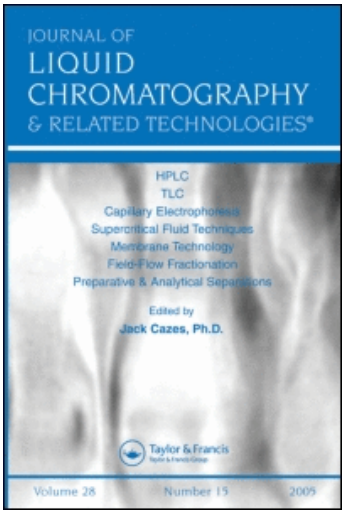

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TLC Separations of Amino Acids on Silica Gel Impregnated Layers

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TLC SEPARATIONS OF AMINO ACIDS ON SILICA GEL IMPREGNATED LAYERS

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ABSTRACT

Copper sulphate and polyamide were tried as impregnants for improving the separation of twenty amino acids on silica gel 'G' layers using a new solvent system MeOH-BuOAc-AcOH-Pyridine(20:20:10:5). Tables are presented to illustrate the improvement in resolution of amino acids on silica gel plates.

INTRODUCTION

Literature survey reveals many TLC systems for the separation of amino acids[1,2]. The use of pyridinium tungstoarsenate for TLC separation of amino acids was reported by Srivastava and coworkers[3]. Recently a comparison of amino acids separation on different layers was reported by Sleckmann and Sherma[4]. The present paper deals with the use of copper sulphate and polyamide mixed silica gel 'G' layers for satisfactory separation of amino acids.

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EXPERIMENTAL

The plates of 0.5 mm thickness were prepared by spreading a slurry of a mixture of silica gel 'G' (SISCO make) and 0.25 % solution of copper sulphate in distilled water. Polyamide mixed layers were made by first homogenising 50g silica gel and 10g of polyamide and then making a slurry with distilled water. The plates were dried at a constant temperature of 60°C for 24 hours.

The aq. solution of amino acids was applied to the layer by glass capillary and the chromatograms developed at 17 ± 1°C with MeOH-BuOAc-AcOH-Pyridine (20:20:10:5). After development the plates were sprayed with 0.1 % ninhydrin in acetone and heated at a temperature of 60°C for one hour.

RESULTS AND DISCUSSION

Table 1 gives hR_f values of amino acids on plain and impregnated silica gel. Tables 2-4 give resolution of amino acids on plain and impregnated layers. The resolution values (R) were calculated by dividing the distance between spot centres by the sum of radius of the two spots. A value of R more than 1.5 was considered as completely resolved and 'R' was placed in the table. A zero value of 'R' indicates that resolution was not possible either due to the tailing of the spot or due to same hR_f value.

It is worthwhile to mention that the hR_f values on impregnated plate is governed not only by the solubility of the product of interaction of amino acid and impregnant in the solvent system employed[5], but also by the adsorption behaviour of the inter-

TABLE 1

hR_f^* Values for Amino Acids

Amino Acid	A	B	C
l-leucine (Leu)	65	63	71
d,l-isoleucine (Ile)	66	72	81
d,l-tryptophane (try)	63	68	75
d,l-methionine (Met)	64	64	72
d,l-valine (Val)	64	60	77
l-lysine·HCl (Lys)	16_T	12	33
l-histidine·HCl (His)	22_T	20	39
d,l-β phenyl alanine (Phe)	64	65	82
d,l-threonine (Thr)	50	51	67
d,l-alanine (Ala)	46	45	64
d,l-serine (Ser)	40	43	56
l-tyrosine (Tyr)	58	61	71
l-glutamic acid (Glu)	41	48	58
d,l-aspartic acid (Asp)	28	25	44
l-arginine·HCl (Arg)	24_T	19	39
glycine (Gly)	36	46	49
l-proline (Pro)	37	36	58
l-cysteine·HCl (Cys)	20_T	17	29
d,l-2 amino butyric Acid (Aba)	51	54	61
l-ornithine·HCl (Ont)	27_T	23	35

* = The values are average of two as more identical runs, 10 cm in 35 minutes. T = Tailing, A = hR_f values on plain silica gel, B = hR_f values on copper sulphate impregnated plate, C = hR_f values on polyamide mixed layers.

TABLE 2

Resolution Data on Silica Gel 'G' Plate

	Ont	Aba	Cys	Pro	Gly	Arg	Asp	Glu	Tyr	Ser	Ala	Thr	Phe	His	Lys	Val	Met	Try	Ile
Leu	R	R	R	R	R	R	R	R	R	R	R	R	0.25	R	R	0.22	0.25	0.5	0.22
Ile	R	R	R	R	R	R	R	R	R	R	R	R	0.5	R	R	0.44	0.5	1.0	
Try	R	R	R	R	R	R	R	R	R	R	R	R	0.0	R	R	0.0	0.0		
Met	R	R	R	R	R	R	R	R	R	R	R	R	0.0	R	R	0.0			
Val	R	R	R	R	R	R	R	R	R	R	R	R	0.0	R	R				
Lys	0.0	R	0.0	R	R	0.0	R	R	R	R	R	R	R	0.0					
His	0.0	R	0.0	R	R	0.0	R	R	R	R	R	R	R						
Phe	R	R	R	R	R	R	R	R	R	R	R	R							
Thr	R	0.22	R	R	R	R	R	R	R	R	R								
Ala	R	1.1	R	R	R	R	R	1.25	R	1.33									
Ser	R	R	R	0.66	0.88	R	R	0.22	R										
Tyr	R	R	R	R	R	R	R	R											
Glue	R	R	R	1.0	1.25	R	R												
Asp	0.22	R	R	R	R	0.88													
Arg	0.0	R	0.0	R	R														
Gly	R	R	R	0.25															
Pro	R	R	R																
Cys	0.0	R																	
Aba	R																		

TABLE 3

Resolution Data on Copper Sulphate Impregnated Plate

	Ont	Aba	Cys	Pro	Gly	Arg	Asp	Glu	Tyr	Ser	Ala	Thr	Phe	His	Lys	Val	Met	Try	Ile
Leu	R	R	R	R	R	R	R	R	R	R	R	R	0.40	R	R	R	0.80	0.80	R
Ile	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
Try	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		
Met	R	R	R	R	R	R	R	R	R	R	R	R	0.5	R	R	R			
Val	R	R	R	R	R	R	R	R	0.40	R	R	R	R	R	R				
Lys	R	R	R	R	R	R	R	R	R	R	R	R	R	R					
His	1.0	R	1.0	R	R	0.40	R	R	R	R	R	R	R						
Phe	R	R	R	R	R	R	R	R	R	R	R	R							
Thr	R	1.2	R	R	R	R	R	R	R	R	R								
Ala	R	R	R	R	R	R	R	1.20	R	0.80									
Ser	R	R	R	R	1.20	R	R	R	R										
Tyr	R	R	R	R	R	R	R	R											
Glu	R	R	R	R	R	R	R												
Asp	0.66	R	R	R	R	R													
Arg	R	R	0.80	R	R														
Gly	R	R	R	R															
Pro	R	R	R																
Cys	R	R																	
Aba	R																		

TABLE 4

Resolution Data on Polyamide Mixed Silica Gel Layers

	Ont	Aba	Cys	Pro	Gly	Arg	Asp	Glu	Tyr	Ser	Ala	Thr	Phe	His	Lys	Val	Met	Try	Ile
Leu	R	R	R	R	R	R	R	R	1.43	R	R	R	R	R	R	0.31	1.33	0.3	R
Ile	R	R	R	R	R	R	R	R	R	R	R	R	0.21	R	R	1.14	R	R	
Try	R	R	R	R	R	R	R	R	1.0	R	R	R	R	R	R	0.50	0.86		
Met	R	R	R	R	R	R	R	R	0.31	R	R	R	R	R	R	1.45			
Val	R	R	R	R	R	R	R	R	R	R	R	R	1.25	R	R				
Lys	0.57	R	R	1.14	R	R	R	R	R	R	R	R	R	R					
His	1.14	R	R	R	R	0.0	R	R	R	R	R	R	R						
Phe	R	R	R	R	R	R	R	R	R	R	R	R							
Thr	R	R	R	R	R	R	R	R	1.14	R	0.86								
Ala	R	0.75	R	R	R	R	R	R	R	R									
Ser	R	1.43	R	0.57	R	R	R	0.57	R										
Tys	R	R	R	R	R	R	R	R											
Glu	R	0.75	R	0.0	R	R	R												
Asp	R	R	R	R	1.43	R													
Arg	1.14	R	R	R	R														
Gly	R	R	R	R															
Pro	R	0.86	R																
Cys	R	R																	
Aba	R																		

action product. It is apparent from tables 3 and 4 that by selection of impregnant a satisfactory separation of amino acids can be achieved. The reported solvent system requires 35 minutes for 10 cm run and thus offers a rapid separation of amino acids on impregnated silica gel layers.

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