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Use of an Optimized 1,2-Indanedione Process for the Development of Latent Prints*

ABSTRACT: 1,2-Indanedione belongs to a class of compounds which have demonstrated great potential in the processing of latent prints, particularly in the area of fluorescence. However, variability in results achieved worldwide has precluded it from being used extensively. In order to isolate the cause of this variability, various components of the formulation were analyzed, including purity level of the indanedione, type of carrier solvent, and the use of $ZnCl_2$ both as a secondary application and incorporated into the reagent. Using a resultant optimized formulation (Ind-Zn), performance comparisons were then made in the areas of visible color development, fluorescence, and degree of substrate staining with those of 1,8-diazafluoren-9-one (DFO) for both fresh and aged prints. Moisture content of the paper substrates on which the prints had been deposited was measured and a correlation found with percentage ambient relative humidity (% RH). Determination of visible color and fluorescence as it corresponded to percentage moisture content allowed for defining critical threshold levels necessary for achieving optimal results. Correlating these values with % RH then allowed for the development of standard operating procedures for obtaining best possible print development. Through this work, it was determined that a 7.4% v/v formulation of Ind-Zn having petroleum ether as a carrier solvent yielded the most optimal results when processing methods optimized for % RH in the laboratory were utilized. Both initial color development and fluorescence were superior to that of DFO; prints developed with Ind-Zn were a minimum of 6.5 units dE* darker and more red than with DFO for all substrates tested. Processing with Ind-Zn on the majority of the substrates examined yielded fluorescence intensity values approximately four times greater than with DFO.

KEYWORDS: forensic science, fingerprints, indanedione, DFO, relative humidity, moisture, fluorescence, visible color

Two of the most sensitive and widely used reagents for visualizing latent prints on paper are ninhydrin (1) and 1,8-diazafluoren-9-one (DFO) (2). The former compound is considered the standard for visible color detection of latent prints, while the latter is the standard for fluorescence detection. Ninhydrin offers many advantages as a reagent, including low cost and good solubility in a range of solvents. Its major drawback is that its reaction product with amino acids, Ruhemann's purple, is not fluorescent, which could limit its ability to aid in the detection of weak prints. DFO produces a weakly colored reaction product with latent print residue that has the advantage of exhibiting strong fluorescence without additional treatment. However, it has several disadvantages, including high cost and poor solubility.

The quest for improving the color and fluorescence obtained from the reaction of ninhydrin and amino acid residue in a latent print began with the work of Almog, et al. in the early 1980s (3). This effort focused on improving the fluorescence intensity of ninhydrin compounds without the need for liquid nitrogen or posttreatment with zinc salt solutions. In the mid-1980s, the U.S. Secret Service began its research program to investigate these new ninhydrin analogs. At that time, a partnership was established with Dr. Madeleine Joullie's research group at the University of Pennsylvania's (UPENN) Department of Chemistry. Over the next

10 years, the Research Section of the Forensic Services Division evaluated nearly 100 compounds synthesized by that group (4). Although some of these compounds showed promise for latent print visualization, their commercial viability was limited by cost.

Although the synthesis of 1,2-indanediones (Ind) had been published before (5,6), these compounds had never before been tested on latent prints. In December of 1995, the first novel Ind compound was received for evaluation from the UPENN. Application of this compound, 6-methylthio-1,2-indanedione, to latent prints produced pale orange color ridge detail that fluoresced moderately. The fluorescence of this new compound was comparable to the best ninhydrin analogs. This fluorescence was significantly enhanced by the subsequent application of a zinc nitrate solution. In early September 1996, the parent compound, Ind, was submitted for evaluation. Application of this compound to both amino acid spots and latent prints on paper produced pale pink initial color ridge detail with moderate fluorescence. Once again, the subsequent treatment of these spots and prints with zinc nitrate resulted in not only enhancement of fluorescence, but also visible color. Given its structural simplicity and its relatively easy synthesis, Ind became one of the most commercially viable of all of the ninhydrin analogs produced up to that time.

Research began to focus on the optimization of the Ind reagent. Initial studies reported that the application of zinc salt solutions significantly enhanced the intensity of the fluorescent reaction product, making it comparable and in some cases better than DFO (7–9). Others reported that the application of zinc chloride had little or no effect on the fluorescence intensity (10,11). The fluorescence of some of the compounds evaluated was found to be superior to that of DFO even without subsequent zinc salt treatment. Other studies found that the performance of DFO was superior to Ind (12–14). Another publication reported that when deciding with which reagent to process porous items (ninhydrin or 1,2-indanedione), the

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latter would be the primary choice (15). Another recent publication reported that sequencing ninhydrin after processing with 1,2-indandeione failed to produce a statistically significant number of additional prints (16). This result raised the possibility of eliminating both ninhydrin and DFO and using Ind exclusively in that laboratory.

The concept of mixing together the zinc salt solution and the Ind reagent had been considered for years, but results indicated few advantages and the disadvantage of destabilizing the combined solution (Bruce Taylor, University of Pennsylvania Department of Chemistry, Philadelphia, PA, personal communication, 1998). The use of a two-step process (with the second step being the zinc salt solution) was found to be too laborious and not practical for high volume casework.

However, a recent publication reported on the successful incorporation of a zinc chloride solution into the Ind reagent. Despite the presence of a small amount of ethanol from the zinc chloride solution, the resulting reagent appeared to be stable and produced comparable color and superior fluorescence when reacted with latent print residue (17). It has been observed that the presence of alcohol solvents in Ind reagents produces hemiketals, which diminish the reactivity of the reagent with amino acids (11,18). Interestingly, the opposite is true with DFO, the reactivity of which increases with the presence of hemiketals in the reagent solution (19).

Upon learning about this new technique in June of 2006, the U.S. Secret Service Forensic Services Division decided to attempt to reproduce and validate the results. The key focus of this effort was to be the investigation and optimization of the development conditions for the Ind-zinc reagent (Ind-Zn). Conditions such as ambient laboratory humidity, moisture content of the paper substrates, temperature, and zinc chloride solution concentration were among the variables to be tested. The ultimate objective of this study was to determine whether or not to incorporate Ind-zinc into our processing procedures for porous items of evidence.

Methods

All comparisons throughout the experiments were conducted using three substrates: Substrate A was a coated business paper from International Paper, Hammermill Color Copy, 28 lb., 105 g/m^2, 98 brightness; Substrate B was a canary yellow legal paper from Ampad, Economy Series, $8.5 \times 14''$, wide-ruled; and Substrate C was a standard copy paper, Xerox Business 4200, 20 lb., 75 g/m^2, 92 brightness. Nine total donors were used for the experiments, comprised of four males and five females. Donors deposited fingerprints on strips of each substrate, two inches in width. Each strip was given a unique identifier and cut in half, with each half being labeled with the procedure to which it would be subjected. For comparison purposes and in order to preclude the possibility of conformational or pressure bias, for each donor, the side of the strip on which a particular procedure was being tested was alternated between samples. After being dipped into the applicable reagent, 10 min were allotted for the drying of specimens.

Two solutions of DFO were first prepared using two different sources of DFO. The first source was Lumichem, lot B7-99 of uncertified purity, while the second was that from Crime Sciences, Inc., certified to be >99% pure. Comparisons were made between these two formulations and the superior was later used for all evaluations with the optimized Ind-Zn solution. The formula for both of these DFO solutions was identical, 0.05% w/v, with the exception of the source of the compound, and involved first dissolving 0.5 g of DFO powder in 100 mL of methanol. Subsequently, 100 mL of ethyl acetate were added to the solution, followed by 20 mL of glacial acetic acid and 780 mL of petroleum ether (PE) for a total volume of 1000 mL. In all experiments, DFO was processed using a Fisher Isotemp 500 laboratory oven with a temperature setting of 100°C and a dwell time of 20 min.

Two different sources of Ind were utilized in order to determine the effect of purity level upon performance of the reagent. The first source was The Casali Institute of Applied Chemistry from which the Ind was of uncertified purity, while the second was that from Crime Sciences, Inc., certified to be >99% pure. In order to prepare an optimized Ind formulation for the development of latent prints, solutions were first prepared in which 1.0 g of Ind was added to 30 mL of dichloromethane. Subsequently, 60 mL of ethyl acetate was added to the solution and stirred, followed by 10 mL of glacial acetic acid. Finally, 900 mL of HFE-7100 was added in order to bring the total volume to 1000 mL. Testing was then performed with these solutions in comparison with others in which the carrier solvent was replaced 1:1 with PE.

To assess the impact of using zinc as a secondary application to the Ind optimized formula, a solution was prepared containing 0.4 g of $ZnCl_2$ dissolved in 10 mL of absolute ethanol, followed by the addition of 1 mL of ethyl acetate, and finally 190 mL of HFE-7100. Testing was performed, as well, in which the carrier solvent was replaced 1:1 with PE. For studies involving the incorporation of zinc into the optimized Ind formula (Ind-Zn), different levels of zinc chloride solution were added, resulting in a comparison among values of 2.0% v/v (2 mL of $ZnCl_2$ per 100 mL of Ind), 3.9% v/v (4 mL of $ZnCl_2$ per 100 mL of Ind), 7.4% v/v (8 mL of $ZnCl_2$ per 100 mL of Ind), 10.7% v/v (12 mL of $ZnCl_2$ per 100 mL of Ind), and 13.0% v/v (15 mL of $ZnCl_2$ per 100 mL of Ind) of the total combined solution.

For the determination of moisture content of the substrates, a Strandberg Engineering M-400 portable moisture meter was used, for which calibration was verified before each use. All percentage relative humidity (% RH) measurements in the laboratory were recorded using a Lufft Certified Durotherm hygrometer. For each % RH value encountered in the laboratory, fingerprint strips, conditioned in this environment and comprised of both cluster prints (index, middle, and ring fingers taken simultaneously) and depletion series (a series of five impressions of an individual finger made in rapid sequence), were measured using the M-400 and the percentage moisture content recorded for each. These samples were then cut in half, with each half being dipped in the same reagent (Ind-Zn, 7.4% v/v). After allowing 10 min for drying, each half was then measured using the M-400, and the resultant percentage moisture content recorded. Subsequently, each half was subjected to one of two processing methods, the laboratory oven method or the humidity chamber method. For oven processing, a Fisher Isotemp 500 series laboratory model was utilized at 100°C with a dwell time of 15 min. For humidity chamber processing, a Sanyo Gallenkamp, model FDC060XHX.E, was used, allowing for user-definable temperature and % RH setpoints; for all experiments, levels of 65% RH and 80°C were utilized.

For comparisons of initial color development and fluorescence, samples were cut into halves, with each half being subjected to processing with either DFO or Ind-Zn, and then subsequently examined. Visual assessment was conducted with illumination of samples under either a cool white fluorescent light source or an alternate light source, a SPEX Crimescope CS-16-400. Excitation energy for this instrument for substrates A and C was provided with a setting of 515 nm, while observation was with orange goggles provided by SPEX. For substrate B, excitation energy was provided with a setting of 555 nm, while observation was with red

goggles provided by SPEX. For quantification of visible color development when comparing the performance of formulas, a HunterLab UltraScan Pro spectrocolorimeter having CIE d/8 sphere geometry was used, with all measurements being taken in reflectance mode using CIE illuminant D65, the 10 degree standard observer field of view, and a 4 mm area view. Additionally, the degree of substrate staining that resulted from use of DFO as compared to the optimized Ind-Zn formulation was quantified using this instrument. Measurements were made of samples of the blank substrate and averaged; the result was assigned as the standard to which the processed samples would be compared. After processing with each of the two reagents, measurements were made of the substrate component of both the DFO and Ind-Zn samples. Results were averaged and reported as a value for each reagent. In order to assess the effect of age of prints on reagent performance, latent prints analyzed ranged in freshness from the same day as evaluation to 11 years of age.

For the measurement of fluorescence when comparing the finalized Ind-Zn formula with DFO, a customized ChemImage Hyperspectral Contrast Imaging System was used. Excitation light for the system was provided by a SPEX Crimescope CS-16-400 Alternate Light Source (ALS) with a setting of 515 nm for substrates A and C and 555 nm for substrate B; the Liquid Crystal Tunable Filters were set to 560 and 640 nm respectively.

In order to ascertain how well the optimized reagent would work with naturally handled specimens, 58 envelopes were evaluated that had been mailed in the fall of 2003. Each envelope constituted two examinations in that both the front and back of each were evaluated.

The samples were first processed using DFO and then photographed, front and back, using a Foster-Freeman DCS-3 system under green light (500–550 nm) with an OG550 barrier filter. The specimens were then processed with Ind-Zn and again photographed using the DCS-3 under the same conditions. The order of processing was subsequently reversed for 20 envelopes, 40 total examinations, such that Ind-Zn was used first, followed by DFO. Again, all specimens were photographed using the DCS-3 with the aforementioned illumination and observation conditions.

Results and Discussion

Purity of Reagents

In comparing results achieved using the two different sources of DFO, that from Lumichem proved superior in both visible color development and fluorescence to that procured from Crime Sciences, Inc. Therefore, DFO powder obtained from Lumichem was utilized in all experiments for comparisons with the optimized Ind-Zn formulation. In comparing results achieved using the two different sources of Ind, performance was found to be equivalent both for visible color development and fluorescence. It was later determined that Crime Sciences, Inc. had actually obtained its Ind directly from The Casali Institute of Applied Chemistry, and therefore, the two were essentially the same. In that there was a greater supply of the chemical from The Casali Institute available at our laboratory, this Ind was utilized for all experiments conducted.

Petroleum Ether as a Carrier Solvent

Formulations in which Ind had been used in the past included HFE-7100 as the carrier solvent. However, the cost per liter of HFE-7100 is approximately 18.6 times greater than that of PE, based upon the pricing available to us. Because of this cost advantage, as well as the fact that it is already utilized in other reagents at the United States Secret Service, a formula containing PE was deemed to be ideal. Advantages realized with the use of HFE-7100 are decreased flammability, as well as a diminished likelihood of running of inks; however, to this agency, no improvement in performance was noted and the benefits afforded by the use of PE for fingerprint development outweighed these.

In comparisons of Ind having HFE-7100 as the carrier solvent versus PE, in all cases, the visible print developed was darker with solutions containing PE. The strength of fluorescence corresponded directly with these visual observations, thus PE was selected as the carrier solvent to be used in all comparisons of DFO with Ind. Findings from an earlier study had indicated that the use of HFE-7100 yielded better results than did PE (16); however, the opposite held true in our evaluation.

Addition of Zinc

The addition of Zn^{2+} to indanedione processing, be it as a post-treatment application or integrated within the solution itself, has proven to enhance the fluorescence results obtained with the reagent. It has been postulated that this may result from Zn^{2+} serving as a Lewis Acid, thereby accelerating the reaction. Additionally, stabilization of the fluorescent dipole results from addition of this metal, increasing the longevity of the print once developed (Dr. Bruce Taylor, University of Pennsylvania Department of Chemistry, Philadelphia, PA, personal communication, 1998). Secondary application of $ZnCl_2$ to a 0.1% w/v Ind solution results in very chromatic, pink visible color development and strong fluorescence. However, integration of $ZnCl_2$ into the Ind solution is desirable in that it decreases processing time and complexity. Therefore, various levels of $ZnCl_2$ were evaluated to determine if any afforded the same strength of color and fluorescence as did the posttreatment process. Levels of 2.0, 3.9, 7.4, 10.7, and 13.0% v/v were added to 0.1% w/v Ind and this combined formulation (Ind-Zn) used in the processing of prints. Comparisons were then made among Ind, Ind with a secondary application of $ZnCl_2$, and the aforementioned combined formulations. Proving to be consistently comparable in both initial color development and fluorescence to the zinc post-treatment was the 7.4% $ZnCl_2$ (Fig. 1). A decrease in performance was realized when this concentration was increased to the 10.7% and 13.0% levels. Therefore, for the subsequent comparisons with
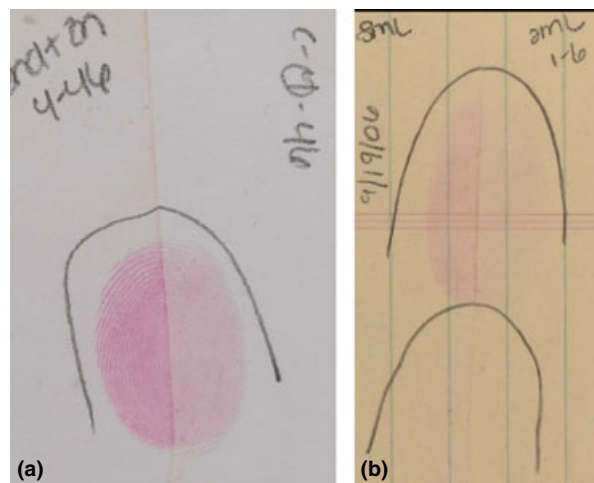

FIG. 1—(a) Comparison of 7.4% Ind-Zn solution with 2.0% Ind-Zn on Substrate C and (b) 7.4% versus 2.0% on Substrate B.

DFO, the 7.4% formulation (8 mL of $ZnCl_2$ per 100 mL of Ind) was utilized. This differed from the findings of Stoilovic et al. in which a secondary application of $ZnCl_2$ produced results that were actually inferior to a combined Ind-Zn formulation containing approximately 2.0% v/v (17).

Of concern with these combined formulations is shelf-life; it is theorized that self-condensation can be catalyzed by the incorporation of zinc, thereby resulting in a decreased half-life for the working solution (Bruce Taylor, University of Pennsylvania Department of Chemistry, Philadelphia, PA, personal communication, 1998). The solutions were tested weekly and began to show some degradation in performance at approximately 14 weeks; therefore, the shelf-life of this formulation could generally be safely stated as 3 months. This is consistent with that reported by Stoilovic et al. wherein a combined Ind-Zn (using HFE-7100 as a carrier solvent) showed no degradation in performance over a 3-month period (17).

Indanedione Performance—Effect of Humidity

1,2-Indanedione has been noted to perform inconsistently among laboratories worldwide. In our own laboratory, the results achieved using this method appeared to be impacted by fluctuations in % RH, which were rather large in magnitude. While the temperature remained relatively constant, the % RH ranged from 51% to 81% during the course of the study. When the % RH was relatively low, prints processed using the laboratory oven method showed little color development, although fluorescence was typically adequate for identification purposes. However, when the % RH was relatively high, samples processed using this same method demonstrated dark color development and strong fluorescence. This finding corresponded with the observations of Wallace-Kunkel et al., in which performance differences were noted concerning two locales, one with a higher % RH than the other (16). The potential impact of humidity exposure to print quality when using Ind as a reagent was mentioned in the work of Azoury et al., to be potentially attributable to a complex phenomenon involving paper, sweat, reagent, and water (20).

In an effort to isolate the cause of this variability in results with Ind-Zn, a Strandberg Engineering M-400 Portable Moisture Sensor was used to quantify the percentage moisture content of the various papers on which the fingerprints were being developed in order to determine if a correlation existed between this parameter and the % RH of the air. Proving to be the critical percentage moisture value was that recorded after exposure to the reagent and subsequent drying. The key variables affecting this appeared to the % RH of the air, as well as dwell time in the reagent. When deviating significantly from the standard dip time of 5 sec or less, differences were observed in moisture content after drying between these samples and control samples otherwise subjected to the same conditions. By following a defined protocol for processing, this variability was eliminated, thereby negating its effect.

The samples were assessed visually to determine strength of initial color development. A chart was developed for each substrate, detailing the maximum percentage moisture content observed and threshold values at which the color was notably lighter or darker for each procedure (fluorescence has proven in these experiments to be in direct correlation with visible color). The ranges of optimal, transitional, and weak color development were demarcated and depicted on the graph as shades of pink representative of the actual color (Fig. 2). In the transitional range, the processing methods resulted in comparable results; it was inconsequential as to which method was used. Therefore, the minimum percent moisture value for this range was defined as the
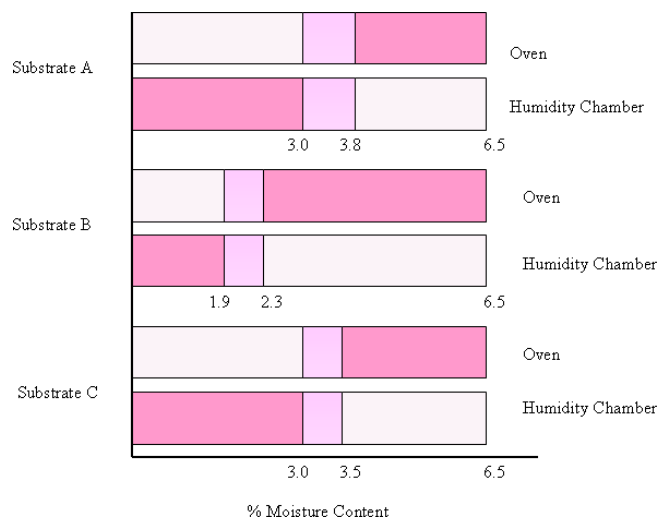

FIG. 2—*Chart depicting resultant color of developed Ind-Zn prints for each of the two methods, oven and humidity chamber processing, for each of the three substrates.*

critical threshold, at which point an alternative processing method needed to be utilized. As demonstrated in Fig. 2, for substrates A and C, the critical moisture level was 3.0%, while for substrate B, the critical level was 1.9%.

While many laboratories have hygrometers, the number having moisture sensors, such as the M-400, is most likely limited. Therefore, in order to define standard operating procedures for optimal processing with Ind-Zn, these percent moisture ranges were then correlated with % RH values. The minimum moisture percentage observed at each % RH level was charted, along with the corresponding standard deviation of all samples measured under those conditions in order to quantify the variability present. A line was then drawn signifying the critical percent moisture threshold, which was, again, substrate-specific, and the corresponding % RH on the graph defined as the critical relative humidity value (Fig. 3). From this, it can be determined that for substrates A and C, this critical % RH is below 69%, while for substrate B, it is interpolated to be approximately 72%. When the % RH is below these threshold values, processing should be conducted in the humidity chamber; above these values, the laboratory oven should be used in order to achieve optimal results.

Comparison with DFO—Visible Color

In order to quantify the amount of visible color development obtained using the combined Ind-Zn formulation versus that with DFO, a HunterLab UltraScan Pro spectrocolorimeter was used. All samples were measured using the CIELab color scale with dE* as a measure of total color difference. With opponent color scales such as CIELab, lightness to darkness is represented on one axis (in this case, L^*), redness to greenness on one axis (a^*), and yellowness to blueness on the final axis (b^*). The more positive each value, the lighter, redder, and/or more yellow is the sample in comparison to a defined standard. Conversely, the more negative each value, the darker, greener, and/or bluer the sample is in comparison to the standard. The calculation for dE* incorporates all three of these values, such that a total color difference is yielded. This value represents the distance or displacement between a sample and a standard in color units. While the sensitivity of the human eye is not constant in different regions of color space,
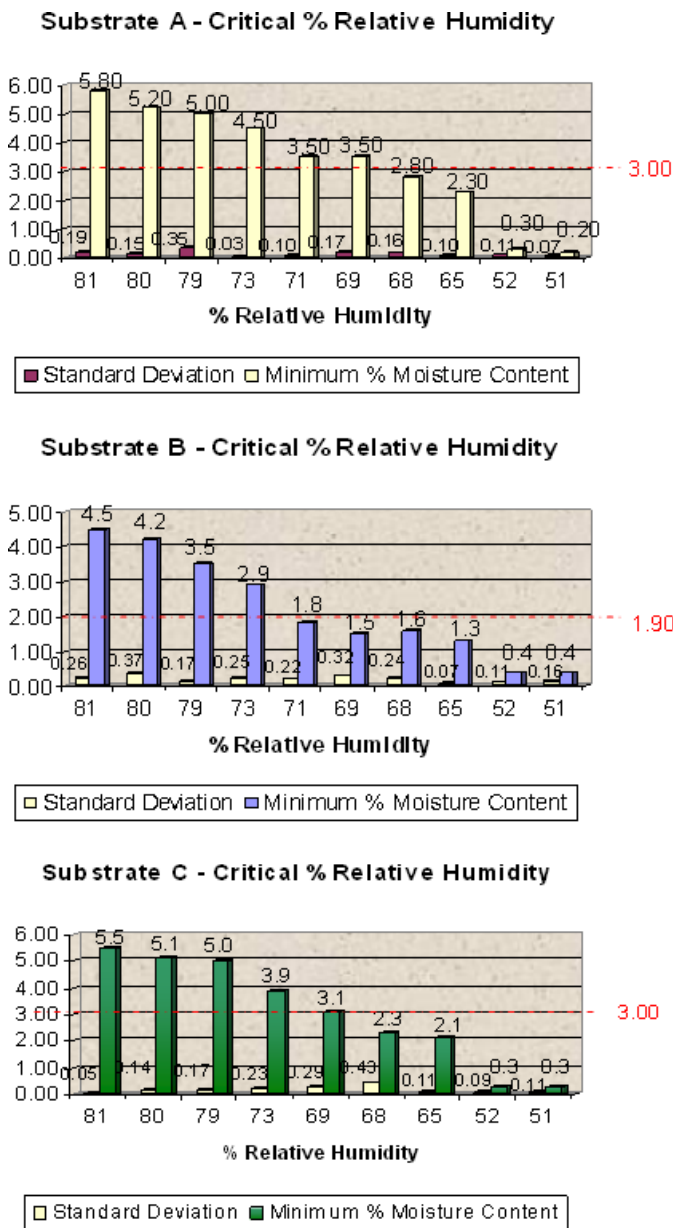
Substrate A - Critical % Relative Humidity

Substrate B - Critical % Relative Humidity

Substrate C - Critical % Relative Humidity

FIG. 3—*For Substrates A–C, charting of minimum percentage moisture at each percentage relative humidity level observed, along with the standard deviation of all measurements at each % RH level. The critical percent moisture content value is depicted in dotted-dashed line. All % RH values below this threshold indicate humidity chamber processing is warranted; all those above indicate the oven method is preferable.*

typically, values less than 1.0 dE* are not discernible to the human eye. This is a general rule, however, in that in regions of the visible electromagnetic spectrum in which the human eye is most sensitive, such as with those yellow in color, samples having values of 0.75 dE* from one another can be distinguished. Additionally, 1976 CIE color space has been optimized such that certain regions have been contracted, while others expanded, in order to more closely approximate visual assessment, with some regions having better correlation than do others. Finally, the human eye is most sensitive to differences in the *b** value of a sample, as represented on this axis is the degree of yellowness of the sample.

For the three substrates and nine donors, specimens processed using DFO for one-half of each fingerprint strip and Ind-Zn for the other half were measured. For all comparisons, the DFO

measurement was assigned as the standard, while the Ind-Zn value was assigned as the sample to which it was being compared. For substrate A, the minimum dE* observed between DFO and Ind-Zn was 6.69 with a maximum of 18.55 (Fig. 4). For substrate B, a wide range was not observed, the average being approximately 6.51 units dE*. For substrate C, the range was 6.54–26.32 units dE*. Thus, it can be concluded that processing with the optimized Ind-Zn formulation resulted in visible color development of latent prints a minimum of approximately 6.5 color units different from those with DFO. Based upon the *L** and *a** values, the difference lay specifically in the Ind-Zn prints being darker and redder than the DFO.

Substrate Staining

In latent print development, processing methods can result in staining of the substrate, which can interfere with the visualization of developed prints. For substrate A, a significant difference was noted, both visually and instrumentally, between the two reagents; DFO was 5.2 units of total color difference farther from the standard than was Ind. The visual effect was that DFO resulted in considerably more yellowing of the substrate, the component of color difference to which the human eye is most sensitive. For substrate C, DFO was 4.22 units of total color difference farther from the substrate, again primarily being more yellow, although not to as great a degree as with substrate A. For substrate B, the canary yellow legal paper, both resulted in slight staining of the substrate; however, the 0.15 unit dE* difference was indistinguishable to the human eye. In that the primary shift in color for the other substrates was in the *b** value, the fact that this substrate is itself yellow most likely resulted in the color difference being of low magnitude.

Effect of Age of Prints on Visible Color Development

In order to ascertain whether the age of prints affected the results obtained with Ind-Zn processing, samples ranging from same-day deposition to 11 years of age were analyzed. As with the previous comparison prints, which ranged in age from 33–69 days, those processed with Ind-Zn were darker and redder than those with DFO. For substrate A, the dE* value for fresh prints averaged 21.79 which demonstrated a statistically significant difference from the range of 6.69–18.55 obtained with older prints. For substrate B, the dE* value for fresh prints averaged 21.86, which showed, again, a significant difference from 6.51 dE*, the average of older prints. However, for substrate C, the average dE* for fresh prints was 16.81, which fell within the range of 6.54–26.32 previously observed for older prints. To help quantify whether the performance would continue to diminish with additional time, a print 11 years of age was then processed and measured. The results fell within these previously observed dE* ranges for more aged samples (Ind-Zn as compared to DFO) for substrates A and C and was not significantly different from the average for those of substrate B.

Comparison with DFO—Fluorescence

In order to quantify the fluorescence yielded with Ind-Zn versus that with DFO, a ChemImage Hyperspectral Contrast Imaging system (HCI) was utilized. Regions of interest of comparable size were defined for each half of each sample, and the average intensity collected (Fig. 5). The results indicated that processing with Ind-Zn yielded intensity values approximately four times greater than that with DFO for both substrates A and C. For substrate B,

Miniumum dE* Between DFO and Ind-Zn – Substrate A
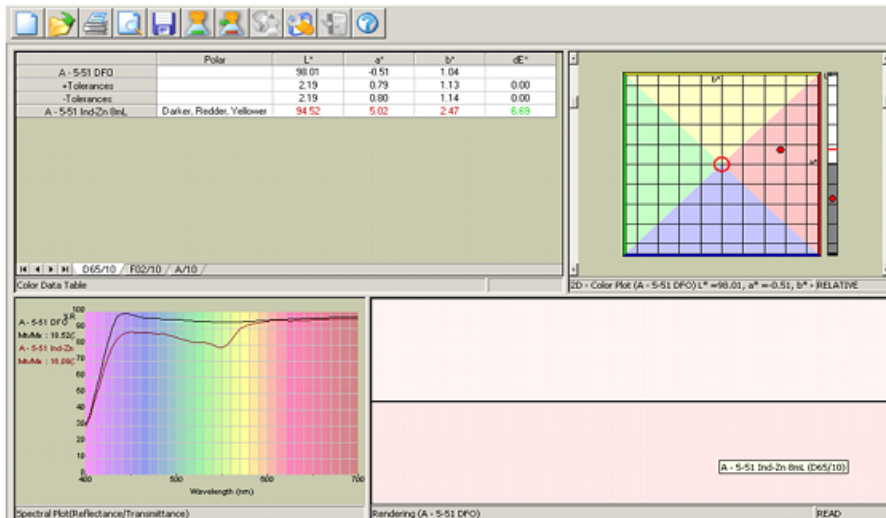
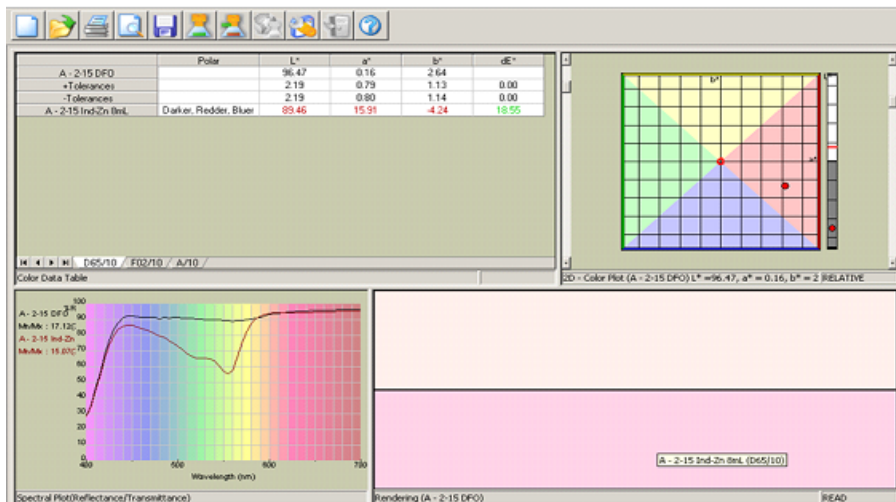Maximum dE* between DFO and Ind-Zn – Substrate A

FIG. 4—For the evaluation of Substrate A, screen captures from HunterLab's EasyMatch QC software depicting four views: moving clockwise around the images, the first contains the absolute colorimetric values for the samples, as well as corresponding dE* value; the second, a two-dimensional color plot representing in color space the relationship between the standard (DFO—the centerpoint of the graphic) and Ind-Zn; the third, a reflectance spectral plot representing both values; and finally, a color rendering representation of the actual color of each sample, with DFO positioned at the top of the view and Ind-Zn on the bottom. (A) Minimum dE* between DFO and Ind-Zn. (A) Maximum dE* between DFO and Ind-Zn.
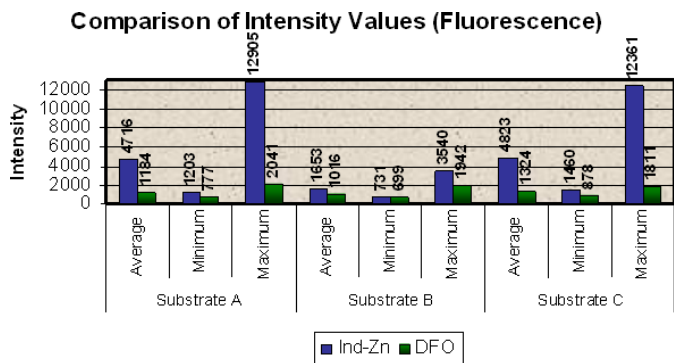

FIG. 5—Relative intensity values of DFO and Ind-Zn samples as reported by the ChemImage Hyperspectral Contrast Imaging System.

use of Ind-Zn resulted in intensity values approximately one and a half times greater; however, because of the added luminescence contributed by the bleeding of the blue lines in this paper with the use of DFO, the intensity values for this reagent were artificially high (Fig. 6).

The samples were then evaluated using the ALS to determine whether a visual correlation existed between the way a latent print examiner would view the prints and the intensity values reported by the HCI system. Seven categories of fluorescence were defined based upon visual observation: Extremely Strong, Very Strong, Strong, Medium, Weak, Very Weak, and Barely Visible. After classifying each sample into the most applicable category, the data was evaluated to determine what intensity values corresponded with each category. A direct correlation was found between measured intensity values and visual perception of fluorescence; generalized
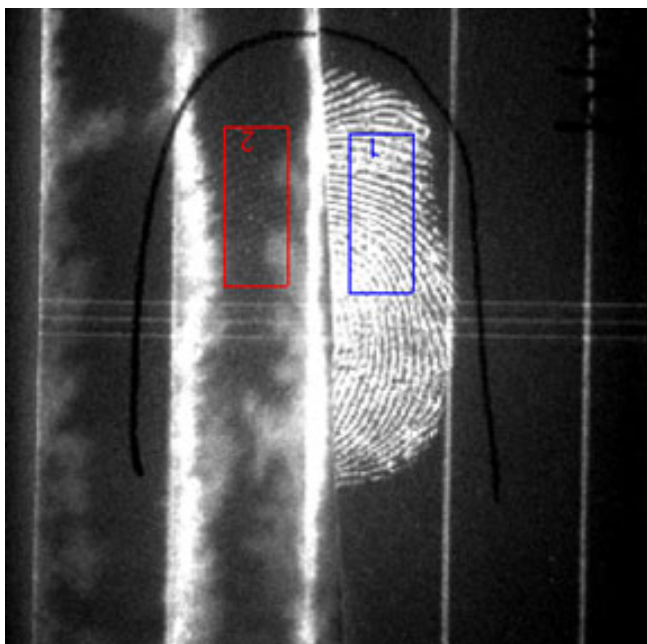
FIG. 6—*Print processed on Substrate B; DFO utilized on the left side; Ind-Zn on the right.*

ranges of these values could be assigned to each of the seven visual categories. Substrates A and C, both white, behaved similarly and the same scale could be used for both, while a separate table had to be generated for Substrate B. A total of 474 samples was developed, one-half in indanedione and one-half in DFO; the total number of prints analyzed was 237. The number of samples falling into each category for each reagent was then charted, and a total number identifiable for each calculated (Fig. 7).

A total of 205 samples (86.50%) of the 237 processed with Ind-Zn were of adequate fluorescence to render identification possible. A total of 10 samples (4.22%) of the 237 processed with DFO were of adequate fluorescence to render identification possible (those categorized as "Weak" or better). While some samples for substrate B were classified as "Medium" or "Weak" on the basis of intensity, in actuality the bleeding of the blue lines caused by the 2% acetic acid concentration resulted in artificially high values. In other words, the regions of interest contained contamination from the fluorescent dye present in the blue lines. Therefore, the total number of prints potentially identifiable with this reagent on this substrate included only a portion of those classified as "Medium" and higher.

Applicability to Casework

For the 58 naturally-handled envelopes evaluated, 116 total examinations, 84 showed development of prints with Ind-Zn that were not detectable with DFO (Fig. 8). For 16 of the specimens, there was additional development with Ind-Zn processing; however, it was not specific to prints and included possible saliva and/or other substances. Ten of these specimens performed equally with both DFO and Ind-Zn; no additional development was observed. When reversing the order such that Ind-Zn was used first, followed by DFO, the level of development of prints when using Ind-Zn was similar to that depicted in Fig. 8. With the subsequent processing using DFO, no additional prints were realized, and in coated areas of the envelope, such as on flaps sealed with tape, prints that had been observed when using Ind-Zn were no longer visible.
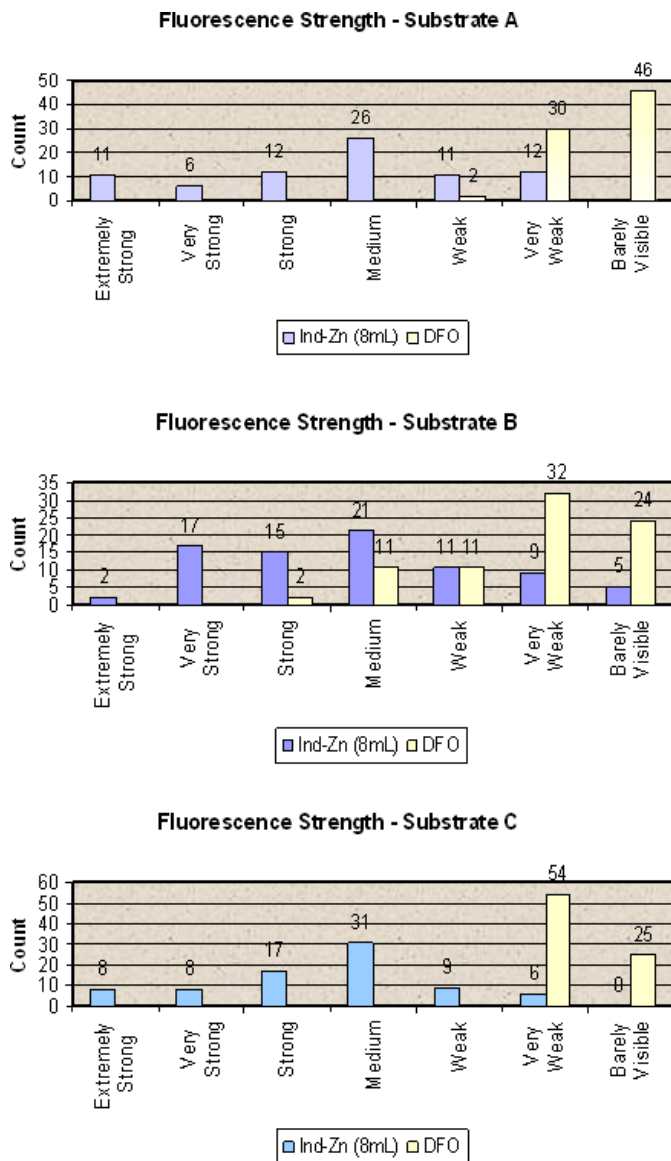

FIG. 7—*Classifications of fluorescence of prints for each substrate derived from correlation of intensity values with visual assessment of fluorescence strength.*

Further Research

It was evidenced in the course of these experiments that the latent print residue of some donors reacted very strongly with Ind-Zn; so much so, that development occurred in levels of high humidity before the samples were even subjected to an oven or a humidity chamber. In attempting to isolate the component potentially responsible for this, various amino acid constituents of these secretions were spotted onto Whatman filter paper and found not only to react weakly with Ind-Zn, but to react less strongly with this reagent than with DFO. Thus, further work lies in determining the exact mechanism of the reaction between Ind-Zn and latent fingerprint residue.

Conclusion

While Ind has proven to yield promising results in the past, and has even been adopted for casework by some agencies, variability
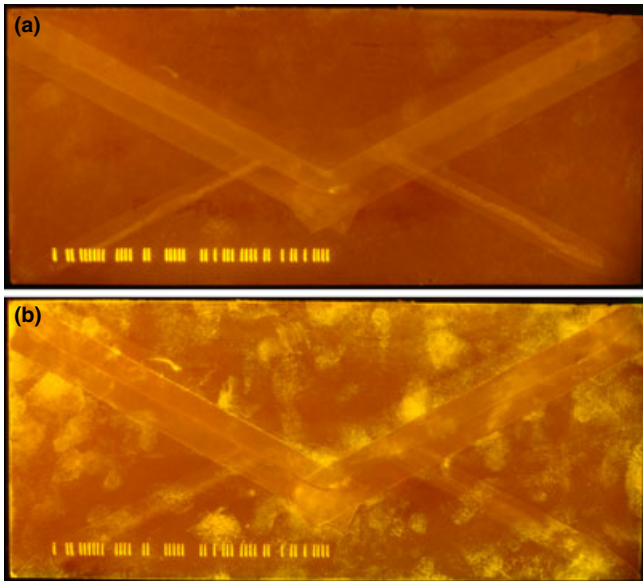
FIG. 8—(a) Comparison of an envelope processed with DFO, and (b) the same envelope then processed with Ind-Zn.

in performance of the reagent experienced by laboratories worldwide has precluded it from widespread use. While it has been noted that incorporation of Zn^{2+} to the formula increases the stability of the resultant complex and reduces the effects of humidity upon its reaction with latent print residue, it was determined in this work that a combined Ind-Zn solution was still sensitive to these effects.

In these experiments, it was found that there exists a direct correlation between the moisture content of the paper on which the prints are deposited and the % RH of the air, such that one can predict the method of processing that will yield the most optimal results based upon these values. The critical moisture value was found to be that measured after the paper had been processed and allowed to dry for a period of 10 min, which was directly impacted by both the dwell time in the reagent and the % RH of the surrounding air. When proper processing protocol was followed, dipping in the solution for a period not to exceed 5 sec, any variability contributed by this component was eliminated, and thus, its effect negated, leaving only the % RH difference impacting the moisture content. If the defined substrate-specific critical % RH value is exceeded, the samples were best processed using a laboratory oven, while if the % RH values fall below this level, most optimal results are achieved using a humidity chamber.

Using the appropriate processing method for the % RH in the laboratory, an optimized Ind-Zn formulation containing PE as a carrier solvent and 7.4% v/v $ZnCl_2$ performed better both in initial color development and fluorescence than did DFO for prints ranging from those freshly deposited to 11 years of age. These values were quantified using a spectrocolorimeter and a hyperspectral contrast imaging system. For visible color development, the optimized Ind-Zn formulation resulted in a minimum of 6.5 units dE* total color difference (darker and redder) than when DFO was used for processing. Correspondingly, intensity values reported by the HCI system were approximately four times greater when Ind-Zn was used for processing versus DFO for both substrates A and C. For substrate B, use of Ind-Zn resulted in intensity values approximately one and a half times greater; however, due to the bleeding of the lines on this paper with the use of DFO, these

values when using this reagent were artificially high. The intensity values were then correlated with visual assessments of fluorescence, the results indicating that 86.50% of prints processed with Ind-Zn were of fluorescence strength sufficient for identification, while only 4.22% of those processed with DFO were. When utilized with naturally handled specimens, use of this Ind-Zn process resulted in development of additional prints on 72.4% of the samples analyzed.

The combined Ind-Zn formulation resulted in a lesser degree of substrate staining than did DFO for white substrates analyzed, perhaps yielding an additional advantage for its use. For substrate B, there was little staining noted and the difference between the two reagents was indistinguishable. In that the majority of this color difference for white substrates was attributable to yellowing, the results obtained with this canary yellow substrate were expected.

The stability of the Ind-Zn reagent was tested at 3 months and found to have the same performance as did a fresh solution. Shortly thereafter, at approximately 14 weeks, some degradation in performance was noted; therefore, the shelf-life for this solution would be limited to 3 months.

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