

Modified Ninhydrin Reagent for the Detection of Amino Acids on TLC Paper

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Abstract

Ninhydrin is the most well known spray reagent for identification of amino acids. Spring with Ninhydrin as a non-specific reagent is well-known and widely used for its remarkable high sensitivity. Using Ninhydrin reagent alone to detect amino acid on thin layer chromatography (TLC) paper is not advisable due to its lower sensitivity. A new spray reagent, Stannus chloride solution (SnCl_2) has been used to detect amino acids on filter paper (whitman paper 14) and TLC paper, silica Gel, 60 F254 TLC Aluminum Sheet 20 x 20 cm Merck- Germany. Al so, modified TLC pre-staining method was used, which only consisted of 3 steps: spotting, separating and color. The improved method was rapid and inexpensive and the results obtained were clear and reliable. In addition it is suitable for screening different amino acid.

Keywords: Amino Acid; Ninhydrin; Modified Ninhydrin Reagent; Stannus chloride Reagent; Thin-Layer Chromatography (TLC) and TLC pre-staining

Abbreviations: TLC: Thin Layer Chromatography; M: Molar; SnCl: Stannus Chloride

Introduction

Identification of amino acid is necessary in the evaluation of protein structure. Thin-layer chromatography (TLC) is an important and inexpensive tool used for amino acid identification. Amino acids are colorless compounds and they can be detected on the chromatogram by using Ninhydrin reagent. Ninhydrin widely used for its remarkable high sensitivity [1,2]. It react with amino acids and produce purple violet color [3] with all amino acids, except proline and hydroxyl-proline, because of the presence of secondary cyclic amine groups [4,5]. In some cases, Ninhydrin reagent may react with amino acid but its detection limits and color contrasts are not so good enough, also certain amino acid chains are degraded. Therefore, separate analysis is required for identifying such amino acids that either react differently or do not react at all with Ninhydrin. In this study, a new spray reagent has been proposed, stannus chloride solution (SnCl_2), for easy identification of various amino acids on thin-layer chromatography plates by developing clear colors. This reagent with Ninhydrin reagent also binds with different amino acids very strongly for the detection of amino acids at trace level. The most significant aspect of this study is that it readily produces strong colors on TLC plates. It is very easy, inexpensive and simple to perform. The detection or identification of amino acids has immense importance in evaluation of protein structure, for determining their occurrence in the Free State in natural products.

Materials and Methods

Standard preparation: 0.1g of each amino acid (glutamine, Leucine, proline, cysteine and cysteine, tryptophan-Sigma, USA) was dissolved in 10ml ethanol and spotted in TLC Aluminum paper.

Reagents preparation

Reagent 1:

- Stannus chloride solution ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$): (This solution freshly prepared) 16 mg of Stannus chloride dissolved in 10 ml (0.2M) of Citrate buffer solution (pH 5.0).
- Citrate buffer solution (pH 5.0): 10g of NaOH dissolved in 100 ml distilled water (2N).

Reagent 2:

Ninhydrin solution: dissolve 0.25 g of Ninhydrin in 12.5 ml of ethanol (95%).

Detection on Plates: Standard amino acids solutions were spotted on the Chromatography plates (silica Gel, 60 F254 TLC Aluminum Sheet 20 x 20 cm; thickness 0.1mm. Merck- Germany) by graduated capillary tubes (capacity: 5 μL volume-Spectrochem). Plates were air-dried and subjected to TLC with butanol- glacial acetic acid and water 12: 5: 3 (v/v) as mobile phase [6]. After development plates were dried and sprayed with the reagent I and then heated at 110°C for 3 min. in an oven. Plates were cooled and then sprayed with reagent II and then heated at 110°C for 4-5 min. Colors were observed visually.

Results and Discussion

A lot of works had been done to develop color reagent for amino acid detection on TLC [7-9]. Successful attempts to modified spray reagents have been developed by several studies [10-12]. The Dichloro-Dicyano-Quinone DDQ [13] and 2, 3-Dichloro-1, 4-Naphthoquinone [14] are the two important reagents that can detect amino acid directly and no need to use Ninhydrin as a detector. Three new sulfur reagents representing three different class of organic compounds thiophenol (aromatic), 2-marcaptoethanol (saturated aliphatic) as well as carbon disulphide (unsaturated aliphatic)] have been developed by [15]. These three sulfur containing reagents are capable of developing various distinguishable colors with many of them, but their detection limited, other reagents developed by [16] and [17] given the same result. Study was done by [18] proposed that introduce p-bromo-benzoic acids the modified spray reagents for the development of color reagents. This reagent is capable of developing various distinguishable colors with many amino acids.

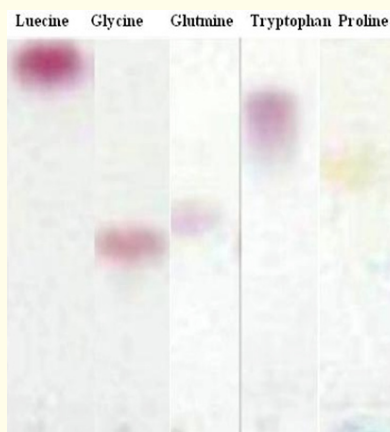


Figure 1: Formation of color by traditional TLC chromatogram of 5 amino acids using Ninhydrin reagent [9].



Figure 2: Formation of color by amino acids using two reagents (2-hydroxy-benzene-carbaldehyde and 4-bromobenzeneecab aldehyde as shown by [19]).

In this study, we proposed the modified spray reagent that contains stannus chloride (SnCl_2) for rapid and easy identification. Thus, our result illustrated in Figure 3 and Table 1 indicated that modified spray reagent give sensitive result by developing specific color. Because the reagents form a variety of colors, they are undoubtedly more effective for detection of the amino acids on TLC plates than Ninhydrin, despite its remarkably high sensitivity [19-21]. The colors were stable for several hours. It is highly sensitive for easy and rapid identification of amino acids on TLC chromate-plates. Furthermore, it shows high sensitivity (0.01-1.0 μg) comparable to Ninhydrin alone.

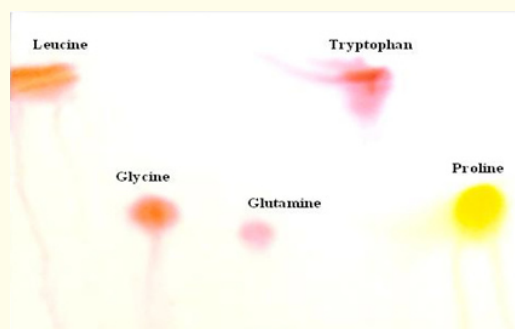


Figure 3: Formation of color by amino acids using stannus chloride (SnCl_2) and Ninhydrin reagents, on silica gel with butanol - glacial acetic acid and water 12. 5: 3 (v/v) as mobile phase.

It was observed that traditional TLC chromatogram used for detection amino acid with Ninhydrin reagent only gives similar colors expect proline, it yield a yellow colored compound. This color can be used to identify it satisfactorily. Our result revealed that Ninhydrin gives various distinguishable colors with amino acids in the presence of reagent 1 after final heating. Color development is not different almost in all the cases after second heating. Such color formations with high sensitivities of this modified spray reagent make it somewhat more useful in the identification of amino acids on TLC plates The profiles of 5 amino acids from this methods were almost identical and the R_f values (see Figure 1 and Figure 2) are not influenced by the modification.

Amino Acids	Color Observation	R _f values
Glycine	Reddish brown	0.40
Leucine	Reddish orange	0.80
Glutamine	Pink	0.33
Proline	Yellow	0.43
Tryptophan	Pinkish violet	0.80

Table 1: Color formation of amino acids on TLC paper with stannous chloride (SnCl_2) and ninhydrin reagents.

The mechanism leading to the color formation may due to the formation of a secondary amide, as proposed by [22], the reaction between stannous chloride with amino acids and the product thus obtained forms coloring complexes with Ninhydrin, color variability depending on the nature of the amino acids. Also, stannous chloride if used alone with Ninhydrin will produce violet-colored which results from the oxidative damnation of a primary amino group by Ninhydrin [23]. Stannous chloride, used in this technique, May served to reduce part of the Ninhydrin and also to prevent oxidation of the colored salt by dissolved oxygen.

In this study, various distinguishable colors have been seen that may due to the presence of either butanol or glacial-acetic acid in a mobile phase. The general requirements of the reducing agent for the Ninhydrin reagent are cheapness, greater solubility in the reagent solution, stable and easy to handle. No harmful reaction for the sensitivity as well as other coloration, inertness to the equipment, and no deposition during use. Advantageously, after extensive investigations and a survey of a desired reducing agent, the present study mentioned that stannous chloride is most promising for the purpose mentioned above.

TLC is widely used as technique. Modified reagent were used in this technique were facilitated identification and sensitivity of amino acid in samples, and give an indicator to the quality of protein in foods for nutritional purposes

Conclusion

The application of this method would be of value in assessment of the amino acid profile/protein quality also in evaluation of protein structure, for determining their occurrence in the Free State in natural products. Furthermore this modified method is rapid, easy, and inexpensive. In comparison with the traditional thin layer chromatography, the modified thin layer chromatography is clean, and reproducible. Amino acids can easily be identified by their visible colors and no need to calculate their R_f value.

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