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Modified Ninhydrin reagent for the detection of amino acids on TLC plates

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Abstract: 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 proline and hydroxy proline. A new spray reagent, *para* bromobenzoic acid has been introduced here which produces distinguishable colors with the amino acids with moderately high sensitivity (0.01-1.0) µg. A probable mechanism for such color formation has also been proposed.

Keywords: Amino acids, Ninhydrin, p-Bromobenzoic acid, Thin-layer chromatography

INTRODUCTION

Identification of amino acids has utmost importance for the evaluation of protein structure and also for the determination of the presence of amino acids in numerous natural products. There are several methods for the determination of amino acids in biological and pharmaceutical samples. Thin layer chromatography (TLC) finds its place when the relatively costly equipment required by other methods is unavailable. Thin layer chromatography is an important tool for the identification of amino acids by various spray reagents (Lorenz and Flatter, 1970; Devaux and Mesnard, 1971; Wolski *et al*., 1980; Distler and Fresenius, 1981; Laskar and Basak, 1988; 1990; Basak and Laskar, 1990; Laskar *et al*. 1991; 2001a,b,c; Basak *et al.*, 1993; Sinhababu *et al*., 1994; 2013; Khawas and Laskar 2003a,b; Khawas *et al*., 2004; Samanta *et al*., 2004; Samanta and Laskar, 2006; Das *et al*., 2010; Sahana *et al*., 2011). Of the reagents in general use, ninhydrin is the most popular because of its remarkably high sensitivity (Stahl, 1969). However, ninhydrin produces the same purple/violet color with most amino acids (only proline and hydroxyproline produce yellow color). In present study, an effort has been made to resolve this color problem by modified ninhydrin reagent for the identification of aminoacids.

MATERIALS AND METHODS

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.). Standard amino acids were obtained from Sigma (U.S.A) and n- propanol from Merck (India): Reagent I: 0.1% p-bromobenzoic acid (Aldrich Chemical Co., U.S.A.) in ethanol, Reagent II: 0.25% ninhydrin (Sigma, St. Louis, MO, U.S.A.) in acetone.

Detection of aminoacids on TLC plates: Standard solutions (1 mg mL^{-1}) of amino acids were prepared in 0.01 mol L^{-1} phosphate buffer (pH 8.0) and spotted on the TLC plates by means of a graduated micropipette (5µL). 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 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). Colors were observed visually. Detection limits for the amino acids after use of ninhydrin reagent alone is also given in Table 1.

RESULTS AND DISCUSSION

To develop color reagents for the detection of amino acids on TLC plates, a lot of work has been done in this context and a good number of modified spray reagents have been developed. The DDQ (Sinhababu *et al*., 1994) and 2,3-Dichloro-1,4-Naphthaquinone (Khawas *et al*., 2004) are the two important reagents that can be used alone instead of ninhydrin in this respect. Some complex reagents have also been developed by other workers (Das *et al*., 2010; Sahana *et al*., 2011) but their detection limits and color contrasts are not so good enough than some other reagents referred here. Recently, we have also introduced some p-haloderivatives as the modified spray reagents (Sinhababu *et al*., 2013). In our continuous effort for the development of color reagents, the present author is now able to introduce one more reagent (p-bromobenzoic acid) with high sensitivities for easy and rapid identification of amino acids on TLC chromatoplates. The reagent is capable of developing

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

Amino acids	Reagent I + Ninhydrin				Detection limit of Ninhydrin[a] (µg)	R_f values[b]
	Cold condition (before second heating)		Hot condition (after second heating)			
	Color observed	Detection limit (µg)	Color observed	Detection limit (µg)		
Glycine	Saffron	0.05	Reddish brown	0.05	0.001	0.32
Alanine	Pinkish violet	0.05	Reddish pink	0.04	0.009	0.37
Valine	Pinkish violet	0.04	Pink	0.03	0.010	0.45
Leucine	Pinkish violet	0.05	Dirty pink	0.04	0.010	0.55
Isoleucine	Pinkish violet	0.04	Pinkish violet	0.04	0.200	0.53
Serine	Dirty pink	0.20	Pink	0.05	0.008	0.35
Threonine	Pink	0.20	Reddish pink	0.10	0.050	0.37
Aspartic acid	Gray	1.00	Grayish violet	0.10	0.100	0.33
Asparagine	Straw yellow	0.40	Deep yellow	0.10	0.100	0.14
Glutamic acid	Deep pink	0.60	Pink	0.10	0.040	0.35
Glutamine	Reddish pink	0.20	Dirty pink	0.10	0.100	0.15
Lysine	Pinkish violet	0.01	Pinkish violet	0.01	0.005	0.03
Histidine	Pink	0.20	Brown	0.10	0.050	0.20
Arginine	Pinkish violet	0.04	Reddish pink	0.04	0.010	0.02
Phenyl alanine	Pink	1.00	Saffron	0.50	0.050	0.58
Tyrosine	Pale rose	0.80	Pale rose	0.50	0.030	0.57
Tryptophan	Pink	1.00	Gray	1.00	0.050	0.62
Cysteine	Pink	0.10	Light pink	0.10	0.020	0.38
Cystine	Reddish pink	0.50	Pale cream	0.30	0.010	0.32
Methionine	Reddish pink	0.40	Reddish pink	0.20	0.010	0.51
Proline	Lemmon yellow	0.40	Brownish yellow	0.10	0.100	0.26
Hydroxy proline	Pinkish violet	0.10	Rosy pink	0.10	0.050	0.34

[a]Stahl (1969); [b]n-propanol : water = 70:30 (v/v)

various distinguishable colors with many amino acids and also shows high sensitivity (0.01-1.0 µg) comparable to ninhydrin alone.

It was observed that ninhydrin gives various distinguishable colors with amino acids in the presence of reagent I before and after final heating. The detection limits (µg spot^{-1}) are substantially low (0.01-1.00 µg) which is quite comparable to ninhydrin alone. Color development is somewhat different almost in all the cases after second heating. In most cases the detection limits are either equal or a little higher than those obtained after final 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 (Table 1).

The mechanism leading to such color formation is uncertain but a possibility may be ascertained. One possibility is the formation of a secondary amide by the reaction between p-bromobenzoic acid with amino acids and the product thus obtained forms coloring complexes (charge transfer) with ninhydrin, colors of which are variable depending on the nature of the amino acids (Foster, 1969).

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REFERENCES

Basak, B. and Laskar, S. (1990). Spray reagent for the detection of amino-acids on thin-layer Plates. *Talanta*, 37:1105-1106.

Basak, B., Bhattacharya, U.K. and Laskar, S. (1993). Spray reagent for the detection of amino acids on thin layer chromatography plates. *Amino Acids*, 4:193-196.

Das, D., Sahana, A., Saha, R., Kundu, P. and Laskar, S. (2010). Anthracene-anchored derivatized methionine: A new ligand for detection of amino acids, and estimation of binding constants. *Journal of Planar Chromatography*, 23:255-259.

Devaux, G. and Mesnard, P. (1971). Mechanism of the color reaction between ascorbic acid and amino acids. *Bulletin of the Society of Pharmaceutical Sciences Bordeaux*, 110:145-151.

Distler, W. and Fresenius, Z. (1981). 1-Chloro-7-nitrobenzoic-1, 2, 5 oxadiazolein Spruhreagenszur Fluoreszenz-Detection

von Aminosauren auf Dunnschicht-Chromatogrammen. *Analytical Chemistry*, 309:127-128.

Foster, R. (1969). *Organic charge transfer complexes*, Academic Press, New York.

Khawas, S. and Laskar, S. (2003a). A reagent for the detection of amino acids on thin layer chromatography plates. *Asian Journal of Chemistry*, 15:512-514.

Khawas, S. and Laskar, S. (2003b). Two new spray reagents for the detection of amino acids on thin- layer plates. *Journal of Planar Chromatography*, 16:165-166.

Khawas, S., Panja, D. and Laskar, S. (2004). Identification of amino acids on TLC plates with a new reagent. *Journal of Planar Chromatography*, 17:314-315.

Laskar, S. and Basak, B. (1988). Detection of amino acids on thin-layer plates. *Journal of Chromatography*, 436:341-343.

Laskar, S. and Basak, B. (1990). Identification of amino acids with ninhydrin in acid media. *Journal of Planar Chromatography*, 3:535-536.

Laskar, S., Bhattacharya, U. and Basak, B. (1991). Modified ninhydrin spray reagent for the identification of amino acids on thin-layer chromatography plates. *Analyst*, 116:625-626.

Laskar, S., Sinhababu, A. and Hazra, K.M. (2001a). A modified spray reagent for the identification of amino acids on thin layer chromatography plates. *Journal of Indian Chemical Society*, 78:49-50.

Laskar, S., Panja, D. and Sinhababu, A. (2001b). A spray reagent for identification of amino acids on thin-layer chromatography plates. *Journal of Planar Chromatography*, 14:462-463.

Laskar, S., Sinhababu, A. and Hazra, K.M. (2001c). A modified spray reagent for the detection of amino acids on thin layer chromatography plates. *Amino Acids*, 21:201-204.

Lorentz, K. and Flatter, B. (1970). Staining of amino acids with benzoquinone in paper chromatography. *Analytical Biochemistry*, 38:557-559.

Samanta, T.D., Nag, M. and Laskar, S. (2004). A new spray reagent for identification of amino acids on thin-layer chromatography plates. *Asian Journal of Chemistry*, 16:1947-1949.

Samanta, T.D. and Laskar, S. (2006). New reagent for detection of amino acids on TLC plates. *Journal of Planar Chromatography*, 19:252-254.

Sahana, A., Das, S., Saha, R., Gupta, M., Laskar, S. and Das, D. (2011). Identification and interaction of amino acids with leucine-anthracene reagent by TLC and spectrophotometry:experimental and theoretical studies. *Journal of Chromatographic Science*, 49:652-656.

Sinhababu, A., Basak, B. and Laskar, S. (1994). Novel spray reagent for identification of amino acids on thin-layer chromatography plates. *Analytical Proceedings Including Analytical Communications*, 31:65-66.

Sinhababu, A., Basak, B., Dey, H. and Laskar, S. (2013). Identification of amino acids with modified ninhydrin reagents on thin layer chromatography plates. *Journal of Planar Chromatography*, 26:26-30.

Stahl, E. (1969). *Thin layer chromatography: A laboratory handbook* (2nd ed) Springer, New York.

Wolski, T., Golkiewicz, W. and Rompola, A. (1980). 4-N, N-dimethyl aminoazobenzene-4-sulfonyl chloride-a high sensitive reagent for the spectrophotometric and chromatographic analysis of amino acids. *Chemical Analysis (Warsaw)*, 25:583-590.