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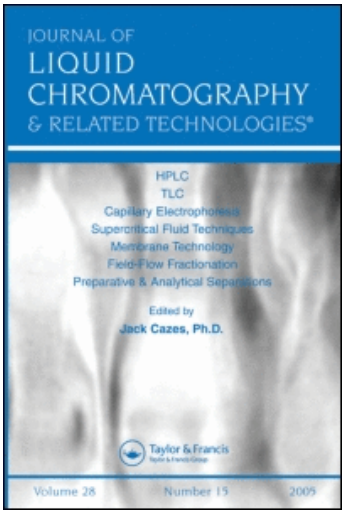

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RESOLUTION OF PTH-AMINO ACIDS IN THREE NEW SOLVENTS ON Zn, Cd, Hg IMPREGNATED SILICA PLATES

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ABSTRACT

A ten component mixture of PTH-amino acids has been resolved by TLC using impregnated silica plates. Three new solvent systems, $CHCl_3$-H_2O-EtOAc(28:1:1), CCl_4-AcOH(19:1) and $CHCl_3$-MeOH-Benzene(19:1:5) were developed and used. The effect of 0.1, 0.2 and 0.5 % concentrations of each of the impregnant (Zn^{++}, Cd^{++}, Hg^{++} ions) on R_F values was studied.

INTRODUCTION

The principle of phenylisothiocyanate method for identification of 'N' terminal amino acids in a protein or a peptide lacks many of disadvantages of DNP method and the former can be applied for a step by step degradation of peptides and proteins during sequence analysis. Of the several methods employed,

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thin layer Chromatography is one of the wide spread techniques used for the separation and identification of PTH-amino acids. Different Chromatographic methods and several TLC systems for separation and identification of PTH-amino acids are reviewed by Rosmus and Deyel(1). Most of the reported systems are successful for at the most quaternary mixtures of PTH-amino acids and it takes more than an hour(or 2-3 hours) for the development of chromatograms. It was therefore, considered worthwhile to develop new rapid TLC systems for resolution of ten or more component mixture of PTH-amino acids. To the best knowledge of authors attempts have not been made to separate PTH-amino acids using impregnated TLC plates. The present paper reports the TLC separation of 10 component mixture of PTH-amino acids on thin silica gel layers impregnated with three different concentrations of Zn^{++}, Cd^{++} and Hg^{++} metal ions employing the same three new solvent systems for each concentration.

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EXPERIMENTAL

All the amino acids, reagents and solvents used were of SISCO Research Lab and B.D.H.(England),A.R.grade. The PTH-amino acids were prepared and their UV spectra were recorded to ensure their purity(2-4). 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 0.1, 0.2 and 0.5% concentrations of each. The samples of individual PTH-amino

acids and their mixture were applied at 500 ng level (1 μ l) using a 10 μ l Hamilton Syringe. The Chromatograms were developed at 20°C ± 1°C in paper lined rectangular glass chamber had been pre-equilibrated with solvent for 15 minutes.The solvent systems $CHCl_3$-H_2O-EtOAc(28:1:1); CCl_4-AcOH (19:1) and $CHCl_3$-MeOH-Benzene(19:1:9) were used for developing the chromatograms for three concentrations of each metal ion. The PTH-amino acids were located in iodine chamber as yellow brown spots. The R_F and size of each spot were measured.

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RESULTS AND DISCUSSION

Three new solvents were developed and used for the successful resolution of 10 component mixture of PTH-amino acids. These solvents provided good resolution even on plain silica gel, however, the use of metal ion impregnated plates resulted into more compact spots without tailing.The PTH-amino acids, solvent systems and R_F values on control plates, without any impregnation are recorded in table -1. In all, 27 systems were employed for the TLC of 10 PTH-amino acids. The effect of different concentrations of each metal ion on hR_F values is ploted in figure I-III for the three solvent systems. It is evident from the figures that 7-9 PTH-amino acids were separated from 10 component mixture. The R_F values in these impregnated systems were in general found to increase when compared to those on plain silica gel. The time required for developing the impreg-

TABLE 1

hR_f values on control plates without Impregnation

S.N.	PTH-amino acid	Solvent Systems		
		I	II	III
1.	Gly	25	13	54
2	Ala	40	28	43
3	Tyr	12	7	34
4	Ile	68	51	65
5	Leu	70	48	63
6	Met	35	35	36
7	Glu	80	48	47
8	Try	90	57	36T
9	Phe	64	40	77
10	Val	75	47	82

I : $CHCl_3$ - H_2O - EtOAc (28:1:1)

II : CCl_4 - AcOH (19 : 1)

$CHCl_3$ - MeOH-Benzene (19:1:5)

nated chromatograms was 25-30 minutes in comparison to 2-3 hrs for plain silica gel(3).

The resolution possibilities of each pair of acids in these systems were calculated(5) in usual manner (6,7) by dividing the distance between spot centres by one-half of the sum of the diameter of the two spots. The higher the resolution

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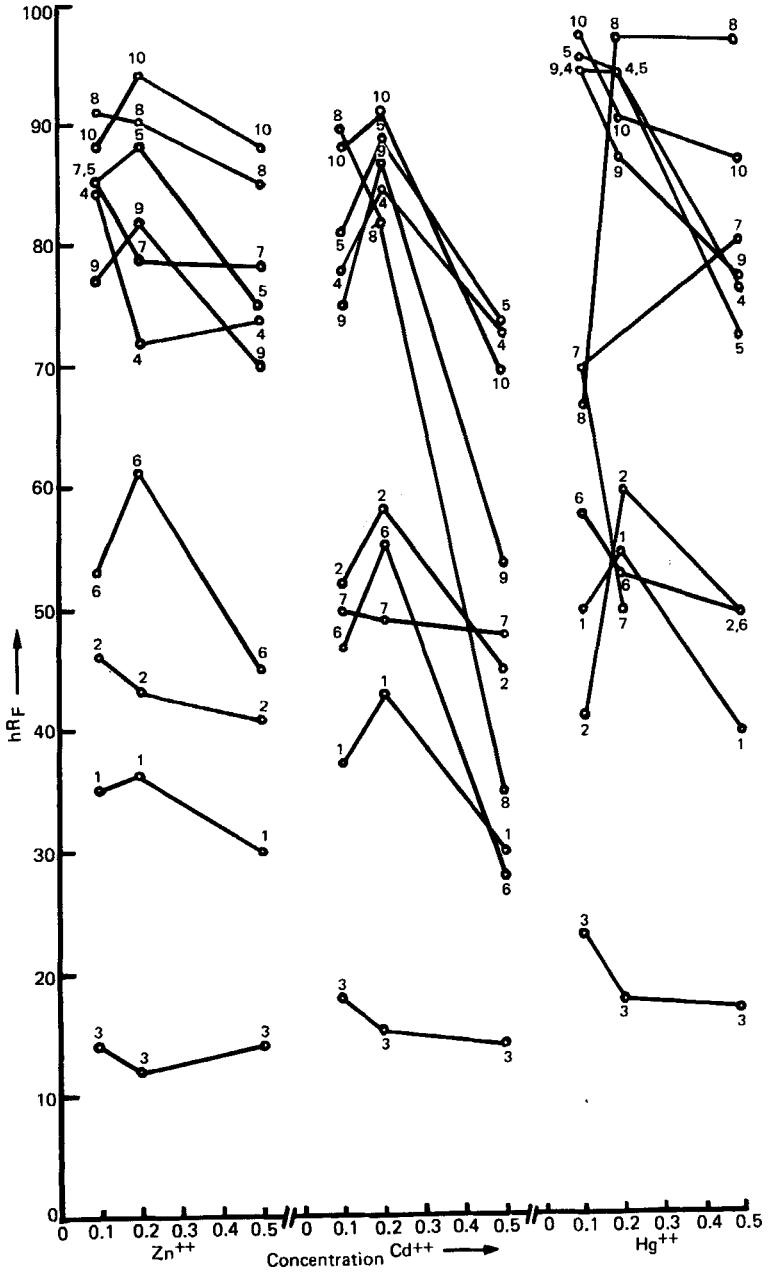

FIGURE 1. Effect of Concentration of Zn^{++}, Cd^{++}, and Hg^{++} on hR_F in Solvent I.

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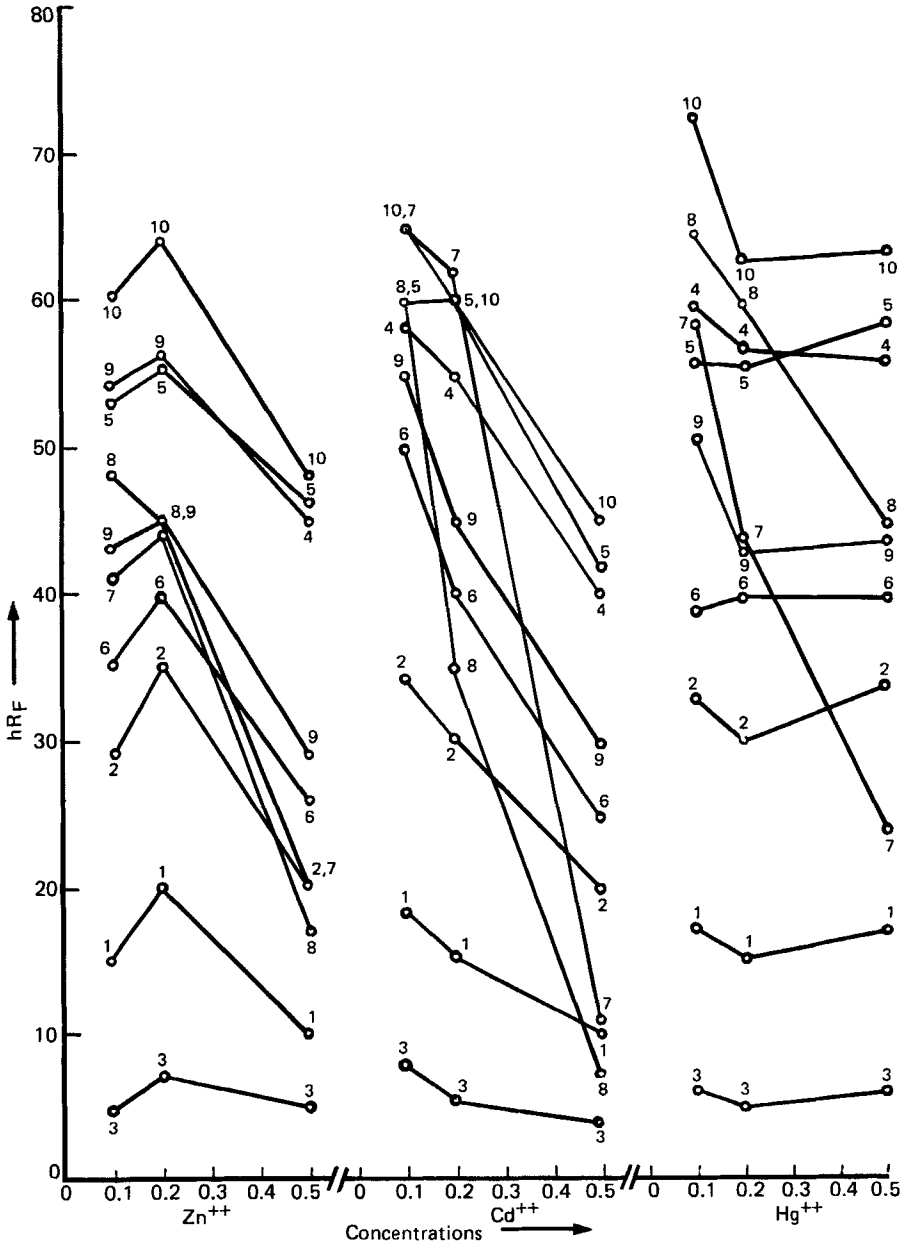

FIGURE 2. Effect of Concentrations of Zn^{++}, Cd^{++}, and Hg^{++} on hR_F in Solvent II.

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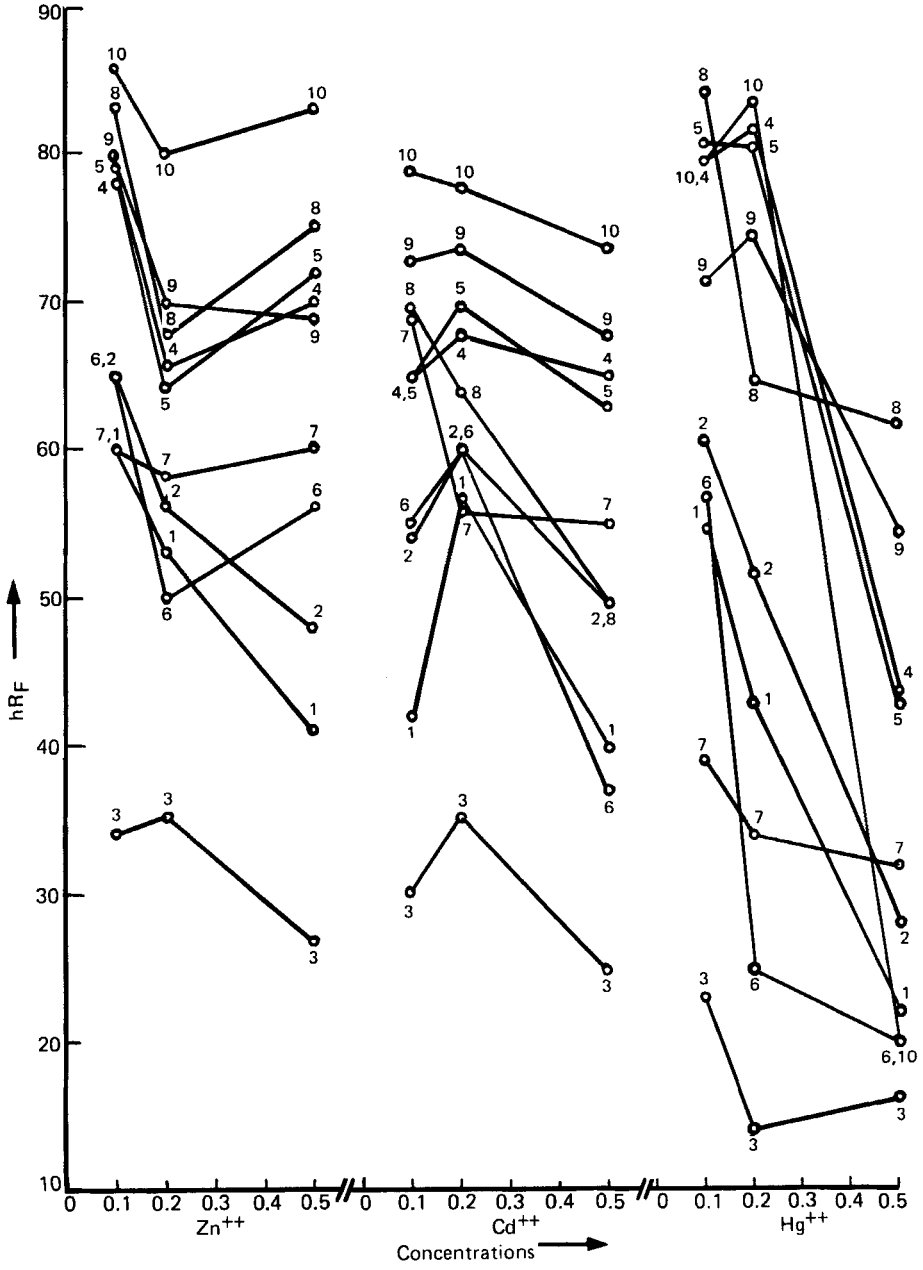

Figure 3. Effect of Concentrations of Zn^{++}, Cd^{++}, and Hg^{++} on hR_F in Solvent III.

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value, the greater was the separation obtained between the two components. When calculated value was greater than 1.50, the compounds were considered to be completely resolved while the value below 1.0 indicated incomplete resolution. The resolution data is not given here to keep the printed matter short. However, the results of separation from resolution data and those presented in fig I-III were in full agreement.

The adsorption phenomenon on metal ion impregnated layers is most likely effected by the complex formation between metal ion and PTH-amino acids. The metal ions of zinc group have a greater ability to form complexes(8) and the complex formation may be due to electron donation from 'N' or 'S' or oxygen atom of PTH-amino acids or (pi) electron donation from aromatic ring. The increase in R_F value on impregnated plates may thus be interpreted in terms of the movement of complex having higher solibility in the solvent system and larger adsorption energy of amino group to the silica gel(9) as there are two amino nitrogens in the molecule.

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