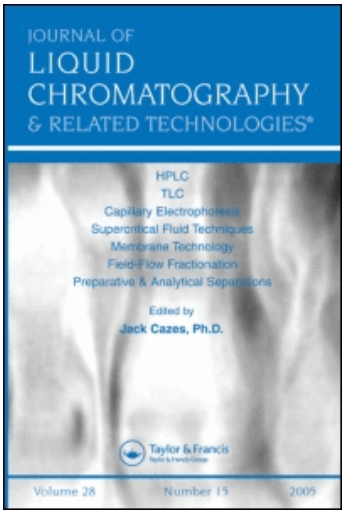

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TLC Resolution of Amino Acids in a New Solvent and Effect of Alkaline Earth Metals

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TLC RESOLUTION OF AMINO ACIDS IN A NEW SOLVENT AND EFFECT OF ALKALINE EARTH METALS

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ABSTRACT

A new solvent system for the resolution of 15 component mixture of amino acids and the effect of alkaline earth metals on the resolution is reported, the TLC was carried out using plain and impregnated silicagel plates and, solutions of amino acids in tris buffer and pretreated with metal hydroxides.

INTRODUCTION

Amino acids may be classified as non-polar, polar, basic and acidic depending upon the structure of the side chain 'R'. The charge on amino acid molecules, their pk_a values and hydrophobic interactions due to non-polar side chains are reported to be affecting their chromatographic behaviour [1,2]. The difficulties inherent in altering the variables that affect chromatographic behaviour often prohibit extensive experimentation with a system, and therefore the theory of

amino acids separations has received less attention in the literature than the technical aspects. The literature survey [3-6] and our earlier TLC studies of amino acids on impregnated silicagel layers [7,9] indicated that attempts could be made to study some aspects of theory of amino acid separations taking advantage of their zwitterionic nature. Since amino acids exist as anions above their isoelectric points (pI) and may combine with a cation (metal ion) which may thus influence chromatographic behaviour, experiments were carried out to study the effect of alkaline earth metals under different conditions on thin layer chromatographic behaviour of amino acids; and the results are reported in the present paper. A new solvent system for the TLC resolution of fifteen component mixture of amino acids was developed and used for these studies.

EXPERIMENTAL

Reagents, chemicals, silicagel and amino acids used were of SISCO Res.Lab, or BDH (England). The pH meter was of Electronics Corporation of India model pH5652. The TLC plates (20 x 20cm x 0.5mm) were prepared by spreading a slurry of silicagel (50g) in water (100ml) with a Stahl type of applicator. The solutions of amino acids were 10^{-3}M, and were spotted at 500ng level using a 25µl Hamilton Syringe. Following different systems were used.

[A] plain plates spotted with solutions of amino acids in 70% ethanol (control run).

[B] plain plates spotted with solutions of amino acids in 70% ethanol pre-treated with aq.solutions (0.1M) of hydroxides of Mg,Ca,Sr and Ba.

[C] plates impregnated (5%) with Mg, Ca, Sr and Ba hydroxide separately, spotted with solutions of amino acids in 70% ethanol.

[D] plates impregnated as in [C] spotted with solutions of amino acids in Tris buffer of pH7.5

All the chromatograms were developed in n-Butanol-formic acid-ethanol, 3:1:1, in paper-lined, pre-equilibrated rectangular glass chambers, sprayed with ninhydrin (0.2% in acetone) and heated at 70°C for 10 minutes when the characteristic colors of amino acid spots were visible.

RESULTS AND DISCUSSION

The hR_f values and the time required for the development of chromatograms in systems [A], [B] and [C] are recorded in table 1. The spots, in general, were very compact and 9-10 amino acids were resolved from a 15 component mixture in systems [A] and [C] while system [B] showed resolution of 7-8 amino acids. There was much tailing and poor resolution in system [D] therefore R_f not given. The resolution possibilities for each pair of amino acids was calculated as reported earlier [7,8] by dividing the distance between two spot

TABLE 1

hR_f Values of Amino Acids Showing the Effect of Alkaline Earth Metals

Name of amino acids	A	B				C			
		Mg	Ca	Sr	Ba	Mg	Ca	Sr	Ba
Gly	50	55	50	50	49	40	50	45	50
Ala	60	64	62	59	60	55	60	65	57
Val	71	74	74	79	77	60	75	75	75
Ile	80	86	84	84	85	80	80	75	76
Leu	83	86	85	86	85	84	85	77	80
Tyr	83	85	85	84	84	78	80	70	76
Pro	40	40	40	40	35	30	40	41	35
Thr	58	56	55	55	55T	50	58	60	54
Cys	65T	65T	63T	60T	58	55T	61T	65T	60T
Met	74	80	79	80	76	75	75	68	75
Try	80	85	85	84	84	85	85	81	83
Phe	78	84	84	85	85	85	86	80	85
Asn	35	44	40	40	40	34	35	41	30
Ser	54	60	55	55	54	59	48	55	45
Asp	62	65	63	60	59	58	55	63	50
Time	62	62	62	62	62	70	72	85	93

Solvent System:n-Butanol-Formic acid-Ethanol (3:1:1)
Room Temperature: 17 ± 2°C
A,B,C, as given in experimental

centres with the sum of two spot radii, and a value of 1.5 was taken as a measure of complete resolution.

The results clearly show that alkaline earth metals are influencing the chromatographic behaviour of amino acids as it can be observed that hR_f values increased in system [B] as compared to those in [A], although the resolution became poorer in system [B] i.e.when amino acids were spotted on plain plates in the form of their metal salts and chromatographic properties (solubility and adsorption coefficient) were those of this salt rather than pure amino acid. On the other hand in system [C], the impregnation by alkaline earth metal hydroxides might be effecting the characteristics of the adsorbent (silicagel) which inturn influenced the chromatographic behaviour of amino acids. There may be some resistance to the movement of amino acids, may be due to the blocking of capillaries by the metal ions on the chromatograms. Further analysis of data shows that R_f values increases with increase in branching of side chain 'R' in the amino acids (i.e.gly, ala, val, ile, leu), which suggests that some other forces contribute to the resolution pattern [2] and these may be hydrophobic interactions between non-polar side chains and the silicagel layer having an adsorbed water layer. The TLC of amino acids buffered at pH 7.5 over impregnated plates (system D) also clearly indicated the influence of metal ions, as there was tailing and the poorest resolution. The

results, thus provide a new system for the resolution of amino acids, however, these studies are being extended to determine precisely the effect of metal ions on theory of amino acids separation.

REFERENCES

1. Blackburn, S., Ed.Amino Acid Determination-Methods and Techniques, Marcel Dekker, New York, 1978, pp.48.

2. Stein, W.H., and Moore, S., Cold Spring Harbor Symp.Quant .Biol.,**14**, 179 (1950).

3. Kirchner, J.G., Thin Layer Chromatography, 2nd Ed., Wiley Interscience, N.Y.,(1981).

4. Zweig, G. and Sherma, J.,Anal.Chem.,**48**, 66R (1976); **50** 50R(1978); **52**, 276R(1980).

5. Sherma, J. and Fried, B., Anal.Chem.,**56**, 48R(1984).

6. Sherma, J., Anal.Chem.,58, 59R (1986).

7. Srivastava, S.P., Bhushan, R., and Chauhan, R.S., J.Liquid Chromatogr.,**7**, 1359 (1984).

8. Bhushan, R.,Chauhan, R.S., Reena and Ali, I., J.Liquid Chromatogr.(1987).

9. Bhushan, R., and Ali, I., Chromatographia (1986) In Press.