Technical Note

Fingerprint Visualization and Spectroscopic Properties of 1,2-Indanodione-alanine Followed by Zinc Chloride or Europium Chloride

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Abstract: We investigated the reaction product of 1,2-indanedione with alanine in methanol at room temperature using absorption, excitation, and emission spectroscopy. We observed that the pale pink color of 1,2-indanedione-developed fingerprints on papers is also present in the 1,2-indanedione-alanine methanol solution at an appropriate concentration. The addition of zinc and europium salts to the solution and to 1,2-indanedione-treated fingerprints was presented and discussed. We confirmed the laser-induced fluorescence enhancement when adding zinc to the 1,2-indanedione-alanine solution and also on 1,2-indanedione-treated fingerprints after post-treatment with zinc. However, no emission enhancement was observed with the addition of europium, even though we observed the formation of a 1,2-indanedione-alanine-Eu complex.

Introduction

The reaction mechanisms and some of the spectroscopic properties of 1,2-indanediones with some amino acids have been previously reported [1–3]. The reaction of 1,2-indanedione and

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Received July 29, 2010; accepted October 27, 2010
an amino acid is unlike the formation of Ruhemann’s purple (which is independent of the amino acid used), but seems to be dependent on the amino acid. That is, there is an “R dependence” on the product where R comes from the general form of a primary amine, R-NH₂. The structures of colored fluorescent elements formed in these reactions were partially confirmed.

1,2-Indanedione was first synthesized as an intermediate for the preparation of substituted ninhydrin [1], and its capability of visualizing latent prints [4–6] was proven. 1,2-Indanedione is a single-step fluorescence method of developing latent prints on porous surfaces. Under visible laser excitation, 1,2-indanedione-developed fingerprints glow yellow. Even though 1,2-indanedione does not produce strongly colored initial print development (typically, a pale pink color), it produces strongly fluorescent detail under suitable blue-green light excitation.

In a recent study by Spindler et al. [7], the spectral variations for reaction products of nine major amino acids with four detection reagents (ninhydrin, indanedione, indanedione-zinc, and DFO) were reported. Their results provided insights about these reagents with respect to amino acids left by fingerprints on three types of cellulose-based surfaces. Alanine, present in the fingerprint residue, is one of the targeted amino acids to react with 1,2-indanedione. Preliminary results showed that alanine reacted with 1,2-indanedione (in methanol) at room temperature and was consequently considered as the amino acid of choice for this study. The concentration of amino acids in fingerprint sweat has been reported [8] to be between 0.3 to 2.6 mg/L. This value corresponds to an average amino acid content of about 0.3 µg per print. Alanine is the fourth by decreasing abundance after serine, glycine, and ornithine. Small concentrations of amino acids in sweat are sufficient for development on paper.

Using 1,2-indanedione, we tried to redevelop a six-year-old fingerprint that had been previously developed with 1,2-indanedione. No colored prints were observed after the redevelopment. Also, no fluorescence was seen under laser excitation. We concluded that the first 1,2-indanedione solution reacted with almost 100% of the amino acids present in the fingerprint and no further material was left for any further reaction. Once a fingerprint is completely developed, it slowly photodegrades [9].

Previous investigations [10, 11] with Ruhemann’s purple show that the reaction product of ninhydrin with glycine forms complexes with Zn(II). The zinc complex shows fluorescence when excited with laser radiation via intermolecular energy
transfer and enhances the visualization of faint, developed ninhydrin fingerprints. The addition of zinc chloride to the 1,2-indanedione fingerprint’s processing yields controversial results. Stoilovic et al. [12] reported results that were inferior compared to a combined 1,2-indanedione-zn formulation, and Bicknell and Ramotowski [13] expanded on these findings. For some experimental conditions – zinc chloride (ZnCl$_2$) concentration in particular – they reached similar findings. 1,2-Indanedione formulations and fingerprint development procedures were also reported by others [12–14] and compared with respect to the initial color, fluorescence, the concentration of the reagent, the effects of humidity, and so forth. Our work will focus on the spectroscopic properties (absorption, excitation, and fluorescence spectra) of the reaction product(s) of 1,2-indanedione with alanine (a representative of the targeted amino acids of fingerprint residue) in methanol, at room temperature. We will contribute to previous controversial work regarding the enhancement of the fluorescence of 1,2-indanedione-developed fingerprints when zinc is added by quantitatively measuring the fluorescence of the 1,2-indanedione-alanine solution when adding zinc chloride. We will also present our results on the fluorescence of our 1,2-indanedione-treated fingerprints when adding a secondary ZnCl$_2$ treatment to the half side of the sets of fingerprint samples.

Eu(III)-RP complex [15–17] shows emission enhancement of the lanthanide ion (red phosphorescence) via intramolecular energy transfer from the ligand (RP) to the ion (Eu$^{3+}$). Complexing the lanthanide ion with a ligand that absorbs light well and then transfers that excitation energy intramolecularly to the lanthanide ion of the complex will probably increase the lanthanide luminescence. We attempted to see whether we can enhance the luminescence of either the lanthanide or the organic ligand formed by the reaction product of 1,2-indanedione with alanine (powder form) in methanol or as an addition to the 1,2-indanedione-developed fingerprints. The results are presented in this contribution. We also examined the prospect that complexation of 1,2-indanedione-alanine with europium (EuCl$_3$) might yield enhanced Eu$^{3+}$ emission, which would be useful in concert with time-resolved imaging for background fluorescence suppression [18, 19].
Materials and Methods

Preparation of Solutions

We worked with two types of 1,2-indanedione: (1) a purchased powder (indan-1,2-dione, 97%) (Sigma-Aldrich, St. Louis, MO) and (2) a sample synthesized in the laboratory (the nuclear magnetic resonance spectrum showed a purity of more than 99%). We started to work with ethyl acetate as a solvent, but because it is prone to hydrolysis and trans-esterification, we switched to methanol because of its high polarity and the possibility of dissolving the amino acid alanine. A solution of $4.5 \times 10^{-3}$ M of 1,2-indanedione in methanol was prepared as follows: 0.066 g of 1,2-indanedione was dissolved in 100 mL of methanol at room temperature. This volume was divided into two parts: Solution A ($V_A=50$ mL) and solution B ($V_B=50$ mL). Solution A was reacted with an excess of alanine. The final concentration in the reaction was $4.5 \times 10^{-3}$ M (we are assuming a 1:1 stoichiometry). The solution was left at room temperature for one day for the reaction to reach completion, because of the low solubility of alanine in methanol. (Heat may accelerate the reaction.) The color of the reaction product(s) depended on the concentration, but it was close to pink. Solution B was left without adding anything and was used for fingerprint treatments.

Europium and Zinc 1,2-Indanedione-Alanine Complexes Formation

The previously prepared solution ($4.5 \times 10^{-3}$ M) of 1,2-indanedione and alanine in methanol (left for one day at room temperature) was divided into three parts. The first part had nothing added to it. To the second part, a methanol solution of europium chloride ($4.5 \times 10^{-3}$ M) was added, giving the product a pink-red complex (Figure 1). To the third part, a methanol solution of zinc chloride ($4.5 \times 10^{-3}$ M) was added, giving the product an orange-red complex. We shall note that all the reactions took place at room temperature and the volumes were mixed in a 1:1 proportion. Parts of the solutions were used for spectroscopic measurements.
Spectroscopic Measurements

Absorption spectra were obtained using an Agilent-8453 UV-visible spectrometer. The samples’ temperature was 20 °C. Excitation and emission spectra were measured at room temperature, by standard techniques, using a Jobin Yvon Fluorolog 3. All of the measurements were done with the following setting: increment 1 nm, integration time 1s, slits opening 2 nm for the excitation and 5 nm for the emission. Liquid samples were put in quartz cuvettes (4 cm³ Silica quartz cuvette, UV-visible spectrometry 200 nm and up).

Preparation, Treatment, and Observation of the Fingerprint Samples

In this study, different types of papers were used (mainly white office papers). Fingerprints from four donors were either deposited (deposited in a known location) on the papers or collected as touched papers (random samples where the print locations were not known). Two of the donors deposited two sets and touched two sets of samples. The other two donors deposited eight sets and touched four sets of samples (16 sets in total). The samples were deposited over a period of two months. All of the samples were left in open air at room temperature and were treated after at least three to four weeks after deposition.

Figure 1

4.5 x 10-3 Methanol solution of 1,2-indanedione and alanine (solution A, right): complex formed when adding ZnCl₂ to solution A (middle); complex formed when adding EuCl₃ to solution A (left).
The 16 sets of fingerprints were treated with 4.5 x 10^{-3} M solutions of 1,2-indanedione in methanol in a period of three months. The touched papers were sprayed with the solution, and the papers with deposited fingerprints were dipped for few seconds. (The touched papers were sprayed because we did not know precisely where the fingerprints were located. The papers with deposited fingerprints were dipped for a few seconds, because the locations of the prints were known and the surface was small, so dipping was appropriate.) The samples were left to air-dry for a few minutes and were then put in an oven at 50 °C and about 60% relative humidity for 30 minutes. (At room temperature, the reaction could have taken up to a day, because of the low kinetics of the reaction of amino acids with 1,2-indanedione).

The samples were observed with an Argon laser (Coherent Innova I-310 Laser, Santa Clara, CA). The excitation was all lines (475 nm, 488 nm, and 514 nm) radiation, with power ranging from one to two watts expanded into a beam diameter of approximately 5 cm at the sample position. An orange filter (OG 550 Long Pass, Edmund Optics K47-399) was used for visual observation, because the response of the fingerprint samples was a yellow broad emission with a maximum of 560 nm. Photographs were taken with a DCS-4 imaging system (Foster and Freeman, Worcestershire, U.K.).

**Results and Discussion**

*Absorption Measurements*

The absorption spectra were taken at room temperature. The UV-visible spectrum of 1,2-indanedione-alanine showed a broad band in the UV around 320 nm with a half width of about 126 nm, and a lower band in the visible around 500 nm with a half width of about 70 nm, as shown in Figure 2. The visible band (blue-green, 488 and 514.5 nm) is used to excite the 1,2-indanedione-alanine liquid solution and also for visualizing, under laser, the 1,2-indanedione-treated fingerprints. The use of visible light instead of UV is an advantage of 1,2-indanedione-developed fingerprints. The complexes formed by adding ZnCl₂ or EuCl₃ to the 1,2-indanedione-alanine ligand showed a blue shift of 25 nm and 40 nm, respectively, due to the complexation. The two complexes also showed narrower UV bands. The 1,2-indanedione-alanine compounds and their europium or zinc complexes absorption spectra show the same features (Eu and Zn do not absorb much radiation).
Emission and Excitation Measurements

The broadband excitation spectrum (Figure 3) shows that the 1,2-indanedione-alanine liquid solutions are well excited in the visible 420 to 510 nm. Therefore, blue-green laser lines (488 nm and 541.5 nm) are well-suited excitations of the samples in solution and also for fingerprint visualization. The emission spectra of 1,2-indanedione-alanine solution and its Eu and Zn complexes were taken under 488 nm excitation. The three spectra show about 120 nm Stokes shifted. They present a broad band having its maximum around yellow (560 to 570 nm). No emission enhancement was seen in the case of Eu: The observed (qualitative) emission was weaker for the Eu complex under the same concentrations. No quantitative emission measurements of emission yields were done to address fluorescence quenching. The fluorescence spectrum of the Zn complex shows substantial luminescence enhancement with a sharp peak around 560 nm. This confirms that adding Zn to the 1,2-indanedione-alanine enhances the product’s fluorescence. The sharp peak explains the fluorescence enhancement of ZnCl₂ post-treated, faint, 1,2-indanedione-developed fingerprints and the improvement of the fluorescence of the ridge detail.

Figure 2
Absorption spectrum of the reaction product of alanine with 1,2-indanedione in methanol, and its Zn and Eu complexes at room temperature.
Fingerprint Visualization with Laser Induced Fluorescence

Indanedione Treatment

Under room light, some of the samples turned pink and some fingerprint detail could be observed, but other samples did not show any pink color or fingerprint detail. We did not use a forensic formulation of indanedione to perform tests on fingerprints because there are many forensic formulations.

ZnCl$_2$ or EuCl$_3$ Fingerprint Post-treatment

The 1,2-indanedione samples previously described were cut into two parts (left and right). No further treatment was done to the right side of the samples. Half of the left sides of the samples were dipped in 4.5 x 10$^{-3}$ M solutions of EuCl$_3$ and the others were dipped in 4.5 x 10$^{-3}$ M solutions of ZnCl$_2$. The two previous treatments were done under the same conditions. The left sides of the post-treated samples were dried at room temperature. Observation under room light showed a change in the pink color of the left side treated with ZnCl$_2$: the color after post-treatment was pale orange (Figure 4). This color is the same as the Zn complex (Figure 1).
Figure 3
Excitation spectrum of 1,2-indanedione-alanine (left) and emission spectra of alanine-1,2-indanedione in methanol (red), its Eu(III) complex (blue), and its Zn(II) complex (green).

Figure 4
Fingerprint, from set 13, developed with 1,2-indanedione. The left side of each image was post-treated with ZnCl$_2$: (a) seen under room light; (b) seen under blue-green Argon laser.
Figure 5 shows that the samples’ left sides did not show much color change under room light. This is due to the lack of coloration of the sample under room light. But under laser light, the difference between the two sides is well seen.

The samples post-treated with EuCl₃ did not show any color change under room light (Figures 6a and 6b). There was also less luminescence under laser light. This result was also seen in the fluorescence of the solutions. The 1,2-indanedione-alanine-europium complex absorbs in the range 330 to 350 nm (Figure 2), but qualitative observation of the europium post-treated fingerprints did not show any enhancement of the left side of the samples.

Conclusion

The redevelopment with 1,2-indanedione of a six-year-old fingerprint that had been previously developed with 1,2-indanedione did not give any colored prints after the redevelopment. Also no fluorescence was seen under laser excitation. We concluded that the first 1,2-indanedione solution reacted with almost 100% of the amino acids present in the fingerprint and no further material was left for any further reaction. Once a fingerprint is completely developed, it starts to slowly photodegrade.

The reaction product of amino acid alanine with 1,2-indanedione in methanol solution was studied qualitatively by direct observations and quantitatively by spectroscopic techniques. The pictures of the solutions and of the treated fingerprints, under room light, showed color changes when adding ZnCl₂ or EuCl₃. The fluorescence enhancement in solution for the formed complex when adding ZnCl₂ to 1,2-indanedione-alanine solution is confirmed. A sharp and more intense yellow peak in the corresponding fluorescence spectrum was observed. But we observed a slightly weaker fluorescence in solution when adding EuCl₃ (a less intense yellow peak in the corresponding fluorescence spectrum). The same observations were true for fingerprint post-treatment with the two salts. Quantitative emission measurements of emission yields are needed in the case of the 1,2-indanedione-alanine-Eu complex to address fluorescence quenching.
Figure 5
Fingerprint, from set 16, developed with 1,2-indanedione. The left side of each image was post-treated with ZnCl$_2$: (a) seen under room light; (b) seen under blue-green Argon laser.

Figure 6
Fingerprint developed with 1,2-indanedione. The left side of each image was post-treated with EuCl$_3$: (a) seen under room light; (b) seen under blue-green Argon laser.
Acknowledgment

This work was made possible through a grant from the Fulbright Program and the Moroccan-American Commission for Educational and Cultural Exchanges. The corresponding author received a Senior Fulbright Research Scholarship, hosted by Professor M. Joullié (University of Pennsylvania).

Thanks to professor G. Fang and Dr. I. J. Dmochowski (Chemistry Department, University of Pennsylvania, Philadelphia) for the use of the spectroscopic equipment; Dr. S. Berritt (Post doc at Joullié’s laboratory) for useful organic chemistry discussions; J. Lee (PhD student at Joullié’s laboratory) for synthesizing the 1,2-indanedione powder; and Dr. R. Ramotowski (USSS, Forensic Science Division, Washington DC) for useful forensic discussions and for providing some images.

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References


