A LIQUID–LIQUID ION EXCHANGE SYSTEM FOR THE SEPARATION OF AMINO ACIDS

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In view of the importance of amino acids and their more complex derivatives, peptides and proteins, numerous chromatographic systems for their analysis have been developed. The nature of these systems is determined by the strongly hydrophilic character of amino acids, due to their tendency to ionization over the whole pH range, with the formation of zwitterions in the proximity of the isoelectric point, and to the hydrophilic character of amine and carboxylic groups even in the unionized state. Adsorption, ion exchange, as well as partition systems are employed; the latter belong usually to one-phase systems (*e.g.*, the Partridge system, butanol–acetic acid–water, 4:1:5) or are a saturated solution of water in an organic solvent of marked mutual solubility with water (*e.g.*, phenol, collidine, etc.); the polar phase is formed in the paper as a result of conditioning or demixion. Less polar organic solvents, *e.g.* chloroform, extract amino acids to a negligible degree.

In the present communication a system of two liquids is described which are only slightly soluble in each other; nevertheless, the partition coefficients of many amino acids are relatively high in favour of the non-polar phase. It is the system: *n*-hexanol + HDEHP/aqueous buffer solution. HDEHP, *i.e.*, hydrogen di(2-ethylhexyl) orthophosphoric acid, is a solvent belonging to the class of the so-called liquid ion exchangers and has been widely employed in the extraction and chromatography of inorganic substances. In our previous work[1] we have employed HDEHP in paper chromatography of alkaloids, also giving a brief account of earlier related papers.

The solvent system discussed has, besides features interesting from the theoretical viewpoint, the following advantages:

(1) The negligible miscibility of the two liquid phases qualifies the system for application in preparative countercurrent methods, including column liquid–liquid partition chromatography with a continuous recording of the eluate concentration. The latter technique, supplementary to gas–liquid partition chromatography, has been recently drawing the increasing attention of chromatographers and producers of chromatographic equipment.

(2) The feasibility of controlling the partition by variation of pH of the aqueous phase or the composition of the mobile phase (both by the concentration of HDEHP and by the type of diluting solvent), so that gradient elution is readily obtainable.

(3) The system combines the advantages of partition and ion exchange systems; the diffusion of the solutes into the liquid ion exchanger is probably much more rapid compared to diffusion into solid ion exchangers, so that equilibration is facilitated, with resulting higher separation efficiency.

(4) The sequence of amino acids is not identical with those observed for frequently employed chromatographic systems.

One of the remarkable and unusual features of the system reported here is that the buffer solution forms the stationary phase, while the ion exchanger is the mobile phase. On the other hand, however, the system is analogous to the Partridge system (except that both organic solvents, acidic and neutral, are practically immiscible with water) and to the systems of the type: diluting solvent + oleic acid/buffer solution, whose interesting properties have been discussed in an earlier paper[2].

EXPERIMENTAL

In view of the low solubility of water in the organic phase, the polar phase in the paper could not be formed as a result of adsorption from the developing solvent; therefore, the "moist buffered paper" technique was employed (for details and references *cf.* ref. 2).

Whatman No. 1 paper was impregnated with McIlvaine's buffer solutions (0.2 *M* Na_2HPO_4 + 0.1 *M* citric acid), controlling the degree of impregnation so that the strips contained, at the moment of transferring to the chamber, 0.5 ml of buffer solution per 1 g of dry paper.

The chromatograms were developed by the descending technique with solutions of HDEHP in *n*-hexanol, in tanks 24 × 7 × 15 cm.

The chromatographic behaviour of 22 amino acids was investigated, the main variables being the pH of the stationary phase and the concentration of HDEHP in the mobile phase.

The effect of pH (Fig. 1a–c) is reflected by regular bell-shaped curves, the

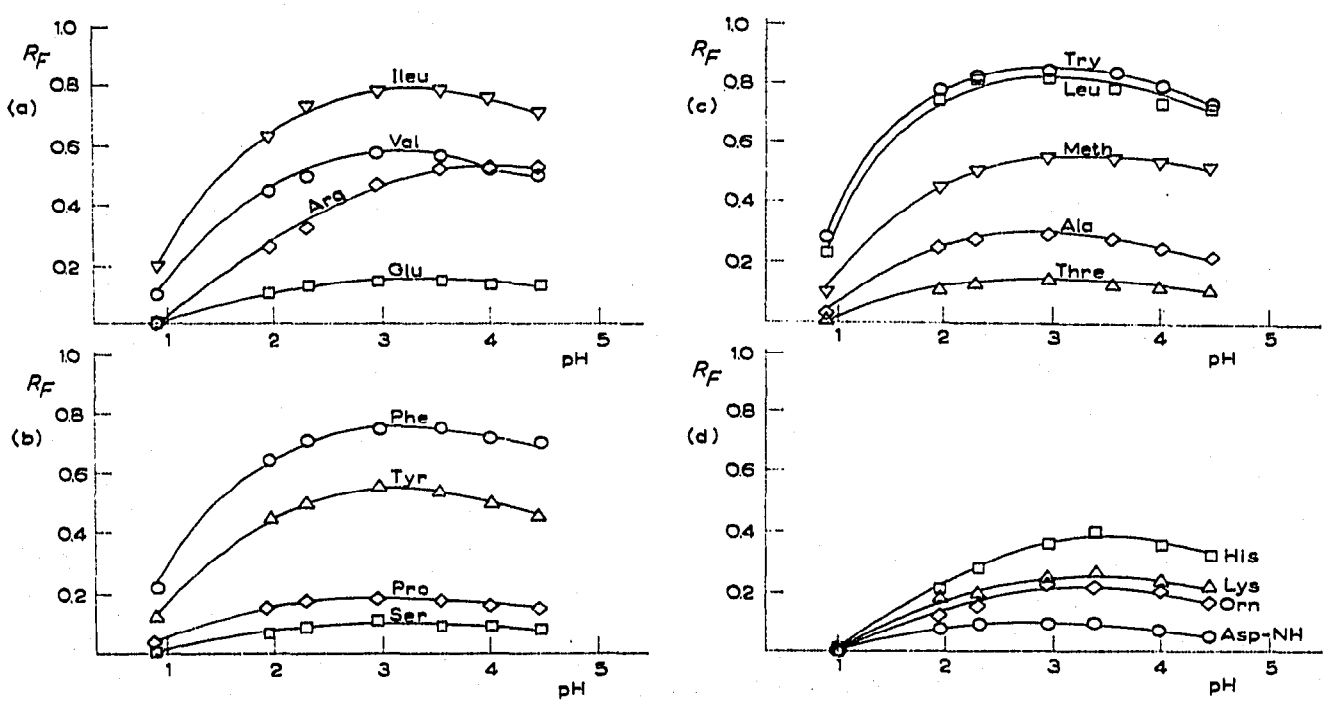


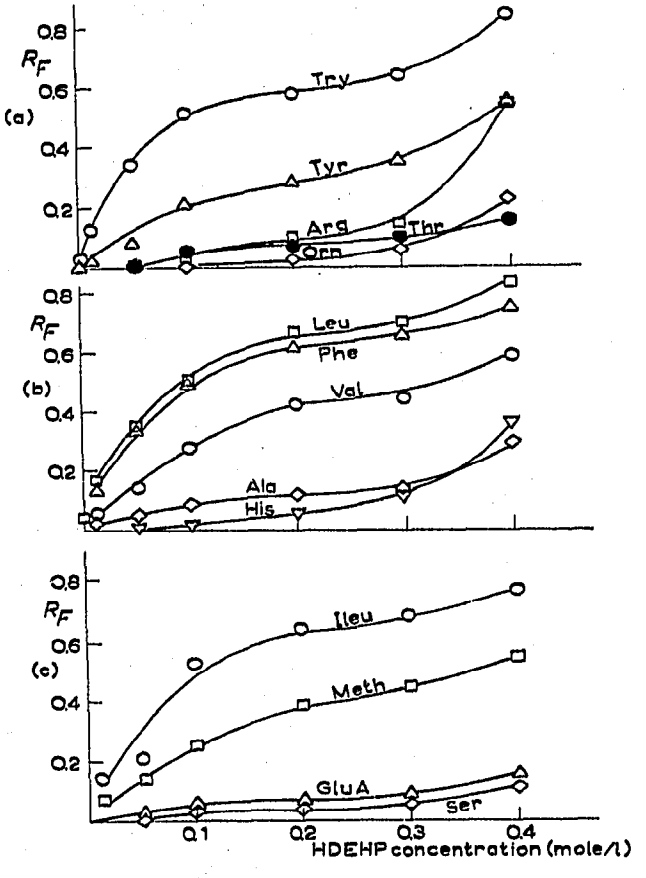
Fig. 1. R_F *vs.* pH relationships of 17 amino acids (cysteine, cystine, hydroxyproline, glutamine and aspartic acid have an $R_F < 0.08$). Mobile phase: 0.4 *M* HDEHP in *n*-hexanol.

maxima of which occur at pH *ca.* 3.0 independent of the ionization constants and isoelectric points of the solutes; thus, the maxima occur in the range of basic ionization of the amino acids, since their isoelectric points are in most cases at higher pH values (except for glutamic acid and aspartic acid, for which pI ≐ 3.0). The initial increase of R_F for decreasing pH values is probably due to increase of the basic ionization of the amino acids, and, possibly, to a decrease in the concentration of the competing sodium ions in the buffer solution. For still lower values of pH, the displacement of amino acid cations by hydrogen ions becomes the decisive factor, according to the equilibrium:

$$H\cdot DEHP_{(org)} + R(COOH)\,(NH_3)^+_{(w)} \rightleftharpoons R(COOH)\,(NH_3)\cdot DEHP_{(org)} + H^+_{(w)}$$

$$K_t = \frac{[R(COOH)\,(NH_3)\cdot DEHP]\,[H^+]}{[H\cdot DEHP]\,[R(COOH)\,(NH_3)^+]}$$

(neglecting the activity coefficients; the brackets denote concentrations expressed in terms of mole fractions. The latter can be substituted by molar concentrations when the solutions are dilute).



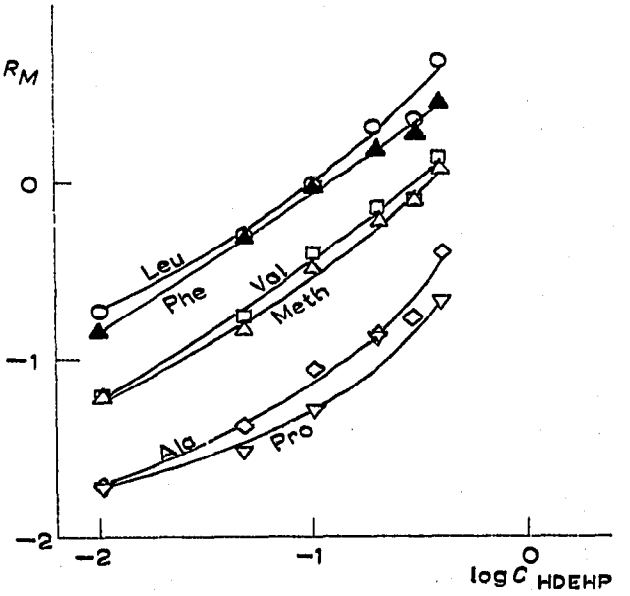


Fig. 2. Effect of concentration of HDEHP in the mobile phase on R_F values of some amino acids. Stationary phase: McIlvaine's buffer solution, pH ≐ 3.

Fig. 3. R_M *vs.* log C_{HDEHP} relationships of some amino acids plotted from data of Fig. 2. $R_M = \log R_F/(1 - R_F) = \log D + \text{const.}$

Also it cannot be excluded that an additional effect may be due to varying extraction of HDEHP from the organic phase by aqueous solutions of different pH.

The effect of composition of the organic phase on R_F values is illustrated in Fig. 2a–c. It can be seen that pure hexanol does not extract amino acids. With increasing concentration of HDEHP in the organic phase (and at a constant pH of the water phase) the partition of amino acids is shifted in favour of the organic phase. Defining the extraction coefficient D as the ratio of concentration in the ion exchanger to that in the aqueous phase, and assuming certain simplifications, we have[2]:

$$D = \frac{[\mathrm{R(COOH)\,(NH_3)\cdot DEHP}]}{[\mathrm{R(COOH)\,(NH_3)^+}]} = K_i \cdot \frac{[\mathrm{H\cdot DEHP}]}{[\mathrm{H^+}]}$$

$$\log D = \log K_i + \log [\mathrm{H\cdot DEHP}] + \mathrm{pH}$$

At higher concentrations of HDEHP the concentrations should be expressed in terms of mole fractions and the R_M values should also be suitably re-calculated for mole fractions; the linear R_M *vs.* log molar concentration of HDEHP relationships can then only be regarded as semi-empirical, even when the activity coefficients are assumed to be constant. As a matter of fact, R_M values plotted against log C_{HEDHP} show marked deviations from linearity (Fig. 3).

The mechanism of partition of amino acids in the system studied is not yet quite clear and its elucidation would require additional bulk extraction experiments. In particular, it is not quite clear whether the "ion exchange" occurs in the bulk of the organic phase or on the liquid–liquid interface with molecules of HDEHP oriented there. With the latter mechanism predominating, the ion exchange capacity and partition behaviour would probably be markedly dependent on the area of the liquid–liquid interface.

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SUMMARY

It was demonstrated that amino acids, in spite of their strongly hydrophilic character, distribute between water and a mixed solvent composed of *n*-hexanol and di(2-ethyl-hexyl) orthophosphoric acid. The mixed organic phase, belonging to the class of liquid ion exchangers, is practically immiscible with water; the solvent system can thus be employed in column liquid–liquid partition chromatography. The partition of amino acids in the system studied can be controlled by pH of the aqueous phase and/or concentration of HDEHP in the organic phase.

REFERENCES

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