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NOTE

A Reagent for the Detection of Amino Acids on Thin-Layer Chromatography Plates

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Detection or identification of amino acids is most important in protein chemistry as they are monomeric units of proteins and also for the determination of their occurrence in natural products. 4-Hydroxyacetophenone-ninhydrin is able to produce various colours when it reacts with amino acids, whereas ninhydrin itself can only give same purple colour with most of them. The detection limits for amino acids for the proposed spray reagent range between 0.1 and 1.0 μg under cold condition and 0.01 and 0.1 μg after heating.

Key Words: Amino acids, 4-Hydroxyacetophenone, Ninhydrin, Thin-layer chromatography.

Thin-layer chromatography is an important tool used for the detection of amino acids using several selective and non-selective reagents[1–16]. Such identification is essential because of the occurrence of amino acids not only as structural units of proteins but also in the free state in numerous natural products and also as the C-terminal units of degraded proteins. It is well known that ninhydrin, a non-selective reagent, is the most popular spray reagent because of its remarkably high sensitivity[2]; however it produces the same purple colour with all amino acids except of proline and hydroxyproline which can produce yellow colouration. An attempt has been made to counter this difficulty (colour problem) using 4-hydroxyacetophenone-ninhydrin as a spray reagent instead of ninhydrin alone. The present communication deals with the aforesaid reagent which can produce various colours when it reacts with different amino acids.

Chromatography plates (20 × 20 cm, thickness 0.1 mm) were prepared using silica gel 'G' (E. Merck, India) and an Unoplan coating apparatus (Shandon, UK). Standard solutions (1 mg/mL) of amino acids (Sigma Chemical Co., USA) were made in 0.01 M phosphate buffer (pH 7.8–8.0).

Reagents: Reagent I: 5% 4-hydroxyacetophenone (Fluka, Switzerland) in acetone (E. Merck, India); reagent II: 0.25% ninhydrin (Sigma Chemical Co., USA) in acetone.

Detection on TLC plates: Standard amino acid solutions were spotted on thin-layer chromatographic (TLC) plates by a graduated micropipette (25 μL). After developing in *n*-propanol -H_2O (70 + 30, v/v)[2], the plates were sprayed with

reagent I, dried in air and sprayed with reagent II. The plates were then air dried and colours were observed after drying. The plates were again heated in an oven at 100°C for 10 min. The colours were again observed just after heating.

The colours obtained and the detection limits for the amino acids in question are presented in Table-1. The proposed reagent (4-hydroxyacetophenone-ninhydrin) produces various colours with many of the amino acids before and after final heating (Table-1). The detection limits obtained by use of the proposed reagent range between 0.1 and 1.0 μg under cold condition and between 0.01 and 0.1 μg after final heating (Table-1). The detection limits under cold condition are either equal to or a little higher than those obtained after final heating. Moreover, the simplicity of use of this reagent with high sensitivities (comparable to ninhydrin itself) and its advantage in rapid identification of amino acids on TLC plates makes it useful for practical purposes.

TABLE-1
COLOUR FORMATIONS OF AMINO ACIDS ON TLC PLATES USING 4-HYDROXYACETOPHENONE-NINHYDRIN AS SPRAY REAGENT

Amino acids	Cold condition*		After final heating	
	Observed colours	Detection limit (μg)	Observed colours	Detection limit (μg)
Glycine	Orange	0.1	Yellowish pink	0.01
Alanine	Pink	0.1	Pinkish violet	0.05
Valine	Pink	0.1	Pinkish violet	0.10
Leucine	Pink	0.1	Pinkish violet	0.10
Isoleucine	Pink	0.1	Pinkish violet	0.10
Serine	Light pink	0.1	Pinkish violet	0.01
Threonine	Rosy pink	0.1	Reddish pink	0.10
Aspartic acid	Light brown	1.0	Violet	0.10
Asparagine	Straw yellow	0.4	Dark yellow	0.40
Glutamic acid	Pink	0.1	Deep pink	0.05
Glutamine	Pink	0.1	Deep pink	0.10
Lysine	Pinkish violet	0.2	Pinkish violet	0.01
Histidine	Dirty pink	0.1	Pinkish violet	0.09
Arginine	Light violet	0.1	Violet	0.09
Phenyl alanine	Dirty pink	0.2	Pinkish violet	0.01
Tyrosine	Light pink	0.1	Pink	0.01
Tryptophan	Dirty pink	0.1	Brownish pink	0.01
Cysteine	Rosy pink	0.1	Rosy pink	0.05
Cystine	Light grey	0.1	Greyish pink	0.10
Methionine	Pinkish violet	0.1	Violet	0.09
Proline	Yellow	1.0	Yellow	0.10
Hydroxy proline	Light violet	0.1	Brownish yellow	0.01

*After spraying reagent II followed by drying in air.

The mechanism leading to such colour development is uncertain. It may be assumed that the carbonyl group of 4-hydroxyacetophenone condenses with α-amino group of an amino acid to form an imine type intermediate which in turn forms a charge transfer complex with ninhydrin.

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