



Fingerprint Detection using Ninhydrin

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Abstract—This study aims to examine the formation of Ruhemann's blue through the reaction between ninhydrin and L-alanine, observing color changes as heat is applied. Additionally, it explores the development and comparison of fingerprints on various paper types using a ninhydrin solution and a water-soluble ink pad.

I. INTRODUCTION

Dating back as early as 1750 B.C., fingerprints have long been used for identifying individuals. Initially, they were primarily used to confirm identity or ownership, serving as an unforgeable signature. Since the late nineteenth century, however, fingerprints have become integral to criminal forensics. At crime scenes, fingerprints collected from surfaces or items of interest are used to connect suspects to specific locations or objects.

In 1954, the application of ninhydrin revolutionized fingerprint detection. Ninhydrin, a chemical powder soluble in ethanol or acetone at room temperature, reacts with amino acids present in fingerprint residues, producing a dark purple color known as "Ruhemann's purple." The color intensity can also serve as a quantitative indicator of amino acid concentration in the sample.

This experiment primarily aims to evaluate the effectiveness of ninhydrin in revealing latent fingerprints on various surfaces commonly encountered in forensic investigations. By applying ninhydrin to different materials, we seek to assess its ability to produce clear, identifiable ridge patterns, potentially enhancing fingerprint analysis techniques. The results will be compared to those obtained from traditional ink pad-based fingerprints.

II. THEORY

Ninhydrin solution can be prepared in different solvents, such as ethyl, isopropyl, or butyl alcohol. The ninhydrin test is widely used to detect amino acids and proteins and is commonly applied in analyzing amino acid spots in paper chromatography. In forensic chemistry, ninhydrin is frequently used to reveal latent fingerprints on porous surfaces like cloth, paper, and cardboard.

The amino group of a free amino acid reacts with ninhydrin, which serves as an oxidizing agent. Upon application, the amino acid undergoes oxidative deamination, resulting in the release of CO₂, NH₃, and an aldehyde, as well as hydrindantin, a reduced form of ninhydrin. The ammonia then reacts with another ninhydrin molecule to form diketohydrin, also known as Ruhemann's complex, which produces the characteristic deep blue color of the reaction. The blue color intensity is directly proportional to the concentration of amino acids and can vary depending on the specific amino acid present.

For optimal results, conditions such as temperature, pH, and humidity should be precisely controlled. It is also important to note that while some individuals leave clear fingerprints, others may leave only faint smudges; therefore, it is advisable to rub fingers through hair or touch oily areas of the face to improve fingerprint clarity. The type of paper used in fingerprint development affects the clarity and quality of the fingerprints due to differences in porosity and absorbance.

- A4 paper is a good absorbent with uniform thickness and a smooth surface, making it effective for holding and evenly distributing the ninhydrin solution. As a result, it produces distinct fingerprints.

- Blotting paper, being more textured and highly absorbent, causes uneven distribution of the ninhydrin solution, leading to inconsistent fingerprint development.
- Filter paper has a highly porous structure, which causes an uneven spread of the ninhydrin solution. Its high absorbency can lead to over-saturation, resulting in diluted ninhydrin and fingerprints with lower resolution.

A4 paper is preferred for fingerprint development due to its absorbance, porosity and uniform thickness, which improve the clarity and quality of the fingerprints compared to Blotting paper.

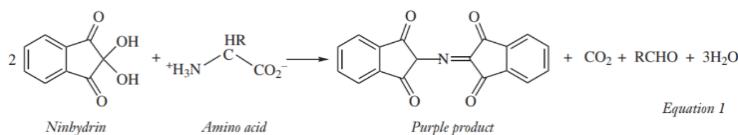


Fig. 1. Chemical reaction between ninhydrine and L-alanine

III. EXPERIMENTAL PROCEDURE

A. Apparatus and Materials Used

- **Test tubes:** For holding and mixing ninhydrin solution with other chemicals.
- **Regular paper (A4) - 2 pieces:** A smooth, non-porous paper that provides even ninhydrin distribution, producing clear and high-resolution fingerprints.
- **Blotting paper - 2 pieces:** Textured and highly absorbent, blotting paper can lead to uneven ninhydrin spread, resulting in less consistent fingerprint quality.
- **Filter paper - 2 pieces:** Due to high porosity and absorbency, filter paper often causes over-saturation, leading to blurry and low-resolution fingerprints, making it the least ideal.
- **Ninhydrin solution:** The primary reagent for fingerprint development by reacting with amino acids.
- **L-alanine solution (25 mmol):** Used as a known amino acid sample to observe ninhydrin's color reaction.
- **Hot plate:** Provides controlled heat to accelerate the ninhydrin reaction.
- **Petri dish:** Contains materials for fingerprint development on a stable surface.
- **Forceps:** A gripping tool used to handle the papers and other materials, preventing contamination from oils on hands.
- **Inkpad, water-soluble:** For creating baseline ink-based fingerprints.
- **Gloves and Goggles:** Safety equipment to protect from harmful chemicals.
- **Droppers:** For precise application of ninhydrin on test surfaces.
- **Water bath:** Provides a gentle and controlled heat source.

B. Methodology

1) Experiment 1

- Calculate the volume of water required to dilute the ninhydrin solution and L-alanine solution to a final concentration of 25 mmol/L.
- Mix the prepared methanol solution of ninhydrin with the L-alanine solution in a 2:1 ratio.
- Cover the test tube immediately with aluminum foil to prevent alcohol evaporation.
- Shake the test tube gently to initiate the reaction, which should produce a light blue color, indicating the initial formation of Ruhemann's blue. Take care not to spill the solution.
- Place the test tube in a hot water bath to allow the reaction to proceed fully. As it heats, the solution will change to a deeper blue, eventually reaching a dark violet color.

2) Experiment 2

- Create fingerprints on sheets of blotting paper, filter paper, and regular A4 paper.
- Wearing gloves, dip each sheet into the ninhydrin solution, allowing it to soak briefly before letting it drain on filter paper. If using a spray method, allow the paper to dry thoroughly before handling with forceps to avoid smudging.
- Once the paper is dry enough, handle it carefully and fan it in the air to allow complete drying.
- Place the paper about 10 cm above a hot plate preheated to 80°C to develop the prints, taking care to avoid scorching. Heat for 2–3 minutes until the fingerprints are visible.
- Observe the appearance of purple prints or spots on the paper.
- Repeat the process on another piece of blotting or filter paper.
- Compare the fingerprints obtained with ninhydrin to those created using the ink pad.

IV. CALCULATIONS

The dilution of solutions is calculated using the formula:

$$M_1 V_1 = M_2 V_2$$

For the ninhydrin solution:

$$100 \times 10^{-3} \times V = 25 \times 10^{-3} \times 4$$

$$V = 1 \text{ ml}$$

Therefore, 1 ml of ninhydrin solution was combined with 3 ml of alcohol to prepare a 25 mmol/L ninhydrin solution.

For the alanine solution:

$$1 \times V = 25 \times 10^{-3} \times 4$$

$$V = 0.1 \text{ ml}$$

Thus, 0.1 ml of alanine solution was mixed with 3.9 ml of water to obtain a 25 mmol/L alanine solution.

V. RESULTS

A. Observation

- 1) *Experiment 1:* The reaction between 25 mmol/L Ninhydrin and 25 mmol/L L-alanine in alcohol produced the Ruhemann's blue complex. Heating the solution in a hot water bath deepened the blue color, confirming the completion of the reaction.
- 2) *Experiment 2:* When fingerprints on blotting, filter, and A4 paper were treated with 100 mmol/L Ninhydrin solution and heated, bluish-purple imprints and spots developed.

B. Explanation

After heating, differences in fingerprint quality were observed across the papers. Fingerprints on regular A4 paper showed high resolution, with clear, detailed ridge patterns. On Blotting paper, the prints appeared with good color intensity but lower resolution. Filter paper yielded the least resolution, with ridges that were less defined and more irregular.

The quality variation is due to material differences between papers. Smooth-surfaced papers like A4 produce clearer imprints, as their uniform texture allows consistent absorption of ninhydrin. Rough, porous papers create less precise prints due to uneven ninhydrin distribution, occasionally forming random spots of Ruhemann's blue. This effect is also impacted by the uneven spread of sweat residues from the fingerprint.

VI. CONCLUSION

In this experiment, Ruhemann's product was successfully synthesized from L-alanine and a methanol solution of ninhydrin, mixed in the correct volumetric ratio. The formation of the product was indicated by the initial light blue solution, which transformed into a deep blue color upon heating in a hot water bath.

The fingerprints made in A4, blotting and filter papers were detected on addition of ninhydrin solution. The fingerprints made in different materials displayed varied properties - intensity and resolution, evidencing the unique absorption properties each material possesses and the ability of the skin oils to react with the amino acids. The inkpad fingerprint matching with that of the obtained results further reinforced the ability of ninhydrin to identify fingerprints.

VII. PRECAUTIONS

- Handle the ninhydrin solution with care, as it is carcinogenic.
- Always wear gloves, safety goggles, and other appropriate protective equipment.

- Keep the paper at a distance of 10 cm above the heating source to avoid the risk of ignition.
- Allow the liquid to dry completely before applying heat, as alcohol solvents are flammable.

VIII. ACKNOWLEDGMENT

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2-Alanine
IP + H₂O

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Date	

Alanine sol.
1M(v)

↓
25 mM, 4mL

$$25 \times 10^{-3} \times 4 \times 10^{-3} = 1 \times x$$

$$x = \cancel{0.05} \text{ mL}$$

Ninhydrine sol.
100 mM(v)

↓

25 mM, 4mL

$$28 \times 10^{-3} \times 4 \times 10^{-3} = 106 \times 10^{-3} \times x$$

$$x = 1 \text{ mL}$$

In Alanine
21/10

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Bleaching paper

A4 sheet

Filter paper



Jyoti

21/10/24