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Enhancement of an Insufficient Dye-Formation in the Ninhydrin Reaction by a Suitable Post Treatment Process*

REFERENCE: Ramminger U, Nickel U, Geide B. Enhancement of an insufficient dye-formation in the ninhydrin reaction by a suitable post treatment process. J Forensic Sci 2001;46(2):288–293.

ABSTRACT: A study of the reaction between ninhydrin and alanine has been carried out taking into account that, adhered on the surface of a dry porous medium such as paper, a quasi-heterogeneous reaction has to take place. Instead of amino acids released from human sweat glands, aqueous solutions of alanine were taken to deliver a given amount of amino acid to the sample. The dye density, obtained after using a standard development process, could noticeably be increased by setting a drop of water on the dye dot, thus indicating that not all the alanine had been used for dye formation during the usually applied process. The incomplete reaction can be explained by the problem of bringing the reactants into contact with each other when both are in the solid phase in the porous surrounding. The temporary presence of water allows a reorientation of the insoluble reactants. With fingerprints an increase in both the rate of development and the final dye density could be obtained when the sample was post treated after the developing process with the blank solvent, thus also the background coloration could be decreased. The ideas presented in this paper may form the basis for a modification of developing processes with ninhydrin in order to increase the proportion of amino acids present (in the sample) used in dye formation without data loss.

KEYWORDS: forensic science, criminalistics, latent fingerprints, ninhydrin reaction

Ninhydrin, as well as some of its derivates, reacts with amino acids to give a purple colored product known as Ruhemann's Purple (1,2). For almost 50 years this reaction has been used for the detection of latent fingerprints on porous surfaces, especially on paper (3,4). However, the results obtained after treating a sample with ninhydrin are often not satisfactory. The reaction rate may be very low and the dye density, which is finally obtained, may be too small to allow reliable interpretation within an acceptable time limit of a few days. Several efforts have been made to try and improve the process and a variety of procedures have been proposed, especially concerning the influence of the temperature and humidity (5–9). Furthermore, some studies on the mechanism of the dye formation in solution were carried out (10–12). However, no attempts have been undertaken to understand the development as a quasi-heterogeneous process, although both the amino acids and ninhydrin are present in the sample in the solid state—apart from a few seconds after dipping the sample into the developing solution.

Usually no dye formation is observed during the few seconds of evaporation of the solvent. Some molecules may react with each other, but the amount of dye formed is too low to be detected visually. In order for the reaction to continue, the reactants ninhydrin and amino acids have to be brought into close proximity to each other and/or be reorientated, which is a difficult process in the absence of any solvent. Therefore, in a very dry atmosphere no further reaction takes place, especially if the reactants are strongly adsorbed on the surface of the cell walls of the cellulose fibers of the paper, such that they cannot adopt a reactive form. High humidity enhances the reaction rate (3) as well as increased temperature. On the other hand, conditions of high humidity and or high temperature are inappropriate as dye formation may be accompanied by strong lateral diffusion of both the reactants as well as the dye resulting in blurred lines and irreparable damage.

A human sweat pore has a diameter of ~0.2 mm (13,14). By touching a porous surface, from each pore up to 3 nL of eccrine sweat can be released on an area of ~0.03 mm^2. This sweat can contain amino acids with a total concentration of up to 1 mM as well as inorganic compounds like sodium chloride, with a concentration of 10 mM (13,14). The more sweat released and the higher the concentration of the components, the more substance transferred to the sample.

For model experiments instead of human sweat, definite amounts of an aqueous solution of alanine or a mixture of several amino acids and sodium chloride may be chosen (13,14). Furthermore, the nL-drop released by a human sweat pore can be substituted by a μL-drop, which is a thousand-fold larger, and on paper covers a hundred-fold larger area and is therefore ten-fold thicker. Thus to transfer the same number of molecules to a given area of the sample, the concentration of the μL drop has to be ten-fold lower than that of the nL drop. After evaporation of the water the components of the solution will get adsorbed on the exterior and interior fibers of the porous material or get crystallized, e.g., sodium choride, on the paper.

The number of molecules of alanine, N_{alanine}, in a 3 μL drop can be calculated from the concentration of alanine, c_{alanine}, (in mol dm^{-3}) and the Avogadro constant N_A (6.023×10^{23} molecules per mol) using the definition for the concentration $c = n/V$ (with n = number of moles and V = volume) and the relation $n = N/N_A$:

$$N_{\text{alanine}} \text{ (in a 3 } \mu\text{L drop)} = 3 \times 10^{-6} \times c_{\text{alanine}} \times N_{\text{A}} = 1.8 \times 10^{18} \times c_{\text{alanine}} \quad (1a)$$

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* Financial support from Fonds der Chemischen Industrie is gratefully acknowledged.

Received 10 Dec. 1999; and in revised form 21 March 2000, 12 May 2000; accepted 26 May 2000.

The molecules are transferred to an area of around 3 mm^2 of the sample. Thus, the number of molecules deposited per mm^2 is:

$$N_{alanine}/mm^2 = 6 \times 10^{17} \times c_{alanine} \qquad \text{(from a 3 } \mu\text{L drop)} \quad (1b)$$

These molecules may be distributed in the area of a dot in the paper (about 0.1 mm thick). Of course, the density should be higher on the top of the paper than at the bottom.

An average of $6 \times 10^5 \times c_{alanine}$ molecules are deposited on an area of only 1 nm^2 ($= 10^{-12}$ mm^2) which corresponds to the size of the Ruhemann's Purple molecule. Thus, at an alanine concentration $>10^{-4}$ M several layers of dye may be formed.

In the same way the number of molecules deposited from nanoliter drops can be estimated:

$$N_{alanine} \text{ (in a 3 nL drop)} = 3 \times 10^{-9} \times c_{alanine} \times N_A = 1.8 \times 10^{15} \times c_{alanine} \quad (2a)$$

As the area covered by a 3 nL drop is only 0.03 mm^2, the amount of alanine deposited on an area of 1 mm^2 is therefore:

$$N_{alanine}/mm^2 = 6 \times 10^{16} \times c_{alanine} \qquad \text{(from a 3 nL drop)} \quad (2b)$$

Thus around $6 \times 10^4 \times c_{alanine}$ molecules are transferred to an area of 1 nm^2. The concentration of alanine has therefore to be ten-fold higher than in corresponding microliter drops to get the same amount of compound per unit area.

While dipping the sample into the developing solution, a large amount of ninhydrin is transferred and, after drying, adsorbed or crystallized inside and outside of the whole sample. The locally adsorbed amino acids may be partially or completely coated by the reagent. The total number of molecules of ninhydrin transferred to a given part of the sample can be estimated from the increase in weight. With a simple dipping process usually 0.5 to 0.8 μg ninhydrin are deposited on 1 mm^2 of a common paper, i.e., many more than 10^{15} molecules per mm^2 or more than a thousand molecules on an area of only 1 nm^2.

In this paper we will first prove by means of a model system the assumption that often a large portion of the amino acid may not be used to form the desired dye. The reasons will be explained from a microscopic point of view. We will then show that a suitable post treatment process may result in a strong increase of the dye density—also for real fingerprints.

Materials and Methods

Compounds

The fundamental experiments were carried out with DL-alanine purum 99% (Aldrich). For the experiments with mixtures of amino acids, the amino acids L-arginine HCl purissimum (SERVA), DL-threonine purum (Roth), histidine HCl purum (Merck) and DL-lysin HCl purum (Fluka) were used. NaCl (Merck) as well as ninhydrin (1,2,3-indantrion × H_2O) (Aldrich) were p.a. quality. Ethanol (no 510, water free, purum) was from the Bundesmonopolverwaltung für Branntwein and petroleum benzine p.a. (40 to 60°C) was from Merck. Usually Xerox Premier TCT 3R91805 paper was chosen as the support medium. For comparison some other papers were used and generally yielded similar results.

Solutions

The aqueous solutions of alanine were prepared by dissolving alanine in distilled water in order to get a final concentration between 0.001 and 4 mM. The aqueous solutions of mixed amino acids were prepared by dissolving alanine, threonine, histidine, arginin, and lysin such that the total was concentration 1 mM. Sodium chloride was then added at a concentration of 0, 0.05, or 0.1 M.

Most often the developer solution was prepared by dissolving 300 mg ninhydrin in 2 mL ethanol and then adding 48 mL petroleum benzine, thus giving a volume ratio ethanol:petroleum benzine of 1:24 and a 0.6% concentration of ninhydrin of 0.033 M. Other compositions were prepared in the same way, varying both the amount of ninhydrin and the organic solvent ratio. Acetic acid was not used.

Deposition of Amino Acids on the Sample

The amino acids were deposited on the porous substrate from aqueous solution with a pipette (1 to 5 μL). The substrate was then left for several minutes to allow the water to vaporize completely and the amino acids to be adsorbed outside and inside the paper. Usually drops of 3 μL were chosen such that dots of around 2 mm diameter resulted on most papers. These then allow an optimum determination of the dye density by means of the densitometer available whose detection surface covers a circle of 2.2 mm diameter.

Development

The development was started by dipping the sample into the developer solution for three seconds. During the subsequent storage of the sample the humidity was controlled by establishing a definite "water vapor pressure" in the air. For most of these experiments 30 to 40% humidity and room temperature were chosen.

Determination of the Dye Density

The dye density was estimated visually for the fingerprints and measured densitometrically for the dots by means of a Digital Transmission Densitometer Cosar 50 (Cosar Corporation, Texas). Usually not only the black and white density was determined, but also the absorbance in the blue, green, and red region of the visible part of the spectra (yellow, magenta, and cyan density). Preferentially, the magenta density was chosen to compare results obtained under varying conditions. The calibration of the densitometer was carried out every day by means of a graded scale. The absorbance of the blank support (around 0.7 for normal paper) had to be subtracted from the measured density to get the dye density. For correct measurements the diameter of the light beam of the densitometer (2.2 mm) had to slightly exceed the diameter of the dots, taking into account a small ring of blank paper.

Results and Discussion

Simple Development of Alanine Dots

In order to get a correlation between the amount of amino acid transferred to a sample and the dye density obtained due to a simple developing process, 3 μL drops of aqueous alanine of a concentration ranging from 0.001 mM to 4 mM were set on paper. After drying these samples in air for at least one day they were dipped for three seconds into a 0.033 M solution of ninhydrin dissolved in ethanol/petroleum benzine (1:24). The samples were then stored at room temperature with a relative humidity of 40%. For the examples displayed on the left hand side of Fig. 1 a solution of 0.011 M ninhydrin was applied, whereas for the development of the dots of the right hand side a 0.033 M solution of ninhydrin was used.

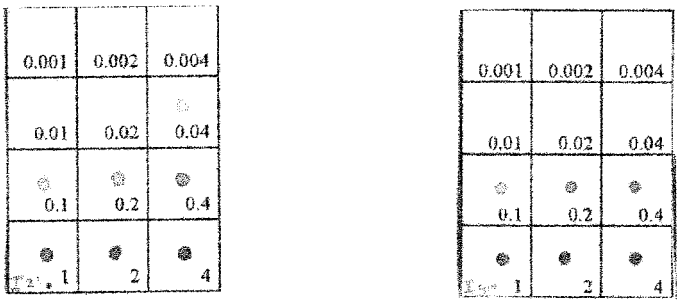

FIG. 1—*Dye-dots obtained by reaction of ninhydrin with alanine which had been deposited from aqueous 3 μL drops on the paper. According to eq (1*a*) the total amount of alanine on each sample ranges from 1.8 × 10^{12} molecules per dot (from 3 μL of a 0.001 mM solution) to 7.2 × 10^{15} molecules/dot (from 3 μL of a 4 mM solution). On the other hand, because of the dipping process in 0.033 M ninhydrin solution around 6.6 × 10^{15}, molecules of ninhydrin are transferred to the area of a 3 mm^2 dot (right hand side example) whereas treatment in 0.011 M developing solution yields around 2.2 × 10^{15} molecules of ninhydrin per dot (left hand side example).*

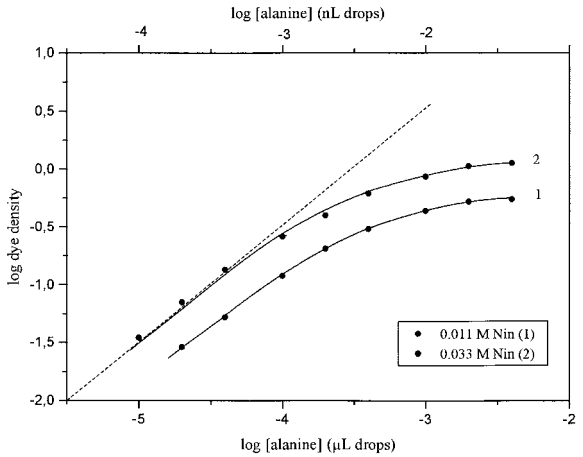

FIG. 2—*Double logarithmic plot of the dye density against the alanine concentration in the applied 3 μL drops. For comparison the corresponding concentration of 3 nL drops is plotted on the upper scale. The dye density was measured two days after developing samples like those shown in Fig. 1. Each curve represents the results of five samples, curve (1) refers to a development process in 0.011 M ninhydrin solution, curve (2) to that in a 0.033 M developing solution. The dashed straight line has a slope of 1.*

In the case of a complete chemical reaction the amount of the obtained dye should equal that of alanine as long as the number of ninhydrin molecules nearby exceeds that of alanine by at least the factor two (10–12). A logarithmic plot of the dye density against the concentration of alanine should therefore give a straight line with a slope of 1. Figure 2 shows two graphs of this kind for results like those presented in Fig. 1. Each point represents the average value of five samples treated in the same way. The dye density was measured two days after the ninhydrin development. The data in the lower curve (1) refers to samples developed in 0.011 M ninhydrin solution, whereas the upper curve (2) contains the results of samples developed in 0.033 M ninhydrin solution.

Both graphs show that only at low concentration of alanine the dye intensity increases linearly with an increasing amount of alanine (dashed straight line). However, the more alanine deposited on the paper, the greater is the deviation from the proportionality. The dye density of the dots developed in the more concentrated ninhydrin solution is higher by a factor of 2.5 over the whole concentration range of alanine (the difference of the logarithm of the dye density of the two curves in Fig. 2 is around 0.4). Further storage of the samples for several weeks under the conditions mentioned above decreased the difference a little but not completely. It is obvious that not all of the alanine has been used for the dye formation (results presented in curve 1) and it is although probable that also the results in curve 2 do not correspond to the maximal possible dye. This assumption is supported by experiments varying the concentration of ninhydrin in the developing solution over the full range possible with the solvent mixture chosen for the experiments displayed in Figs. 1 and 2 as well as for several other ratios of ethanol/petroleum benzine (in order to improve the solubility of the reactant). Usually decreasing the concentration of ninhydrin resulted in decreasing dye density. On the other hand, even a very high excess of ninhydrin could not guarantee the theoretically expected dye density. In order to prove that under the chosen conditions of storage a certain amount of unreacted alanine remains on the sample, further experiments were carried out.

Post Treatment of Developed Samples with Aqueous Ninhydrin or Water

Two days after the development with ninhydrin, from each of both series, i.e., samples developed in 0.011 M ninhydrin solution and in 0.033 M ninhydrin respectively, three sets were selected. One set of samples with a varying amount of deposited alanine remained untreated as a reference (*a*). Another set (*b*) was post treated by setting a 3 μL drop of water on each dot while a 3 μL drop of aqueous ninhydrin was put on the dots of a third set (*c*). This kind of post treatment should not be confused with a second developing process with ninhydrin dissolved in organic solvents. As long as water is present (at least 3 min) both a homogeneous reaction and a reorientation of the rectants can take place. Thus, the formation of further dye with residual alanine may be started. Of course, this procedure cannot be applied for fingerprints, because of the loss of localized information.

On the third day after carrying out the developing process (one day after the post treatment), the new dye density was measured. Figure 3 displays the results for the samples developed in the

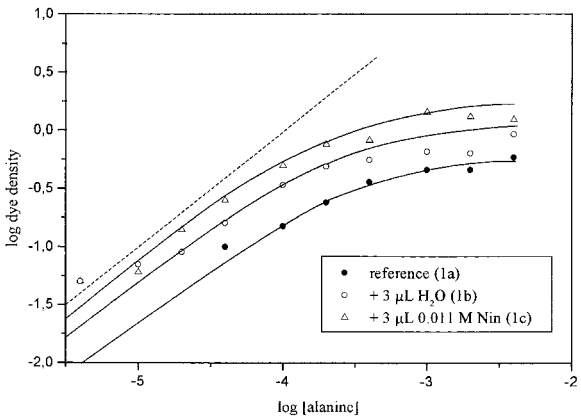

FIG. 3—*Increase of the dye density referring to the results displayed in Fig. 2, curve (1), due of a post treatment process of a sample with water (curve 1*b*) and with an aqueous solution of 0.011 M ninhydrin (curve 1*c*). The density of the single dots was determined one day later, i.e., three days after carrying out the developing process. For comparison the dye density of untreated dots after storage for three days, is also included (curve 1*a*). The dashed line with gradient 1 has the same position as that in Fig. 4.*

0.011 M ninhydrin solution and Fig. 4 the samples treated with the more highly concentrated developing solution. The dye intensity had only increased slightly in the case of set (*a*) but significantly more in the case of sets (*b*) and (*c*).

The results displayed in Fig. 3 show that post treatment with water causes an increase in the dye density by a factor of 2 (increase of log density = 0.30) for each dot in the sample. If aqueous 0.011 M ninhydrin solution is applied instead of water, the density becomes as much as threefold higher (increase of log density = 0.48). Thus, a large amount of so far unreacted alanine could further be coupled with ninhydrin because of the reorientation of the molecules during the temporary presence of water. Additional ninhydrin favors this process. Repeated post treatment with water as well as with aqueous ninhydrin yielded in a slight increase in the dye intensity of dots containing a large amount of alanine but could not further enhance the dye intensity of the dots with low amount of alanine.

The samples developed in 0.033 M ninhydrin carry a higher amount of ninhydrin ($>10^3$ molecules/nm^2). Surprisingly simple post treatment with water gives a stronger increase in dye density than post treatment with an aqueous ninhydrin solution. This result supports the observation that a very high concentration of ninhydrin in the developing solution is disadvantageous, not only because of its poor solubility, but also because of a decrease in the efficiency.

The curve of highest dye density in Fig. 3 almost reaches that in Fig. 4. For a better comparison the dashed straight line with slope 1 was included in both figures. It is obvious that dots containing only a small amount of alanine might be developed almost completely, whereas dots containing a large amount of alanine will remain as a huge amount of unreacted amino acid even after carrying out a post treatment process of this kind.

Bar Diagram of the Results

The large amount of unreacted amino acid which may survive the simple developing process may better be recognized if the dye density is not plotted logarithmically. As an example, in Fig. 5 the results displayed in Fig. 3 are presented in the form of a bar diagram. The first column of each group refers to the reference sample (1*a*), the second one to the sample post treated with water (1*b*), and the third one (1*c*) represents the sample which was post treated with 0.011 M aqueous ninhydrin. If there is a fourth column it presents the results of a cross experiment, i.e., a sample developed in 0.011 M ninhydrin was post treated with 0.033 M aqueous ninhydrin solution.

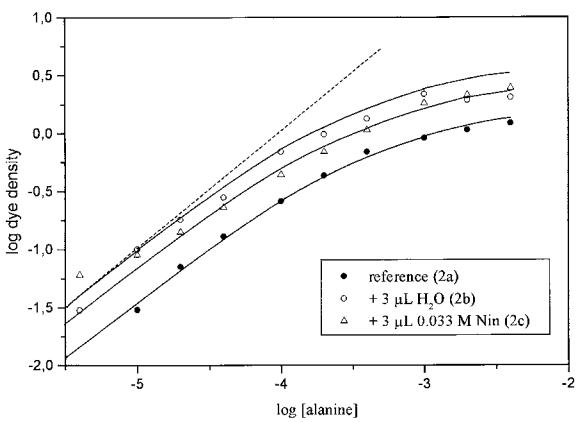

FIG. 4—*Results displayed analogously to those in Fig. 3, but obtained with the samples developed in the more highly concentrated ninhydrin solution. Curve (*2a*) refers to the untreated set of dots, curve (*2b*) to the sample post treated with water and curve (*2c*) to the post treatment process with aqeuous 0.033 M ninhydrin solution. The dashed line with slope 1 may here indicate complete dye formation.*

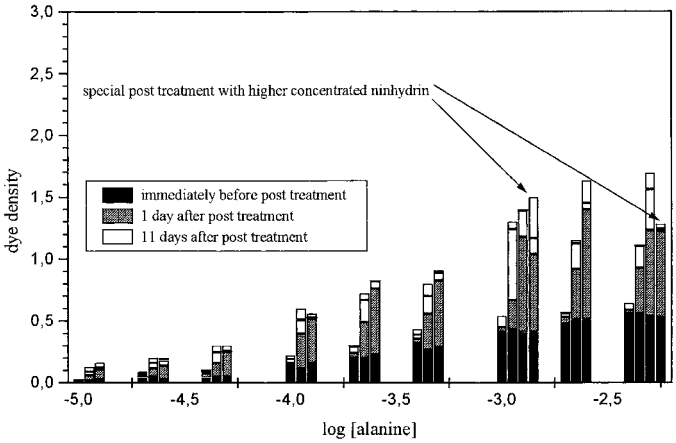

FIG. 5—*Results displayed in Fig. 3 in the form of a bar diagram with a nonlogarithmic plot of the dye density. Additionally the increase in density after up to two weeks storage is included. For details see text.*

Each column has a black basis. This part gives the dye density measured two days after dipping the sample into the developing solution but before carrying out a post treatment process. The shaded part of the column indicates the increase of dye density between the second and the third day. This area is usually very small for the reference samples but large in cases of post treatment processes. The white part gives the additional increase in dye density during subsequent storage for almost two weeks at room temperature and a relative humidity of 40%. During the following weeks almost no further increase in dye density was observed.

Discussion of the Model Experiments and the Post Treatment with Water

What is the reason for the incomplete dye formation after treating the sample in the usual way by dipping it in the developing solution? It is the problem of carrying out a complicated chemical reaction in a nonliquid state. Two molecules of ninhydrin are necessary to give the desired Ruhemann's Purple. On the other hand, from the amino acid only the nitrogen atom is used so an aldehyde as well as carbon dioxide has to be released (10,11). If there is insufficient ninhydrin availabe, a 1:1 reaction between the amino acid and ninhydrin takes place consuming amino acid but not producing the Ruhemann's Purple (15,16). Furthermore, water is both consumed and produced during the reaction and may act as a catalyst (10,11). Often acetic acid is used as it provides the catalytic protons needed in the ninhydrin reaction. Moreover, some water is necessary to allow reorientation or even diffusion of at least one of the reactants. However, too much water may cause diffusion of both the reactants and the formed dye resulting in blurred lines. It is very useful to consider all these reactions and side reactions from a microscopic point of view in order to get an idea how it might be possible to influence the reaction in the desired direction, i.e., a locally fixed but complete use of the amino acid for the formation of the dye.

Each molecule of alanine needs an adsorption area of ~0.3 nm^2. However, irregular surfaces are formed with adsorption or crystallization occurring in several layers while some parts of the surface may remain free from alanine. According to equation (1*b*) from a 3 μL drop a monomolecular layer of alanine might be obtained with a solution concentration as low as 1×10^{-6} M. But both examples displayed in Fig. 2 as well as many other experiments of this kind show that at this low concentration a visually detectable dye may only be formed if alanine is accumulated at the same points. On the other hand, more than a hundred layers of alanine may locally be accumulated in the sample from a drop with a concentration >1 mM.

Dye formation may begin at the border of the reactants but the reaction rate rapidly decreases because of the separation of the reactants by the formed product which may be the dye or only an intermediate. Besides reorientation, either the amino acid or the ninhydrin has to diffuse through the already formed product to meet the other reactant. This diffusion process can only take place in the presence of a solvent. As water may allow diffusion of both reactants, an organic solvent is better because then only ninhydrin becomes mobil. However, the solvent used for the dissolution of ninhydrin rapidly evaporates and consequently the reaction stops.

As mentioned above, water cannot be added directly to a sample carrying a fingerprint because alanine would diffuse rapidly away, resulting in an enlargment of the dot; however, a slight effect of this kind may be advantageous to combine the individual dots produced by separated sweat pores to the desired line. For this reason water is better taken in from the atmosphere, and then the developing process should be carried out in an atmosphere of high humidity. Both the amino acid and ninhydrin can diffuse in the presence of water but again it should be mentioned that it is advantageous to favor the diffusion of ninhydrin in order to avoid over enlarging the alanine dot and subsequent blurring.

It is interesting that the concentration of ninhydrin in the developing solution, i.e., the amount of ninhydrin deposited on the sample, influences the diffusion processes. A thick layer of ninhydrin, inside and on the sample, may hinder water in reaching the zone of possible dye formation.

The entrance of water molecules necessary for the diffusion and reorientation of the reactants may be favored by other compounds not directly involved in the reaction of interest, the most important of which are inorganic ions like halides. For this reason experiments have been carried out with aqueous solutions of amino acids both in the absence and in the presence of sodium chloride. In the presence of salt, the dye formation occurs noticeably faster. On the other hand, here a development at high humidity may cause some problems because the final dye density is reduced. The reason for this effect can not yet be completely explained.

Post Treatment of Both Alanine Dots and Fingerprints with the Organic Solvent

It is well known that repeated dipping of a sample into the developing solution may increase the dye density (17). Also the examples displayed in Fig. 6 show that repeated developing improves the obtainable amount of dye (compare the dots on the sample in the middle with those on the left hand side). Under the conditions of the experiments, however, much more dye was obtained when intermediate washing was carried out (right hand sample). It can therefore be assumed that a second developing process may predominantly be useful because of the reorientation of the reactants and less because of the addition of further ninhydrin. High amounts of ninhydrin are disadvantageous due to strong coloration of the sample.

Experiments of this kind were also performed with fingerprints, some examples are displayed in Fig. 7. Some days after setting the fingerprint the samples were divided and all four segments developed in 0.033 M ninhydrin solution. After briefly drying in air, one sample was dipped again into the developing solution (lower left hand segment), another one was rinsed with the blank solvent, then after drying, dipped again for only one second into the developing solution, and finally rinsed a second time (lower right hand side segment). It is obvious that during the normal developing and stor-

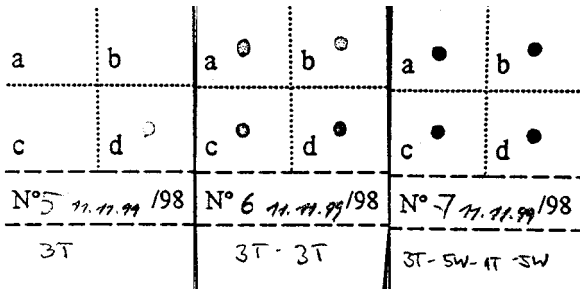

FIG. 6—*Increase of the dye density of four amino acid dots (left hand sample) due to a second developing process (middle sample) and a special post treatment (right hand sample). All samples were first dipped for 3 s into a 0.033 M ninhydrin solution. This process was repeated a few seconds later for the sample in the middle. The post treatment process applied for the right hand sample involved: 5 s washing in blank solvent (petroleum benzine with 4% ethanol), 1 s dipping in the 0.033 M ninhydrin solution and 5 s washing in the solvent. a: 1 mM alanine. b: mixture of five aminoacids (see experimental part) in equimolar quantities and a total concentration of 1 mM. c and d: solution b with 0.05 M and 0.1 M sodium chloride, respectively. T ~ 21°C. $R_0H_0 \leq 30\%$. After 2 days.*

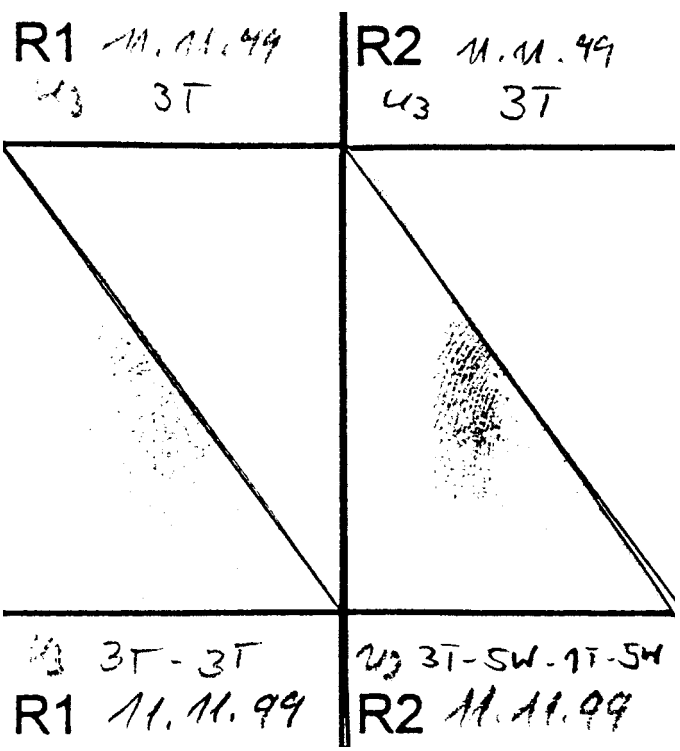

FIG. 7—*Experiments similar to those displayed in Fig. 6, but with fingerprints. For details see text.*

age process not all the amino acid had been used for dye formation, thus only giving partial information. But the post treatment process proposed here resulted in a strong increase in dye density without disturbing coloration of the sample.

Conclusions

The microscopic view of the ninhydrin reaction, combined with suitable experiments, has revealed that under simple developing conditions it is very unlikely to use all the amino acids deposited on a sample for the formation of a colored product that allows the visualization of a fingerprint. The knowledge that after treating a sample with ninhydrin there may remain a large amount of unreacted amino acid, which could still be available to get more information, should encourage studies aimed toward improving the development process while taking into account the reasons for the incomplete reaction. Often a reorientation of the molecules by applying nothing more than an organic solvent may be sufficient to bring ninhydrin into contact with residual amino acids. A very low amount of water, either in the sample, in the solvent, or externally applied during development not only favors the possibility of the reactants to come in direct contact with each other via the diffusion, but also facilitates the chemical reaction. Besides the required humidification during development, it may be advantageous to treat a sample several times alternatively with the blank solvent and the developing solution. It would be most advantageous if the amino acid remains localized, e.g., by strong adsorption, and only ninhydrin is mobilized. Repeated treatment of a sample only in the ninhydrin solution without intermediate washing is not recommended because the layer of ninhydrin becomes very thick. As a result the access of water, which is necessary for the chemical reaction, becomes more and more hindered. Furthermore, ninhydrin will react with some components of the paper yielding strong coloration. This undesired coloration of the whole sample can be avoided when the last process applied is a washing of the sample with the blank solvent.

The experiments described so far have been carried out with relatively large drops. The advantage of μL-drops is the ease of determination of the dye density by means of a densitometer. On the other hand, adsorption as well as some crystallization of amino acids most often does not occur uniformly, especially on slightly hydrophobic surfaces. An excellent agreement with the natural transfer of sweat can be obtained by means of an ink jet printer. The size of the drops from such a printer are in the range of the drops transferred from the sweat pores of a finger onto a porous surface, i.e., in the nanolitre range. The first such experiments have been successfully carried out.

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