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TABLE OF CONTENTS

1. Learning Outcomes
2. Introduction
3. Preparation and Properties of Ninhydrin
4. Ninhydrin as a Fingerprint Reagent
5. Post-treatment of Developed Fingerprints
6. Pre-treatment of Developed Fingerprints
7. Ninhydrin Analogs
8. Summary

1. Learning Outcomes

After studying this module, you shall be able to know –

- The significance of ninhydrin method of fingerprint detection.
- The chemical and physical parameters involved when ninhydrin is being used as a fingerprint reagent.
- The post-treatment and pre-treatment procedures, which enhance the quality of ninhydrin-developed fingerprints.
- An assessment of ninhydrin analogs vis-à-vis the parent compound as fingerprint reagents.

2. Introduction

A latent fingerprint is formed when the sweat pores of the papillary ridges leave a deposit of perspiration on a surface with which the finger has been brought into contact. Human skin possesses three types of sweat glands: Ecrine, apocrine and sebaceous, the secretions of which contribute to fingerprint deposit.

Ecrine glands are widely distributed throughout the body and are particularly numerous on the palms of the hands and the soles of the feet. Besides the water content, eccrine sweat contains up to 1% of other substances of which about one-half are inorganic ions. The remaining half consists of organic derivatives like urea, sugars, lactic acid, fatty acids and amino acids/proteins.

The amino acid content of sweat residue may be fixed by treating the latent fingerprint with a solution of ninhydrin reagent. The amino acid-ninhydrin reaction produces a purple compound, called Ruhemann's purple, which becomes deposited along the ridges, making the latent prints visible.

Ninhydrin is particularly useful for developing fingerprints on porous and absorbent surfaces like paper, paper products, cardboard and wood. Whereas the inorganic ions and most of the organic ingredients of sweat deposit tend to interact with the cellulose content of paper or wood, the amino acids remain inert. Moreover, with passage of time the amino acids do not migrate within the capillaries of the substrate.

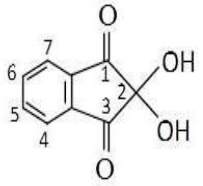
Therefore, the ninhydrin method makes it possible to develop fingerprints that are many years old.

3. Preparation and Properties of Ninhydrin

Ninhydrin (I) was synthesized by Ruhemann in 1910 in an attempt to oxidize 1-hydrindone (II) to 1,2-diketohydrindene (III). However, a di-substituted hydrindone (IV) was obtained, which was subsequently hydrolyzed to ninhydrin.

Ninhydrin crystallizes as pale yellow prisms from ethanol solution. On heating in the solid state it changes to pink or red color at 125-130°C, becomes deep purple at 130-140°C, and finally melts with decomposition at 241°C. When exposed to sunlight, ninhydrin turns red. Therefore, it is stored in a cool, dark place.

Normally, organic compounds having two hydroxyl groups on the same carbon are unstable. However, ninhydrin is stable. It owes its stability to intramolecular hydrogen bonding, as shown in structure (V). When heated in vacuum or when treated with thionyl chloride, ninhydrin dehydrates to 1,2,3-indanetrione (VI). Compounds, such as (VI), having a vicinal tri-ketonic function are also useful for developing fingerprints, although the results are not as good as of ninhydrin.



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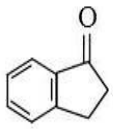

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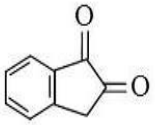

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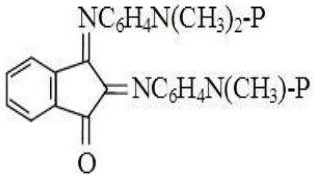

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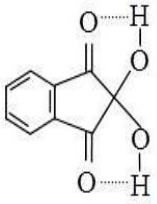

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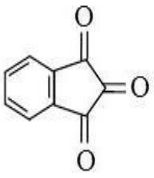

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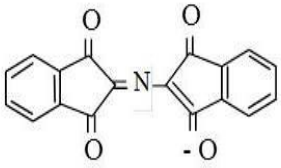


(VII)

The most useful reaction of ninhydrin is with amino acids. A purple colored compound is formed as a result of this reaction. This product has come to be called Ruhemann's purple or RP (VII). The reaction between ninhydrin and a representative amino acid is displayed in Fig. 1.

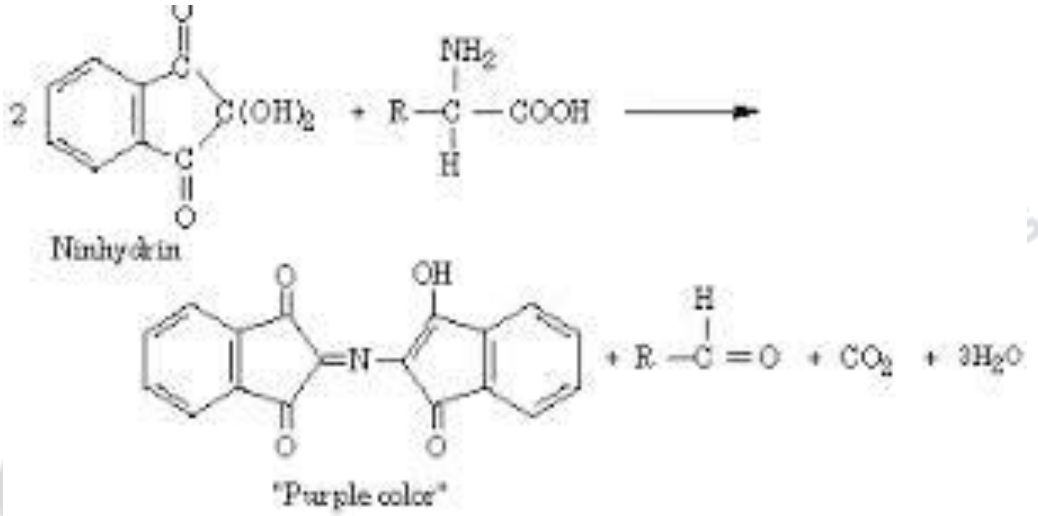


Fig. 1 The reaction between ninhydrin and an amino Acid to yield Ruhemann's purple

The amino acid-ninhydrin reaction has proved to be useful in several analytical procedures. Incidentally, this very reaction makes ninhydrin a reagent for fingerprint development.

4. Ninhydrin as a Fingerprint Reagent

Ninhydrin was first used as a fingerprint reagent in 1954, when two Swedish scientists, S. Oden and Von B. Hofsten developed latent imprints on paper, cardboard and similar materials with its aid. A year later, Oden patented the procedure.

Broadly, the procedure for developing latent fingermarks involves spraying the ninhydrin solution on the surface containing the impression, from a distance of about 6 inches. After the solvent evaporates, the solution is re-sprayed. The surface is then heated for a short time, without allowing it to come into contact with the heat source. If quick development is not required, the item may be allowed to dry at room temperature. Better results are obtained by natural drying. A sample fingerprint developed by ninhydrin method is shown in Fig. 2.

Fig. 2: A fingerprint developed by ninhydrin method

During experimentation, there are several parameters that need to be adjusted for adequate and satisfactory development of fingerprints. There are chemical parameters, such as the concentration of ninhydrin, the nature of solvent and the acidity of the formulation, as well as physical parameters, such as the mode of application, heating temperatures and relative humidity.

a. Chemical parameters

Concentrations of ninhydrin varying from 0.2 to 1.5% have been found suitable for fingerprint development. Various types of solvents have been tried out for preparing ninhydrin solutions. The reagent dissolves in most organic solvents, such as methanol, ethanol, acetone, diethyl ether and naphtha. The choice of the solvent depends, in part, on whether the document is scripted or not. Most organic solvents tend to despoil the calligraphic script.

Freon-113 (also called fluorisol) proved to be the most remarkable solvent for dissolving ninhydrin. It does not interact with ink. It is absolutely non-reactive and therefore there is no chance of the surface becoming degraded. It is non-toxic and therefore poses no occupational hazard to the user. Above all, it is non-flammable and therefore quite safe to use in a laboratory. As a result of this last characteristic, the Freon-based composition has come to be referred to as non-flammable ninhydrin or NFN.

The NFN develops fingermarks on a wide range of paper items. It provides a minimal amount of background effects. Moreover, it is effective on surface-coated papers, such as currency notes, as well as the gummed side of postage stamps.

It is, however, being feared that it may not be possible to continue with NFN in the years to come. Freon-113 belongs to a class of compounds called chlorofluorocarbons. These compounds are being banned out under the terms of Montreal protocol on phasing out of ozone depleting substances. There is therefore, a need for a suitable replacement of Freon-113. Petroleum ether provides an alternative, as it too does not interact with ink.

However, petroleum ether is highly inflammable and explosive in nature and is therefore considered hazardous. Supercritical carbon dioxide is being considered a possible substitute of Freon-113.

b. Physical Parameters

Ninhydrin is applied to the surface impinged with the latent fingerprints generally by spraying, swabbing or dipping. Of these, dipping operation is most extensively used. However, fragile paper items, such as paper napkins, tear apart on dipping and therefore spraying procedure is followed (Fig. 3). Swabbing action tends to smear the ink and is therefore avoided on scripted documents. Less conventional methods include exposure to ninhydrin fumes, dusting with ninhydrin powder or placing a paper dipped in ninhydrin solution over the fingerprint impression.

` **Fig. 3 A ninhydrin spray can**

If development time is not a consideration, ninhydrin-sprayed documents should be allowed to develop at room temperature. This may take a few days, but the results are excellent. Normally however, the surface is heated 80° C so as to accelerate the development process.

The mode of heating is also important. Heating may be carried out by a steam iron; better results are obtained if the iron is moved 1-2 cm above the surface. Most forensic science laboratories have adopted this procedure (Fig. 4). Others have shifted to microwave ovens for heating and steaming. In the absence for these equipments, the object may be waved over a flask containing boiling water.

Fig. 4 Steam iron as a heat source for developing fingerprints

In either case, heating has to be carried out in a humid atmosphere. Optimum development of fingerprints occurs at a relative humidity of 65-80%. Modern fingerprint laboratories are endowed with ninhydrin acceleration chambers, one variety of which is shown in Fig. 5.

Fig. 5 A ninhydrin acceleration chamber

There is a provision to control temperature, as well as to maintain humidity in these chambers. Paradoxically, if the developed prints are kept in humid atmosphere, they fade out at an accelerated pace. Light and heat are other factors which cause fading of imprints. When ninhydrin-developed prints are made fluorescent by post-treatment with zinc chloride (see next section), they fade out on exposure to sunlight or xenon arc light. In presence of moisture or high humidity, the fluorescent character is lost-and so is the clarity of the prints. To extend their lifetimes, it is recommended that the ninhydrin-developed prints be stored in a dark place and at a low relative humidity.

5. Post-treatment of Developed Fingerprints

Although the ninhydrin method is considered to be a standard procedure for developing latent fingerprints, it suffers from a number of demerits. The chemical reaction between ninhydrin and the amino acid content of sweat deposit (Fig. 1) is quite slow and therefore, the developing time is quite long.

Many surfaces interact with ninhydrin, giving a strong background coloration that masks the developed prints. Further, the concentration of amino acids in perspiration is quite low and hence the developed prints usually do not show a sharp contrast. Moreover, the method is not applicable to non-absorbent and dark surfaces. Lastly, on many items, the ninhydrin-developed prints tend to fade out within a very short span of time.

In order to avoid these problems, and to improve the working performance of ninhydrin reagent, the procedure for fingerprint development may be modified. The modifications involve the conversion of non-fluorescent Ruhemann's purple (VII) deposited along the ridges, into a fluorescent derivative by post-treatment of the developed prints with a suitable metal salt. The fluorescent deposition shows a marked enhancement in clarity and sharpness when illuminated by a light source.

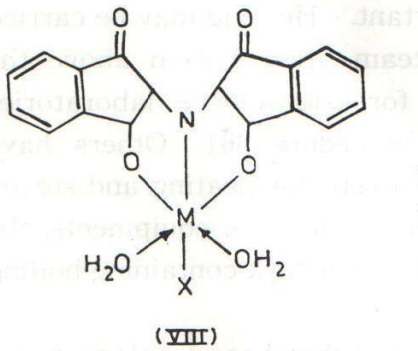
a. Post-treatment with metal salts in concert with arc lamps

Ruhemann's purple (RP), the reaction product of ninhydrin with amino acids, forms coordination compounds with many metals. The metal-RP complexes are generally red or orange in color. Some of these are fluorescent in nature. This reaction may be used to overcome the contrast problem of ninhydrin-developed prints, as well as to improve the stability of fingermarks.

The reaction of Ruhemann's purple with a Group 12 metal salt, like halides or nitrates of zinc(II), cadmium(II) or mercuric(II), in an ethanol-Freon-113 solvent mixture, produces a photo-luminescent complex [VIII; M = Zn (II), Cd (II) or Hg (II); X = halide or nitrate ion].

However, invariably, zinc chloride is the reagent of choice for post-treatment operations. After the solvents evaporate, the sample is dipped in liquid nitrogen so as to bring down the temperature to -200° C.

When examined under an arc lamp, intense fluorescence is produced. A xenon lamp is the most common source for illuminating prints developed by ninhydrin and post-treated by zinc chloride. A Watkin's indium lamp, a Kent's Quaser lamp or a Unilite lamp may also be used for illuminating the fingermarks.



(VIII)

This procedure offers several advantages. The post-treatment may be carried out only when it is deemed essential. If a sufficient degree of contrast is produced with ninhydrin alone, the subsequent steps may be avoided. No expensive equipment like a laser is required. Further, prints may be photographed using conventional fingerprint cameras.

b. Post-treatment with metal salts in concern with lasers

The arc lamp induced fluorescence, is no doubt a cost-effective method for developing latent fingerprints, it suffers from a demerit. The fact that the post-treatment has to be carried out at -200°C, rules out the examination of larger exhibits removed from the scene of crime, as it is difficult and impractical to store cryogenic nitrogen in big tanks.

The problem may be obviated by substituting the conventional arc lamp by sophisticated laser equipment. With lasers there is no need to plunge the sample into liquid nitrogen, except in cases requiring special handling. Moreover, the ridge deposit produces a stronger fluorescence with a laser than with an arc lamp. Laser excitation enables detection of very old prints after these have been developed with ninhydrin and post-treated with zinc chloride.

Fingerprints developed by ninhydrin-zinc chloride method become highly fluorescent under the argon laser light. The 480 nm line of argon laser is appropriate for excitation of the orange zinc-RP complex that absorbs at 485 nm and emits at 560 nm (Fig.6).

Further, with zinc chloride post-treatment and argon laser examination, the ninhydrin method may be extended to the detection of latent fingerprints on non-porous surfaces, like glass and stainless steel.

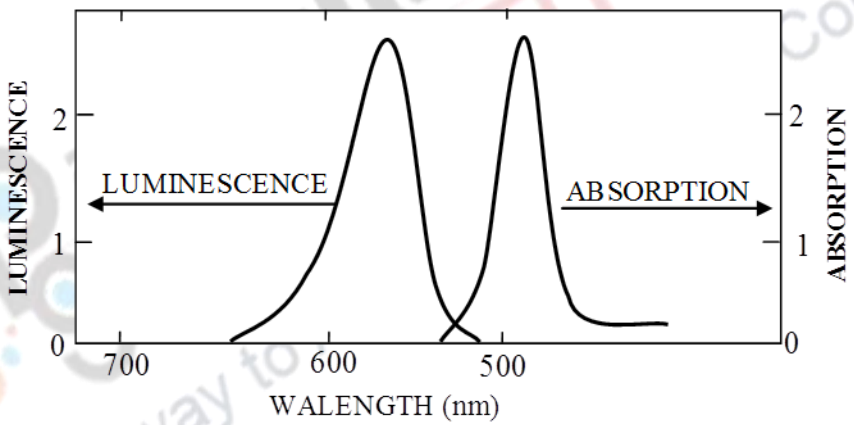


Fig. 6 Absorption and fluorescence spectrum of Ruhemann's purple-zinc chloride complex

The complexes formed by combination of Ruhemann's purple with europium(III) or terbium(III) salts too have been found suitable for fingerprint development, particularly if the background surface of the evidence is fluorescent in nature. These complexes show enhancement of the lanthanide luminescence via intramolecular energy transfer. Moreover, the lifetime of the luminescence is much longer than of the usual background fluorescence. The lanthanide complexes are, therefore, suitable for time-resolved luminescence imaging.

There are a few disadvantages associated with lasers. The casework has to be carried out at the fingerprints bureau. Portable lasers, which may be carried to the scene of crime, are not yet available. Laser equipment is quite costly. When optical spectroscopic techniques are used in combination with lasers, the operational procedures become tedious and complex. However, the end results obtained with lasers are so remarkable that one becomes inclined to believe that their merits outweigh the demerits.

6. Pre-treatment of Developed Fingerprints

The amino acid content of sweat deposition is quite small and therefore ridge details are often not visualized clearly with ninhydrin, even after post-treatment with zinc chloride and laser examination. To improve the sharpness of the developed prints, pre-treatment with hydrolytic enzymes like trypsin may be tried. These enzymes hydrolyze the protein content of perspiration to amino acids, thereby increasing the chances for enhancement of developed fingerprints. The hydrolysis reaction is shown in fig. 7.

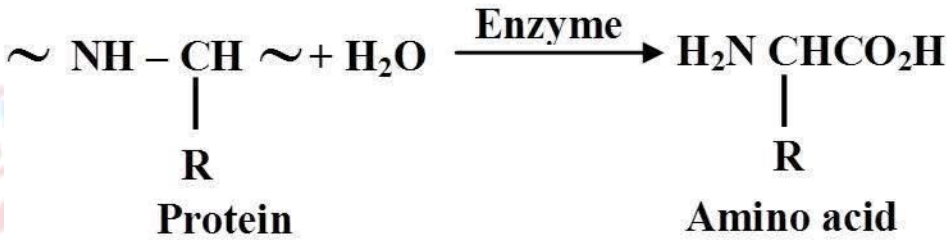


Fig. 7 Enzyme-catalyzed hydrolysis of proteins to amino acids

Initial attempts to pre-treat the fingerprints samples proved futile because the enzymes were dissolved in non-aqueous solvents in which they denatured. Subsequently, however, the dusting of powdered enzymes before the ninhydrin treatment was tried. This gave pronounced enhancement of latent fingermarks, especially those that were 2 weeks to 3 months old.

The methodology involves the following steps: The enzyme is applied by dusting over the fingermark and the sample is incubated for about 40 minutes at 35-40° C under a relative humidity of 80-90%. The article is then dried and excess enzyme is blown off.

This is necessary since the enzymes too react with ninhydrin giving colored compounds. The dried, enzyme-free sample is treated with ninhydrin and re-incubated for about 18 hours. If ridge details are still not clear, further treatment with zinc chloride, followed by laser examination may be carried out.

Enzyme pre-treatment method cannot be applied to routine samples. It requires a long developing time. It is unsuitable for fieldwork. The technique may be tried out over articles bearing extremely weak fingerprints.

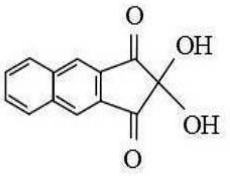
7. Ninhydrin Analogs

Till the early 1980s, it was endeavored to improve the ninhydrin method by altering the solvents, the composition or by pre-treatment / post-treatment of developed prints.

Later, a different approach was adopted: Several derivatives of ninhydrin were synthesized and tried out for developing latent fingerprints. It was anticipated that the derivatives of ninhydrin, possessing the di-ketone function, would react with amino acid content of sweat in the same way as the ninhydrin itself. With several ninhydrin analogs, this expectation turned out to be true.

The most promising ninhydrin analog is benzo [f] ninhydrin (IX). This reagent develops dark green colored fingerprints with excellent resolution. Improved sharpness was observed on treating benzo [f] ninhydrin-developed prints with zinc chloride, followed by examination under an argon laser. However, still better results were obtained with the frequency-doubled neodymium: yttrium aluminum garnet (Nd: YAG) laser. The emission wavelength of the latter device (532 nm) closely matched with the absorption band of the benzo [f] ninhydrin-amino acid-zinc chloride complex. An isomer of benzo [f] ninhydrin, benzo [e] ninhydrin (X) gives good quality, pink colored prints.

Another ninhydrin derivative, which develops latent fingerprints with appreciable degree of sensitivity, is 5-methoxyninhydrin (XI). On paper, this reagent produces fingerprints as efficiently as ninhydrin. Yet, fluorescence after zinc chloride treatment is considerably stronger that with ninhydrin, particularly when examination is carried out by a copper-vapor laser. 5-methoxyninhydrin has also proved useful for developing fingerprints on counterfeit currency.



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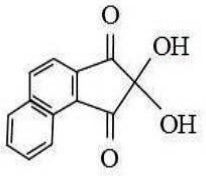

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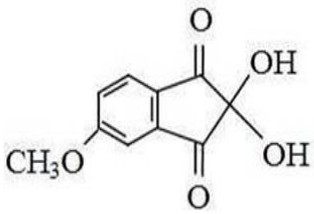

(XI)

Benzo[f] ninhydrin and 5-methoxyninehydrin offer operational advantages over ninhydrin. Prints developed with either of these reagents showed stronger room temperature luminescence after zinc(II) or cadmium(II) treatment in comparison with similar prints developed with ninhydrin. These were also effective for lifting fingerprints on cardboard, where background luminescence precluded the successful enhancements of ninhydrin developed prints.

8. Summary

Ninhydrin is one most suitable reagent for revealing latent fingerprints on absorbent surface like paper, cardboard, raw wood and plasterboard. It is effective in visualizing both the fresh and the old imprints. Its handling requires only a meager degree of skill and experience. It poses no occupational hazards, provided some simple safety precautions, like using gloves, splash-proof goggles and fume cupboard are observed.

Ninhydrin is a versatile reagent in the sense that its formulations are amenable to modifications. Results may be improved by changing the concentration, solvent, heating temperature, heating mode or by pre-treatment of imprints. Enhancement may also be achieved by post-treatment of developed prints by metal salts in combination with arc light or, better still, with lasers. Even if this methodology fails, there is always the option of using a ninhydrin analog for fingerprint development. It is, therefore, not surprising that ninhydrin has been nicknamed as the *latent print examiners chemical workhorse*.