

# Role of Sulfur Compounds in the Detection of Amino Acids by Ninhydrin on TLC Plate

B. Basak, D. Bandyopadhyay, M. Patra, A. Banerji, A. Chatterjee\*, and J. Banerji

Center of Advanced Studies on Natural Products, Department of Chemistry, Calcutta University, 92 A.P.C. Road, Kolkata – 700009, India

## Abstract

Three new sulfur reagents for specific identification of amino acids on thin-layer chromatography plates have been introduced. These three sulfur containing reagents are capable of developing various distinguishable colors with many of them. A probable mechanism for such color formation is proposed.

## Introduction

Several specific and nonspecific reagents have been reported in thin-layer chromatography (TLC) for the detection of amino acids (1–9). Such identification is useful because of the occurrence of these compounds as monomeric units of proteins, in the free state in numerous natural products (seeds and leaves) and as the C-terminal determination of degraded proteins. Ninhydrin, a well-known nonselective color reagent for amino acids, is best used in neutral conditions and produces a purple violet color with all amino acids, except proline and hydroxyproline, because of the presence of secondary cyclic amine groups. The present communication deals with the use of ninhydrin as a spray reagent in presence of three sulfur reagents representing three different class of organic compounds [i.e., thiophenol (aromatic), 2-mercaptoethanol (saturated aliphatic) as well as carbon disulphide (unsaturated aliphatic)]. The most significant aspect of this study is that it readily produces different distinguishable colors on TLC plates. It is very easy and simple to perform.

## Experimental

### Chemicals and chromatographic equipment

Chromatography plates (0.1 mm) were prepared using silica gel G (E. Merck, Mumbai, India) and an Unoplan apparatus

(Shandon, London, U.K.). The samples were spotted on the plates with graduated capillary tubes (5  $\mu$ L) (Spectrochem, Mumbai, India). *n*-Butanol was used as mobile phase. The standard amino acid solution was made in 0.01M phosphate buffer (pH 7.8–8.0).

### Sample preparation

Reagent I consisted of (a) 1% thiophenol (Sigma, St. Louis, MO) in acetone, (b) 1% 2-mercaptoethanol (Sigma) in acetone, and (c) 1% carbondisulphide (E. Merck) in acetone. Reagent II consisted of 0.25% ninhydrin (Sigma) in acetone.

## Results and Discussion

Standard amino acid solutions (Sigma) were spotted on TLC plates by graduated capillary tubes (capacity: 5  $\mu$ L volume) (Spectrochem) and, after TLC, the plates were sprayed successively with the reagents as follows:

The plates were developed in *n*-butanol, dried, and sprayed first with reagent I (a, b, and c differently). After heating at 110°C for 5 min in an oven, the plates were cooled and then sprayed with reagent II. The plates were then air-dried and the colors were observed immediately thereafter. The plates were then heated again at 110°C for 5 min; in the case of reagent I (b), (i.e., 2-mercaptoethanol), the heating time was 10 min. The developed colors were recorded in Table I. Colors developed by various amino acids when thiophenol was used as the spray reagent are shown in Figure 1 (see page 3A). The exact reaction pathways are uncertain, but it may be presumed that at first amino acids formed an additional compound with different sulfur-containing reagents. This additional compound reacted with ninhydrin to form cyclic compounds that were responsible for the development of such different colors. The limit of detection (LOD) of all reagents was 1 ng for all amino acids, except for aspartic acid (in cold), asparagine, and proline, which had LODs of 2 ng when thiophenol–ninhydrin was used as the spray reagent. For carbondisulphide–ninhydrin, the LODs for asparagine and cysteine were 2 ng, whereas for aspartic acid and cystine they were 3 ng.

\* Author to whom correspondence should be addressed.

**Table I. Color Reactions of Amino Acids on Silica-Gel Thin-Layer Plates with Three Spray Reagents\***

| Amino acid     | Thiophenol-ninhydrin            | 2-Mercaptoethanol-ninhydrin | Carbonylsulfide-ninhydrin       |
|----------------|---------------------------------|-----------------------------|---------------------------------|
| Glycine        | No color                        | No color                    | No color                        |
| Alanine        | No color                        | No color                    | No color                        |
| Valine         | No color                        | Light rose                  | No color                        |
| Leucine        | Deep violet (deep violet)       | Rose                        | Purple (grey)                   |
| Isoleucine     | Deep violet (violet)            | Pinkish grey                | Deep purple (grey)              |
| Serine         | Deep blue (deep violet)         | Pinkish grey                | Deep purple (violet)            |
| Threonine      | No color                        | No color                    | No color                        |
| Aspartic acid  | Violet (saffron)                | Light grey                  | Reddish purple (pink)           |
| Asparagine     | Saffron (dirty orange)          | Light grey                  | Light orange (orange)           |
| Glutamic acid  | Violet (yellowish saffron)      | Light saffron               | Saffron (violet)                |
| Glutamine      | Deep grey (deep grey)           | Rose                        | Saffron (deep violet)           |
| Lysine         | Light grey (light grey)         | Grey                        | Light grey (purple)             |
| Histidine      | Light violet (yellowish purple) | Deep grey                   | Deep grey (purple)              |
| Arginine       | Grey (purple)                   | Violet                      | Grey (grey)                     |
| Phenylalanine  | Yellowish violet (saffron)      | Rose                        | Yellowish violet (deep violet)  |
| Tyrosine       | Light violet (violet)           | Faint grey                  | Light rose (light grey)         |
| Tryptophan     | Deep grey (grey)                | Light grey                  | Violet (violet)                 |
| Cysteine       | Pinkish red (light saffron)     | Yellowish grey              | Light red (reddish grey)        |
| Cystine        | Deep pink (yellowish saffron)   | Light violet                | Light violet (yellowish violet) |
| Methionine     | Deep violet (deep grey)         | Violet                      | Purple (violet)                 |
| Proline        | Lemon yellow (pale yellow)      | Lemon yellow                | Lemon yellow (lemon yellow)     |
| Hydroxyproline | Dirty grey (deep grey)          | Orange                      | Light saffron (deep rose red)   |

\* Colors in parenthesis were observable before the TLC plates were heated.

## Conclusion

This method is rapid, easy, and inexpensive by which almost all amino acids can easily be identified by their easily-detectable colors. Moreover, it responded quite well for protein hydrolysate.

## References

1. *Thin Layer Chromatography: A Laboratory Handbook*, 2nd ed. E. Stahl, Ed. Academic Press Inc., New York, 1990, pp. 404–17.
2. A. Bhattachary. Labelling of nucleosides with a fluorescent dye. *J. Indian Chem. Soc.* **64**: 243–45 (1987).
3. S. Laskar and B. Basak. Detection of amino acids on thin layer plates. *J. Chromatogr.* **436**: 341–43 (1988).
4. S. Laskar and B.K. Basak. Identification of amino acids on thin layer chromatography plates. *J. Planar Chromatogr.* **3**: 275–76 (1990).
5. S. Laskar and B.K. Basak. Identification of amino acids with ninhydrin in acid media. *J. Planar Chromatogr.* **3**: 535–36 (1990).
6. B. Basak and S. Laskar. Spray reagents for the detection of amino acids on thin layer plates. *Talanta* **37**: 1105–1106 (1990).
7. S. Laskar, U. Bhattacharya, and B. Basak. Modified ninhydrin spray reagent for the identification of amino acids on thin layer chromatography plates. *Analyst* **116**: 625–26 (1991).
8. B. Basak, U. Bhattacharya, and S. Laskar. Spray reagent for the detection of amino acids on thin layer chromatography plates. *Amino Acids* **4**: 193–96 (1993).
9. A. Sinhababu, B. Basak, and S. Laskar. Novel spray reagent for the identification of amino acids on thin layer chromatography plates. *Analytical Proc. Including Anal. Comm.* **31**: 65–66 (1994).

Manuscript received April 14, 2004.