts develop latent fingerprints on paper as colored impressions that also fluoresce in the visible domain.
2. The quality of the developed prints in both modes is not inferior in any way to the quality of prints that are developed by the two-stage process: ninhydrin followed by metal salt.
3. Cooling the exhibits to liquid nitrogen temperature significantly enhances the intensity of the prints' fluorescence.
4. Reducing the ninhydrin concentration in the formulation to 0.05% turns the solution to a purely fluorogenic fingerprint reagent. The quality of the developed prints is similar to that obtained with the more concentrated solution.

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