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R. Bhushan<sup>a</sup>; R. S. Chauhan<sup>a</sup>; Reena Ali<sup>a</sup>; Imran Ali<sup>a</sup>

<sup>a</sup> Department of Chemistry, University of Roorkee, Roorkee, India

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## A COMPARISON OF AMINO ACIDS SEPARATION ON Zn, Cd, AND Hg IMPREGNATED SILICA LAYERS

R. Bhushan\*, R. S. Chauhan, Reena Ali,  
and Imran Ali

*Department of Chemistry  
University of Roorkee  
Roorkee-247 667, India*

### **ABSTRACT**

TLC separation of amino acids on silica gel impregnated with different concentrations (0.1, 0.2 & 0.5%) of Zn, Cd and Hg ions, belonging to same group in periodic table, in a new solvent system BuAc-MeOH-HoAc-Pyr (20:20:5:5) is compared

### **INTRODUCTION**

Separation and identification of amino acids by TLC has received significant attention in view of its importance in biochemistry, medicine and chemistry. A large number of TLC systems are available in the literature, including the ones using impregnated layers (1-6). The present paper

describes results of the comparative study of TLC separation of amino acids with Zn, Cd and Hg metal ions as impregnants, belonging to same group in the periodic table, using three different concentrations of each but the same solvent system.

### EXPERIMENTAL

All the amino acids, reagents and solvents used were of SISCO Res Lab., and B.D.H.(England) A.R.grade. The TLC plates prepared were of 20 x 20 cm x 0.5 mm size. The impregnants used were zinc-acetate, cadmium nitrate and mercuric chloride in 0.5, 0.2 and 0.1% concentrations for each. The samples of individual amino acids and their mixtures were applied at 500 ng level (1  $\mu$ l) using a 10  $\mu$ l Hamilton Syringe.

Chromatograms were developed at  $17 \pm 1^{\circ}\text{C}$  in paper-lined rectangular glass chambers that had been pre-equilibrated with solvent for 15 minutes. The solvent system BuAc-MeOH-HoAc-Pyr (20:20:5:5) was used for developing all the chromatograms. The plates were sprayed with 0.1% ninhydrin in acetone and heated at  $60^{\circ}\text{C}$  for 1 hr.

### RESULTS AND DISCUSSION

Table 1 gives  $hR_F$  values of amino acids in ten TLC systems, but the same solvent. The resolution of amino acids was verified by calculating resolution values as reported earlier (5,6). When the resolution value was greater than 1.50, the amino acids were considered to be completely resolved while the values less than 1.0 indicated incomplete

TABLE 1

hR<sub>F</sub> values of 15 Amino Acids on Silica Gel in Systems A-J.

	A	B	C	D	E	F	G	H	I	J
Thr	25	55	42	41T	35	36	42	33	50	40
Ser	12	38	39	28T	32	29	31T	15	40	31T
Gly	10	35	29	23T	28	25	28	16	35	27T
Lys	03	13	07	05	51	08	05	04	10	05
Ala	30	48	40	31	38	36	38	20	45	35
Tyr	60	60	52	50	48	45	51	62	55	56
Ile	55	67	56	52	50	48	54	50	60	53
Leu	50	65	55	55	52	50	56	47	65	55
Cys	00	00	00	00	00	00	00	00	00	00
Met	45	62	48	48	48	42	48	39	54	45
Glu	18T	43	38	36T	34	27	38T	18	36	34T
Try	57	60	53	51	51	44	54	45	60	47
Phe	54	67	57	55	55	46	57	58	68	52
Val	50	63	45	50	52	42	56	47	57	45
Arg	07	19	13	13	09	11	11	10	15	08

Solvent system : Butylacetate-Methanol-Acetic acid-Pyridine

(20:20:5:5)

Developing time: 30 minutes.

Detection limit:  $10^{-4}$  M.

Solvent front: 10 c.m.

A = Plain silica gel

B,C,D,0.5%,0.2%,0.1% Zn<sup>+2</sup> impregnated respectively.E,F,G,0.5%,0.2%,0.1% Cd<sup>+2</sup> impregnated respectively.

H,I,J,0.5%, 0.2%,0.1% Hg impregnated respectively.

resolution. The  $R_F$  values for these amino acids were generally found to increase in comparison to those on plain silica gel, however, it was interesting to note that  $R_F$  value of tyr decreased in six and  $R_F$  of ile decreased in five out of nine systems. The time required in each system was 30-35 minutes for 10 cm run.

The impregnants selected were from one group of transition series in periodic table. The metal ions of zinc group has a much greater ability to form complexes and the reactivity decreases  $Zn \rightarrow Cd \rightarrow Hg$ , and the complexes of  $Zn^{++}$  and  $Cd^{++}$  with  $NH_3$  and amines ( where N is the donor atom) have formation constants smaller, by many order of magnitude, than those for  $Hg^{++}$  (7). This is probably reflected in the observation that almost same set of amino acids is resolved in system A and system G and H (0.5 and 0.2 %  $Hg^{++}$  respectively).

Thus the systems reported can be considered as more useful being inexpensive and rapid.

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