Notes

Paper chromatography using liquid ion exchangers

The use of paper impregnated with liquid ion exchangers as a vehicle for the separation of various species of compounds has been described[1,2], and the separation of amino acids *via* paper chromatography using an ascending technique in a variety of solvent systems is a widely used technique[3]. In the present study, a combination of these procedures, *i.e.* using a liquid ion exchanger[4] as the ascending solvent on filter paper, resulted in an effective separation of several amino acids. In addition, unlike the problems observed with several of the commercially available ion exchange-impregnated papers, using a dilute solution of the ion exchanger in an organic phase as the ascending solvent permitted a direct application of ninhydrin reagent to develop the color of the amino acid spots without appreciable paper discoloration.

Experimental

Preparation of solvent and paper. For those data reported in Table I, the ascending solvent was prepared by mixing one volume of a 5 % solution of Amberlite LA-2* in

TABLE I

R_F VALUES OF SOME AMINO ACIDS IN AN LA-2–*n*-BUTYL ALCOHOL SYSTEM

Solvent system: a sample of 5 ml of Amberlite LA-2 liquid ion exchanger was dissolved in 95 ml of *n*-butanol, and the resulting solution was saturated with 100 ml of 0.5 *M* phosphate buffer, pH 6. The organic phase was decanted and used as the ascending solvent. The chromatograms were developed with 0.2 % ninhydrin reagent dissolved in acetone, and allowed to dry at room temperature.

Amino acid	R_F	*Amino acid*	R_F
Alanine	0.13	Isoleucine	0.46
Arginine	0.01	Leucine	0.50
Asparagine	0.04	Lysine	0.01
Aspartic acid	0.42	Methionine	0.33
Cysteine	0.03	Phenylalanine	0.53
Cystine	0.03	Proline	0.17
Glutamic acid	0.51	Serine	0.09
Glutamine	0.05	Threonine	0.14
Histidine	0.06	Tryptophan	0.28
Glycine	0.08	Tyrosine	0.23
		Valine	0.31

n-butanol and one volume of 0.5 *M* phosphate buffer, pH 6. After a brief equilibration period, the phases were separated and the organic layer freed of water droplets by filtration through paper. Two microliters of 0.01 *M* solutions of the various DL-amino

* N-Lauryl-N-trialkylmethylamines.

TABLE II

EFFECT OF pH ON THE R_F VALUES OF SOME AMINO ACIDS

Solvent mixture: 50 ml of appropriate 0.5 *M* phosphate buffer, 47.5 ml of *n*-butanol, and 2.5 ml Amberlite LA-2 liquid ion exchanger.

pH of buffer	*R_F of amino acid*			
	Glutamic acid	*Glutamine*	*Aspartic acid*	*Asparagine*
3	0.48	0.06	0.49	0.04
4	0.54	0.08	0.47	0.06
5	0.55	0.07	0.49	0.05
7	0.39	0.04	0.27	0.03
8	0.12	0.04	0.09	0.03
9	0.03	0.03	0.03	0.03

acids in water were applied to the origin (1 in. from the bottom edge) of rectangles of Whatman No. 1 filter paper (the particular size of the paper was determined by the number of samples to be assayed concurrently, and the desired distance of travel of the solvent front). The paper was made into a cylinder by means of stapling the long edge of the paper, and it was then placed in a jar containing a sample of the organic phase described above and allowed to develop at about 30° using the general technique of WILLIAMS AND KIRBY[3]. The amino acid spots were visualized on the dried chromatograms by dipping the paper into a 0.2 % solution of ninhydrin in acetone, and finally allowing the paper to dry at room temperature for several hours.

Study of operational variables. For purposes of determining the effect of pH and resin concentration on the R_F of the amino acids as determined in this ion exchange solvent system, glutamic acid, aspartic acid, and the corresponding amides were used as model compounds. Using the procedure described above for obtaining the organic phase to be used as the solvent, the effects upon the R_F in several solvent systems are presented in Tables II and III.

TABLE III

EFFECT OF ION-EXCHANGE RESIN CONCENTRATION ON THE R_F VALUES OF SOME AMINO ACIDS

Solvent mixture: 50 ml of 0.5 *M* phosphate buffer (pH 6), and 50 ml of the appropriate concentration of Amberlite LA-2 liquid ion exchanger in *n*-butanol

Concentration of resin in butanol, %	*R_F of amino acid*			
	Glutamic acid	*Glutamine*	*Aspartic acid*	*Asparagine*
0	0	0.04	0.01	0.03
2	0.40	0.05	0.34	0.05
4	0.54	0.06	0.45	0.06
6	0.62	0.08	0.45	0.06
10	0.56	0.09	0.51	0.06
20	0.53	0.09	0.44	0.07

Results and discussion

These studies were initially undertaken in an effort to find an efficient and reproducible paper chromatographic solvent system which could be used to distinguish between

the α-amino-dicarboxylic acids and their corresponding amides. In preliminary experiments, the distribution coefficients of aqueous solutions of glutamic acid were determined in the presence of a number of organic solutions of the liquid anion exchanger Amberlite LA-2. The solvents used included aliphatic, chlorinated-aliphatic and aromatic hydrocarbons, and aliphatic alcohols. The larger distribution coefficients observed were with aliphatic alcohols in the C_4–C_6 range, and *n*-butanol was accepted as the solvent of choice. Subsequently, a number of studies concerning the effect of resin concentration, pH, buffer concentration, height of solvent front, and temperature of the system on the R_F values of the amino acids were carried out. In general, the apparent optimum conditions lay over a relatively broad range of values; thus, minor errors in preparing the solvent system should not greatly affect the values observed. However, as is true in most paper chromatographic systems, standard samples should always be assayed concurrently with the unknown material to eliminate minor variations in R_F values.

From the data presented in the experimental section, a single set of conditions was chosen as being representative of this system, and the R_F values of 21 amino acids were then determined for comparative purposes. These R_F values were not appreciably affected by a change in temperature of operation between 7° and 35°, nor by increasing the height of travel of the solvent front from 15 to 30 cm. This type of solvent system appears to possess certain advantages over that of the typical organic–aqueous procedures[3] in that certain biologically comparable derivatives, such as the α-amino-dicarboxylic acids and their corresponding amides, are significantly separated from one another. Of further interest is that this solvent system also separates various dipeptides in an efficient manner, and further studies with these types of compounds are presently in progress.

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