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A study of fluorescence emission intensity of reaction products between selected amino acids and DFO, 1,2-indanedione and 1,2-indanedione – zinc chloride

Summary

The study shows the results of fluorescence emission intensity measurements of the reaction products of selected amino acids with DFO, 1,2-IND and 1,2-IND – zinc chloride on absorptive surfaces. The conducted research addressed the following variables: type of developing reagent, type of surface, type of amino acid and sample storage time. It was confirmed that the above factors affected the fluorescence intensity of the developed fingerprints. Furthermore, the studies proved that the fluorescence intensity of the reaction products between amino acids and 1,2-IND stored for 1 or 7 days increased for the majority of samples upon addition of zinc chloride to the developing reagent. For samples stored for 4 months the highest fluorescence emission intensity was observed for DFO. No significant differences were found in fluorescence spectral characteristics of the tested compounds, depending on the type of surface.

Keywords DFO, 1,2-indanedione, 1,2-indanedione – $ZnCl_2$, hyperspectral imaging, amino acids, absorptive surfaces.

Introduction

Amino acids are already well established in visualization of fingerprints as a component of the traceable substance which is a substrate of chemical reactions used by forensic laboratories. In the 1950's, ninhydrin dissolved in acetone was used for the first time as the reagent reacting with amino acids. As a result of chemical reaction, a colored product is created that allows for visual detection of latent fingerprints [1]. Despite the fact that the fluorescence-based fingerprint development methods involving the use of dactyloscopic powders containing anthracene – zinc chloride formula, activated by UV radiation, have been in use since the 1930's, the use of the products of the reaction between amino acids and specific fluorescent chemical compounds dates back to the beginning of the 1990's. At that time, the study results on the use of DFO (1,8-diazafluoren-9-one) in the detection of latent fingerprints were published [2, 3, 4]. Earlier literature evidence indicated the possibility of obtaining the fluorescent complex composed of the product of the reaction between ninhydrin and amino acids with zinc chloride or cadmium nitrate. However, the complexation was secondary in relation to ninhydrin-amino acid reaction [4]. At the end of the 1990's, a new component named 1,2-IND (1,2-indanedione) was applied, which reacted with amino acids and with DFO, whereby the reaction product exhibited fluorescent properties [5, 6]. The follow-up scientific activity within this scope was focused on the optimization of solution components and the use of new solvents [7, 8, 9, 10, 11]. A remarkable achievement of the works towards increased sensitivity of the detection methods was the inclusion of zinc chloride ($ZnCl_2$) into the working solution of 1,2-IND [12].

This article presents the results of the studies on fluorescence intensity of the products of the reaction between nine selected amino acids and DFO, 1,2-indanedione and 1,2-indanedione – zinc chloride, deposited onto absorptive surface in the form of printing paper and filter paper. At the same time, the present work is a follow-up on previous studies on fluorescence spectral characteristics of amino acids reacting with DFO, 1,2-indanedione and 1,2-indanedione – zinc chloride deposited onto absorptive surface, over time [13].

Aim of the study

The aim of the conducted studies was to compare the fluorescence emission intensity of the products formed in the reaction between the selected amino acids and DFO, 1,2-IND and 1,2-IND – zinc chloride. It is assumed that the intensity of emitted fluorescence is influenced by such variables as: type of developing reagent, type of surface, type of amino acid and sample storage time. The spectral data obtained will allow to determine whether the type of surface in the form of printing paper and filter paper has a significant effect on spectral characteristics and to verify the thesis that the emission of fluorescence increases upon using 1,2-IND – zinc chloride formula. The measured level of fluorescence emitted by developed fingerprints will enable the determination of the effect by amino acids contained within the traceable substance on fluorescence intensity.

Materials and methods

Chemical reagents

DFO (1,8-diazafluorene-9-one), 1,2-IND (1,2-indanedione) were obtained from BVDA (the Netherlands). Zinc chloride ($ZnCl_2$) and ethanol were obtained from Stanlab (Poland). Amino acids: alanine aspartic acid, glycine, histidine, leucine, lysine, serine, threonine and valine (L configuration) were obtained from Sigma-Aldrich (Poland). Ethyl acetate, light petroleum and methanol were obtained from POCH (Poland). All the chemicals used in this study were of analytical grade and were used without subsequent purification.

Preparation of amino acid solutions

Amino acids were dissolved in demineralized water using a magnetic stirrer. The concentrations of prepared solutions, shown in Table 1, were in line with those used in earlier studies on determination of spectral characteristics, based on information from the literature. It was assumed that the concentrations of the solutions should approximately reflect the average amino acid composition of the natural eccrine containing traceable substance [13, 16].

Preparation of DFO solution

As a first step, DFO concentrate was prepared by dissolving 0.5 g DFO in 100 ml of methanol, and subsequently adding 100 ml of ethyl acetate and 20 ml of acetic acid. Next, all the components were mixed using magnetic stirrer until DFO has dissolved completely. The working solution was prepared by combining 22 ml of DFO concentrate with 78 ml of light petroleum and mixing for 15 minutes.

Table 1 Concentrations of amino acids in aqueous solutions

Amino acid	Demineralized water
Alanine (Ala) – 73 mg	250 ml
Aspartic acid (Asp) – 37 mg	250 ml
Glycine (Gly) – 147 mg	250 ml
Histidine (His) – 37 mg	250 ml
Leucine (Leu) – 25 mg	250 ml
Lysine (Lys) – 98 mg	250 ml
Serine (Ser) – 245 mg	250 ml
Threonine (Thr) – 37 mg	250 ml
Valine (Val) – 25 mg	250 ml

Preparation of 1,2-IND solution

0.5 g of 1,2-IND was dissolved in a mixture consisting of 5 ml glacial acetic acid, 45 ml ethyl acetate and 450 ml light petroleum. Next, all the components were mixed using a magnetic stirrer until 1,2-IND has dissolved completely.

Preparation of 1,2-IND – zinc chloride solution

The zinc chloride containing visualizing reagent was obtained in three steps. As a first step, zinc chloride concentrate solution was prepared by dissolving 5 g of zinc chloride in the mixture containing 80 ml methanol, 80 ml ethyl acetate and 10 ml acetic acid. All the components were mixed using a magnetic stirrer until zinc chloride has dissolved completely. The second step involved preparation of a working solution by combining 20 ml of zinc chloride concentrate with 98 ml of light petroleum, and mixing for 15 minutes using a magnetic stirrer. In a third step, 1,2-IND – zinc chloride solution was obtained. To this end, 10 ml of 1,2-IND solution (the formula described above) was combined with 2 ml of zinc chloride solution and mixed on a magnetic stirrer for 15 minutes.

Preparation of samples

The studies included absorptive surfaces such as printing paper (density of 80 g/m^2) showing high UV-induced luminescence and laboratory filter paper characterized by low UV-induced luminescence. Squares of 5 cm side length were cut out from printing paper and filter paper sheets. Amino acid solutions were applied onto the square surfaces using automatic pipette and allowed to dry at room temperature. A total of 1 782 of printing paper samples and the same number of filter paper samples were prepared and analyzed. The samples were divided into 3 groups that were stored for 1 day, 7 days and 4 months, respectively, at room temperature and a relative humidity of 80%. Following this period, the samples

representing each of the amino acids spotted onto printing paper and filter paper, respectively, were taken one at a time, and exposed to DFO, 1,2-IND and 1,2-IND – zinc chloride, respectively. For the purpose of measurement, narrow stripes were cut out around the sites where amino acids reacted with developing reagent, and subjected to fluorescence emission tests. In one measurement cycle, one of the prepared samples at a time was analyzed under the same induction conditions for each method of development and for both types of surfaces, respectively (Fig. 1 Exemplary image of measurement-ready samples — visible fluorescence emission in the areas where amino acids reacted with developing reagents; see Polish version).

Conditions of reaction between amino acids and DFO, 1,2-IND, 1,2-IND – zinc chloride

The samples of surfaces with applied amino acid solutions were exposed to developing reagents after the time periods predefined in this study. The samples were heated for 20 minutes at a temperature of approximately 90°C.

Measurements of fluorescence emission spectra and data analysis

The measurements of the intensity of the fluorescence emitted by the surfaces were performed by hyperspectral imaging, using a CONDOR Macroscopic Chemical Imaging System™ (ChemImage, USA) equipped with a liquid crystal tunable filter (LCTF). Quadrangle-shaped, reacted areas were selected for measurements. Visualization and analysis were performed using ChemXpert software (Fig. 2). While selecting the areas for measurement, the edges of the reacted spots were avoided due to the amino acids' tendency to crystallize near the rim, which could lead directly to the increased amino acid concentration and stronger signal. The data were collected within the range between 550 nm and 720 nm, at spectral resolution of 7 nm, concurrent with excitation of fluorescence at 515 nm using a Mini Crimescope forensic illuminator (Ybon, USA).

Averaged results of five measurement series were subject to analysis. The numerical data corresponding to the measured areas were transferred to Microsoft Excel spreadsheet for initial verification based on calculation of standard deviation. It was assumed that the results that depart from the arithmetic mean by the value exceeding the standard deviation will be rejected.

In order to identify the difference in spectral characteristics of the fluorescence emitted by the studied compounds depending on the type of surface, the values of correlation coefficient were calculated and presented in tabular form. Figure 2 presents Example of the outlined measurement areas corresponding to the sites where amino acids reacted with developing reagents, with a corresponding emission spectrum; see Polish version).

Results and Discussion

Alanine (Ala)

The measurement results presented as fluorescence intensity charts in a function of wavelength of alanine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 3 (Fluorescence spectra of reaction products: alanine (Ala) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

In the case of printing paper, the intensity of fluorescence emitted by day 1 and day 7 samples was comparable for all the used methods, however, in the case of filter paper, a significant derogation consisting of higher emission by samples reacted with 1,2-IND – zinc chloride was reported. Month 4 samples showed significantly stronger emission of surfaces reacted with DFO. An addition of zinc chloride to 1,2-indanedione solution caused significant increase in fluorescence, also in the case of month 4 samples.

Aspartic acid (Asp)

The measurement results presented as fluorescence intensity charts in a function of wavelength of aspartic acid reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 4 (Fluorescence spectra of reaction products: aspartic acid (Asp) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

For both, printing paper and filter paper, the highest intensity of fluorescence emitted by day 1 and day 7 samples was observed for samples reacted with 1,2-IND – zinc chloride. With regard to this method, the filter paper samples showed significantly wider ranges of emission intensities than the printing paper samples. Month 4 samples showed significantly stronger emission of surfaces reacted with DFO. An addition of zinc chloride to 1,2-indanedione solution caused mild increase in fluorescence only in the case of month 4 printing paper samples.

Glycine (Gly)

The measurement results presented as fluorescence intensity charts in a function of wavelength of glycine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 5 (Fluorescence spectra of reaction products: glycine (Gly) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

For day 1 and day 7 day samples, the best effect was obtained with regard to the 1,2-IND – zinc chloride

method. Distinctively worse effect was observed for the same time point samples analyzed by the 1,2-IND method on filter paper. Printing paper showed slightly lower emission when treated with 1,2-IND than when treated 1,2-IND – zinc chloride solution. Month 4 samples showed significantly stronger emission of surfaces reacted with DFO. An addition of zinc chloride to 1,2-indanedione solution caused minimal increase in fluorescence only with regard to the month 4 printing paper samples.

Histidine (His)

The measurement results presented as fluorescence intensity charts in a function of wavelength of histidine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 6 (Fluorescence spectra of reaction products: histidine (His) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

Printing paper with applied histidine showed the best results when reacted with DFO. While day 1 and day 7 samples showed only slightly lower emission, month 4 samples yielded a significant difference. Filter paper emitted noticeably higher fluorescence in day 1 and day 7 samples treated with both, 1,2-IND and 1,2-IND – zinc chloride. In contrast to other samples, the addition of zinc chloride to 1,2-IND induced a slight decrease in fluorescence in month 4 and day 7 printing paper samples.

Leucine (Leu)

The measurement results presented as fluorescence intensity charts in a function of wavelength of leucine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 7 (Fluorescence spectra of reaction products: leucine (Leu) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

The highest fluorescence emission intensity of printing paper with leucine was recorded for the samples reacted with DFO (day 1, day 7, month 4), whereas in the case of filter paper surfaces, the best effect was observed for 1,2-IND – zinc chloride solution (day 1 and day 7 samples). The emission intensity of day 7 samples on both types of surfaces was similar, regardless of the method used. Month 4 samples showed significantly stronger emission of surfaces reacted with DFO. As distinct from month 4 printing paper samples, the addition of zinc chloride to 1,2-IND solution did not affect the emission intensity of filter paper samples.

Lysine (Lys)

The measurement results presented as fluorescence intensity charts in a function of wavelength of lysine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on on Figure 8 (Fluorescence spectra of reaction products: lysine (Lys) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

Printing paper dripped with lysine showed the highest emission when reacted with DFO, for all time points tested. Filter paper samples stored for 1 and 7 days featured the most intense fluorescence when treated with 1,2-IND – zinc chloride. Month 4 filter paper samples showed significantly stronger emission when reacted with DFO. The addition of zinc chloride to 1,2-IND solution revealed no effect on fluorescence intensity of day 1 printing paper samples and month 4 printing and filter paper samples.

Serine (Ser)

The measurement results presented as fluorescence intensity charts in a function of wavelength of serine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 9 (Fluorescence spectra of reaction products: serine (Ser) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

Serine containing samples stored for 1 day showed clearly the strongest fluorescence when reacted with 1,2-IND – zinc chloride. Filter paper day 1 and day 7 samples reacted with 1,2-IND and DFO showed similar fluorescence intensity. Unlike other amino acids studied, month 4 filter paper samples containing serine reached similar fluorescence intensity upon treatment with DFO and 1,2-IND – zinc chloride. The addition of zinc chloride generally did not affect the fluorescence of printing paper samples stored for 4 months.

Threonine (Thr)

The measurement results presented as fluorescence intensity charts in a function of wavelength of threonine reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 10 (Fluorescence spectra of reaction products: threonine (Thr) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength: 515 nm; see Polish version).

Printing paper surfaces with threonine showed no significant differences with regard to the treatments with 1,2-IND solution, with and without zinc chloride. The above two methods yielded highest fluorescence intensity in samples stored for 1 or 7 days. However, in the case of month 4 printing paper and filter paper samples, the highest emission was obtained by treatment with DFO. For day 1 and day 7 day filter paper samples, the best effect was obtained with 1,2-IND – zinc chloride solution.

Valine (Val)

The measurement results presented as fluorescence intensity charts in a function of wavelength of valine

reacted with DFO, 1,2-IND and 1,2-IND – zinc chloride on the two types of surfaces are shown on Figure 11 (Fluorescence spectra of reaction products: valine (Val) with DFO, 1,2-IND and 1,2-IND – $ZnCl_2$ on printing paper and filter paper. Excitation wavelength 515 nm; see Polish version).

Printing paper samples containing valine showed the highest emission intensity when treated with DFO, for all time points tested. However, the differences were significantly smaller for the first two time points. Filter paper samples stored for 1 and 7 days exhibited the highest fluorescence intensity upon treatment with 1,2-IND – zinc chloride, while for month 4 samples, the highest intensity was registered with DFO.

Further studies did not indicate general differences in spectral characteristics of fluorescence emitted by the compounds studied, depending on the type of surface. The highest variability was obtained for day 1 samples characterized by the lowest value of the correlation coefficient, including DFO-Asp (0.56), DFO-His (0.60), DFO-Leu (0.82), and 1,2-IND-Leu (0.81) (Fig. 12 Values of correlation coefficient for printing paper and filter paper samples stored for 1 day; see Polish version). For day 7 samples, the lowest value of the correlation coefficient was obtained for DFO-His (0.81), whereas for DFO-Gly, DFO-Ser, DFO-Thr and 1,2-IND – $ZnCl_2$-Ser the values did not exceed 0.9 (Fig. 13 Values of correlation coefficient for printing paper and filter paper samples stored for 7 days; see Polish version). In the case of month 4 samples, the correlation coefficient reached the lowest values for 1,2-IND –$ZnCl_2$-Ser (0.81) and DFO-Ser (0.87) (Fig. 14 Values of correlation coefficient for printing paper and filter paper samples stored for 4 months; see Polish version). For all other samples, the correlation coefficient did not exceed 0.9.

The results obtained indicate major differences in fluorescence emission intensities in relation to the three methods studied. The fluorescence intensity depends on the following variables: type of amino acid, type of developing solution, type of surface, sample storage time.

The analysis of the obtained results led to the conclusion that the 1,2-IND – zinc chloride developing method generates a high fluorescence intensity in fresh samples. All day 1 filter paper samples showed the highest emission intensity. In the case of printing paper samples, the obtained results were not as explicit.

Month 4 samples were most effectively induced by DFO. Only in the case of serine, the month 4 samples on filter paper surface reached comparable fluorescence intensities upon treatment with DFO and 1,2-IND – zinc chloride.

The addition of zinc chloride to 1,2-IND developing solution caused a significant increase in fluorescence intensity. Zinc ions are likely to stabilize the reaction product, thus conferring strong fluorescent properties upon it [14].

It should be emphasized that the results depended extensively on the type of surface, with particularly strong effect caused by printing paper surface. Lower absorbency of printing paper led to longer persistence of amino acid solution on the surface. Gradual dessication resulted in the tendency by amino acids to accumulate around the edges of the spot and to form a specific crown-like rim. These areas exhibited clearly higher fluorescence emission upon reaction with developing reagents. Despite the fact that the central area of the spot was designated for measurement, a high variability in the quantity of amino acid particles available to the reagents cannot be excluded. This phenomenon was less noticeable on filter paper surface due to its greater absorbency.

Conclusions

The conducted study proved that the fluorescence intensity of developed fingerprints is affected by the following factors: developing reagent, type of surface, type of amino acid and sample storage time.

It was experimentally confirmed that in the case of most surfaces and amino acids stored for 1 or 7 days, the addition of zinc chloride to 1,2-IND developing solution resulted in a significant increase in the intensity of emitted fluorescence.

The studies revealed that surfaces stored for 4 months exhibited the highest fluorescence emission intensity when developed with DFO solution. The only exception was reported for serine (Ser) deposited on filter paper, where the fluorescence intensity obtained for 1,2-IND – zinc chloride was comparable to that observed for DFO.

No significant differences were observed in terms of fluorescence spectral characteristics of the tested compounds, with regard to the type of surface.

Source

Figures 1–14: authors
Table 1: own elaboration