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NOTE

A New Spray Reagent for Identification of Amino Acids on Thin-Layer Chromatography Plates

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Identification of amino acids is extremely important in evaluation of protein structure for determining their occurrence in the free state in numerous natural products and also for the determination of C-terminal units of degraded proteins. Several specific and non-specific reagents have been reported to identify amino acids on thin-layer chromatography plates. Among these, ninhydrin is the most popular due to its high sensitivity. However, ninhydrin produces the same purple/violet colour with most of the amino acids. A new spray reagent (2,3-dichloro-1,4-naphthoquinone) with high sensitivity for easy and rapid identification of amino acids on thin-layer plates has been introduced.

Key words: Amino acids, 2,3-Dichloro-1,4-naphthoquinone, Thin-layer chromatography.

Detection of amino acids is a prime necessity in the evaluation of protein structure, as they are the structural units of proteins and also for the determination of C-terminal units of degraded proteins. Thin-layer chromatography is an important tool used for such purposes using several selective and non-selective reagents[1–18]. It is well known that ninhydrin, a non-selective reagent, is most widely used because of its remarkably high sensitivity[2], but it gives same purple/violet colour with all amino acids except proline and hydroxy-proline which produce yellow colouration. An attempt has been carried out to encounter this colour problem using 2,3-dichloro-1,4-naphthoquinone as a spray reagent. The reagent in question affords some distinguishable and stable colours (more or less from about 24 to 48 h) with many of the amino acids with high sensitivity.

Reagents: Standard amino acid samples were obtained form Sigma Chemcal Company (USA), 2,3-dichloro-1,4-naphthoquinone from Aldrich (USA), n-propanol from Merck (India).

Thin-layer chromatography plates (20 × 20 cm, thickness 0.1 mm) were prepared using silica gel G (Merck, India) and an Unoplan coating apparatus (Shandon, London, UK)

Detection on TLC plates: Stock solutions of (1 mg/mL and 5 mg/mL) amino acids were prepared in n-propanol : water 20 : 80. Sample solutions were spotted

on thin-layer chromatography (TLC) plates with a graduated micropipette (1 μL) and the plates were air-dried for 1 h and then subjected to TLC using n-propanol-water (70 + 30, v/v)[2] as mobile phase. After development followed by complete evaporation of solvents, plates were sprayed with 0.25% 2,3-dichloro-1,4-naphthoquinone in ethanol. The plates were dried in air until solvent of the reagent (ethanol) was completely evaporated off and colours were observed at this moment (cold condition). The plates were then heated in an oven at 110°C for 10 min. The colours were observed after heating. The observed colours before and after heating and the detection limits for the amino acids investigated are presented in Table-1.

TABLE-1
COLOURS FORMED BY AMINO ACIDS ON TLC PLATES AFTER USE OF 2,3-DICHLORO-1,4-NAPHTHOQUINONE AS SPRAY REAGENT

Amino acid	Cold condition		After final heating	
	Observed colours	Detection limit (μg)	Observed colours	Detection limit (μg)
Arginine	Creamy orange	10.0	Bright orange	0.5
Cysteine	Slate colour*	2.5	Greyish lavender	2.0
Cystine	—	>10.0	Light pale rose	0.25
Histidine	Dirty orange	8.0	Yellowish orange	0.8
Isoleucine	Reddish cream	>10.0	Orangish red	3.5
Glutamine	Reddish cream	>10.0	Dull orange	1.0
Lysine	Pinkish orange	10.0	Reddish orange	0.4
Asparagine	Orangish cream	8.0	Dull orange	0.8
Phenylalanine	Dirty reddish cream	8.5	Orangish red	5.0
Serine	Orangish cream	>10.0	Orange	0.5
Threonine	Organish cream	>10.0	Orange	0.6
Alanine	Light creamy orange	>10.0	Dull orange	2.5
Glutamic acid	Light pale rose	>10.0	Light orange	2.4
Valine	Light creamy orange	>10.0	Reddish orange	1.5
Methionine	Creamy orange	10.0	Dull orange	5.0
Aspartic acid	Light pale rose	>10.0	Light pale rose	5.0
Tyrosine	Light pale rose	>10.0	Light pale rose	1.0
Leucine	Off white	>10.0	Pinkish orange	2.5
Glycine	Creamy orange	8.0	Bright orange	1.0
Proline	Pale rose	5.0	Reddish pink	0.5
Hydroxyproline	Pale rose	5.0	Reddish pink	1.0
Tryptophan	Pinkish brown	5.0	Brownish pink	2.0

*Colour changes first from yellow to brownish orange to brown to slate colour.

It has been found that the reagent, 2,3-dichloro-1,4-naphthoquinone is able to produce distinguishable colours with many of the amino acids after heating, and even before heating (Table-1). The reagent enables good sensitivity detection, the detection limit was within 0.25 μg to 5 μg for all the amino acids after heating but it was higher before heating (2.5 μg to >10 μg). It may be noted here that before heating the reagent gives different colours which are different from the colours obtained after heating (Table-1). The most striking feature of the reagent is that the colours are stable and can be seen even after 24 to 48 h. Although the detection limit is less than ninhydrin, the proposed reagent is convenient for detection of amino acids on TLC plates because it can form different stable colours and provides a rapid and easy method of detection of amino acids on TLC plates which makes it useful for practical purposes.

The mechanism leading to such colour formation is uncertain; it is likely that the colour reactions are of charge-transfer type.

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