

## Identification of Amino Acids on TLC Plates with Binary Visualization Reagent

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Thin layer chromatography (TLC) is an important tool for detecting amino acids by several spray reagents. Ninhydrin is the most well known spray reagent for identification of amino acids due to its high sensitivity. But, it produces same purple/violet color with all amino acids, except two imino acids proline and hydroxy proline. A new visualization reagent, benzaldehyde has been introduced here which produces distinguishable colors with almost all the amino acids with moderately high sensitivity (0.01-1.0  $\mu$ g) in the presence of ninhydrin. A probable mechanism for such color formation is also predicted.

**Key words:** Thin-layer chromatography; amino acids; benzaldehyde; ninhydrin

Identification of amino acids is utmost important in the evaluation of protein structure, as these compounds are the structural units of protein and also for determination of the C-terminal units of degraded proteins. Thin layer chromatography is an important tool for the detection of amino acids by variety of specific and non specific spray reagents<sup>1-2</sup>. Among the reagents used, ninhydrin as a non specific reagent is the most well known and widely used for its remarkable high sensitivity<sup>3</sup>. But, it produces same purple/violet color with all amino acids, except proline and hydroxy proline (both produces yellow color). A new spray reagent, benzaldehyde reported here produces various distinguishable colors with ninhydrin and enables convenient and easy detection of them on silica gel G TLC plates with moderately high sensitivity (0.01-1.0  $\mu$ g).

## MATERIALS AND METHODS

### Apparatus

Chromatography plates (20×20 cm; thickness 0.1 mm) were prepared with silica gel G (Merck, India) using Unoplan Coating apparatus (Shandon, London, U.K.).

### Reagents

Standard amino acids were obtained from Sigma (U.S.A) and n- propanol from Merck (India). Reagent I: 1% benzaldehyde (SRL, Mumbai, India) in acetone.

Reagent II: 0.25% ninhydrin (Sigma, St. Louis, MO, U.S.A.) in acetone.

### Detection on TLC plates

Standard solutions (1mg mL<sup>-1</sup>) of amino acids were prepared in 0.01molL<sup>-1</sup> phosphate buffer (pH 8.0) and spotted on the TLC plates by means of a graduated micropipette (5  $\mu$ L). The solutions were diluted according to the required spot concentration. Plates were air-dried and subjected to TLC with n- propanol-water, 70:30 (v/v) as mobile phase. After development plates were dried and sprayed with the reagent I and then heated at 110°C

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for 10 min in an oven. Plates were cooled and then sprayed with reagent II and colors were noted (Table 1). Colors were further observed after heating the plates at 110°C for 10 min (Table 1). Detection limits for the amino acids after use of ninhydrin reagent alone is also given in Table 1.

#### Limit of Detection

The Limit of detection of amino acids were determined by spotting a standard solution (1mg mL<sup>-1</sup>) of the concerned amino acid on to the TLC plate, which was developed with mobile phase and spot was visualized using the reagent and ninhydrin as described in the above section. This process was repeated with successive dilution of the standard amino acids solutions until no detection was possible. The amount of amino acid just detectable was taken as detection limit.

## RESULTS AND DISCUSSION

It is observed from Table 1 that ninhydrin gives various distinguishable colors with amino acids in the presence of reagent I before and after final heating. The detection limits are also substantially low before (0.06-1.0 µg) and after second heating (0.01-1.0 µg) which is quite comparable to ninhydrin alone. The detection limits before second heating are higher than those obtained after second heating. Color development is somewhat 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 than ninhydrin spray in the identification of amino acids on TLC plates.

The mechanism leading to such color

**Table 1.** Color formation of amino acids on TLC plates with benzaldehyde-ninhydrin

Amino acids	'Bezaldehyde + Ninhydrin'				Detection limit of ninhydrin <sup>a</sup>	R <sub>f</sub> values <sup>b</sup>
	Cold condition		Hot condition			
	(before second heating)		(after second heating)			
	Color observed	Detection limit (µg)	Color observed	Detection limit (µg)	(µg)	
Glycine	Light brownish pink	1.00	Pinkish orange	0.08	0.001	0.32
Alanine	Reddish pink	1.00	Deep pinkish red	0.06	0.009	0.37
Valine	Reddish pink	1.00	Deep pinkish red	0.04	0.010	0.45
Leucine	Reddish pink	1.00	Deep pinkish red	0.05	0.010	0.55
Isoleucine	Reddish pink	0.50	Deep pinkish red	0.02	0.200	0.53
Serine	Pinkish brown	1.00	Deep reddish brown	0.02	0.008	0.35
Threonine	Pinkish violet	1.00	Deep reddish brown	0.03	0.050	0.37
Aspartic acid	-	-	Gray	0.20	0.100	0.33
Asparagine	Dirty yellow	1.00	Reddish yellow	0.10	0.100	0.14
Glutamic acid	Light violet	1.00	Pinkish violet	0.08	0.040	0.35
Glutamine	Reddish violet	1.00	Pinkish red	0.03	0.100	0.15
Lysine	Reddish violet	0.10	Reddish violet	0.10	0.005	0.03
Histidine	Purple	0.08	Brownish purple	0.06	0.050	0.20
Arginine	Violet	0.10	Deep violet	0.05	0.010	0.02
Phenyl alanine	Brownish red	0.50	Deep pinkish red	0.10	0.050	0.58
Tyrosine	Light pink	1.00	Pink	0.10	0.030	0.57
Tryptophan	Reddish pink	0.08	Deep reddish pink	0.06	0.050	0.62
Cysteine	Pink	0.20	Deep pink	0.08	0.020	0.38
Cystine	-	-	Light purple	1.00	0.010	0.32
Methionine	Brownish red	0.10	Deep brownish red	0.01	0.010	0.51
Proline	Yellow	0.10	Yellow centre with brown ring	0.06	0.100	0.26
Hydroxy proline	Reddish pink	0.06	Brownish yellow	0.04	0.050	0.34

<sup>a</sup>Ref: Stahl (1969)

<sup>b</sup>n-propanol : water = 70:30 (v/v)

formation is uncertain but a possibility may be ascertained as follows. An 'imine' intermediate is first formed by the reaction of amino acids with the reagent I and this then forms coloring complexes (C-T)<sup>4</sup> with ninhydrin, colors of which are variable depending on the nature of amino acids. Another possibility is the reaction of amino acids (unreacted or derived from 'imine' intermediate) with ninhydrin in the usual way to produce Ruhemann Complex (except for proline and hydroxyproline). On the other hand, the 'imine' intermediate may also undergo the reaction with ninhydrin in a different way to produce another complex<sup>5</sup>. Since both complexes are formed in unequal amounts depending on the nature of amino acids, we observed the variation of color contrast of the different amino acids.

### CONCLUSION

The newly introduced spray reagent (benzaldehyde) in combination with ninhydrin turns out to be very effective for detection of different amino acids by producing distinguishable colors on silica gel G TLC plates with high sensitivity.

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