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SEMIEMPIRICAL PREDICTION OF RETENTION OF SOME AMPHOLYTES ON A CARBON ADSORBENT*

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SUMMARY

Experimental data were analyzed for several model ampholytes, and parameters were obtained enabling the prediction of the retention behaviour of these substances in dependence on pH and the ionic strength. The possibility of the semi-empirical optimization of the separation conditions is demonstrated.

The experiments were carried out with a JaDo carbon (prepared by reduction of poly(tetrafluoroethylene)), packed in a relatively short (6 cm) column.

INTRODUCTION

The chromatographic process is frequently discussed in terms of numerical treatment and expression. Many authors have dealt with the behaviour of weak electrolytes in reversed-phase high-performance liquid chromatography (theoretically¹, for ODS²⁻⁵, Amberlite XAD copolymers⁶⁻⁹, a carbonaceous adsorbent¹⁰). In most cases the effects of pH, ionic strength (*I*), the organic phase and sometimes temperature were studied separately. The approach described here is one of semi-empirical procedures, which, however, describes the dependence of the retention behaviour of ampholytes on both pH and *I*. On the basis of experimental data it enables inter- and extrapolation of these dependences over a selected real range of the experimental conditions. The following treatment, which involves the construction of a map in pH and *I* coordinates depicting the overlap of the solutes studied, was chosen to obtain a complete picture of the possibilities of solving the separation problem with a given column, and replaces the tedious trial and error method.

THEORETICAL

The retention behaviour of weak electrolytes is described by a phenomenological equation for acid-base equilibrium. The dependence of the capacity ratio on the eluent pH at a constant *I* is given by

$$\kappa = (\kappa_0 + \sum_{i=1}^n (\kappa_i \prod_{j=1}^i K_j / a_{H^+}^i)) / (1 + \sum_{i=1}^n (\prod_{j=1}^i K_j / a_{H^+}^i)) \quad (1)$$

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where κ is the ampholyte capacity ratio at the given I ;
 κ_i for $i \in \langle 0; n \rangle$ are the limiting capacity ratios of the individual ionization forms at the given I ;
 K_j for $j \in \langle 1; n \rangle$ are the apparent dissociation constants of the acids ordered according to decreasing acidity at the given I ;
 a_{H^+} is the proton activity;
 n is the number of groups that are ionized in the given range of experimental conditions.

To describe the effect of the ionic strength, its effect on the apparent dissociation constant and an indirect effect on the limiting capacity ratios must be expressed. The former dependence was expressed in terms of the relationship derived by Davies¹¹ as modified Debye-Hückel equation:

$$K_j = K_j^0 \cdot 10^{((2j-1) \cdot A \cdot I^{1/2}) / (1 + I^{1/2}) - (2j-1) \cdot 0.1 \cdot I} \quad (2)$$

where K_j^0 is the thermodynamic dissociation constant;
 A is an empirical constant (estimated as $0.5093 \text{ mol}^{-1/2} \text{ l}^{1/2}$);
 I is the ionic strength, mol l^{-1} .

The effect of the surface tension of the mobile phase on the limiting capacity ratios is known from the literature². The linear dependence of $\ln \kappa_i$ on the electrolyte concentration can, under certain conditions, be replaced by a linear dependence on I :

$$\ln \kappa_i = a_i I + b_i \quad (3)$$

where a_i and b_i are constants characterizing the i -th ionization form of the ampholyte. To decrease the error stemming from the above simplification, the components of the buffers used must have similar character (*e.g.* uni-bivalent with a bulky anion) leading to similar changes in the surface tension with changing concentration.

Further, it can be assumed that eqn. 3 gives parallel lines for various ionization forms of a single substance. The number of parameters required for characterization of the chromatographic behaviour of an ampholyte is thus decreased. On substitution from eqns. 2 and 3 into eqn. 1 the capacity ratio is obtained as a function of two variables, pH and I . If experimental points are fitted to a plane by regression analysis, dissociation constants K_j^0 are obtained (or tabulated values can be used), as well as the limiting capacity ratios, which together with slope a , enable the capacity ratio value to be calculated.

EXPERIMENTAL AND CALCULATIONS

A Pye Unicam LC-XP liquid chromatograph was used with a 20- μl Rheodyne sampling loop and a Pye Unicam LC-UV variable wavelength detector. The detection was carried out around 200 nm. A stainless steel column, $6 \times 0.43 \text{ cm}$ I.D., packed with a JaDo 1141/3 adsorbent (sample No. II in ref. 10) was employed at laboratory temperature. The column efficiency used in calculations was $N = 120$ at $L = 6 \text{ cm}$ with linear flow-rate $u = 1.5 \text{ mm sec}^{-1}$. The buffers were prepared from *p.a.* dihydrogen phosphate, hydrogen phosphate and phosphoric acid. The ionic strength was adjusted with Na_2SO_4 . The amino acid solute samples, ornithine, lysine, arginine

TABLE I
DISSOCIATION CONSTANTS OF THE MODEL AMPHOLYTES¹²

<i>Amino acid</i>	pK_1	pK_2
Orn	1.705	8.69
Lys	2.18	8.95
Arg	1.822	8.99
His	1.77	6.1

and histidine, were obtained from Lachema (Brno, Czechoslovakia). The dissociation constant values used in calculations are summarized in Table I.

If, for the sake of simplicity, the column efficiency is considered constant for various capacity ratios and the peaks are assumed to be Gaussian, a theoretical intercept of two peaks can be calculated from the relationship

$$t_k = 2/(1/t_n + 1/t_m) \quad (4)$$

The mean value of the overlap was expressed for symmetrical peaks with a unit height,

$$U = 10 \cdot \left(\sum_{k=1}^p \sum_{i=1}^p \exp(-(1 - t_k/t_i)^2 N) \right) / p \quad (5)$$

where p is the number of peaks.

The Gauss-Newton method was used for the regression analysis and the ionic strength was computed by an iteration method¹³ on a TI 59 calculator. For graphical dependences, a HP 9830A desk-top calculator was used, and for pseudographical dependences an ICL 4-72* computer was used.

RESULTS AND DISCUSSION

The dependence of the behaviour of four model ampholytes on the eluent pH and I was investigated. The treatment was based on the capacity ratios measured over a pH range from 2 to 6 and $I \in \langle 0.05; 0.20 \rangle$. Figs. 1(a-d) depict sections through the $\kappa = f(\text{pH}, I)$ plane at constant I . In the measuring range given, this function increased monotonically with increasing I . Table II summarizes the parameters characterizing the individual ampholytes.

A map (Fig. 2) was constructed from these data, in which peak overlap is represented by the isobases. The height scale is non-linear; the overlap of two, three or four peaks is given by heights 5, 15 or 30, respectively. For example, transition of height 5 represents a change in the elution order of two peaks. Abbreviations composed of the initial letters of the solutes express the elution order and are written in the appropriate valleys.

In this way it can be found how the elution order of the amino acids changes on transition from one valley to another. The dot and dash lines denote the regions

* The program written in FORTRAN is available on request.

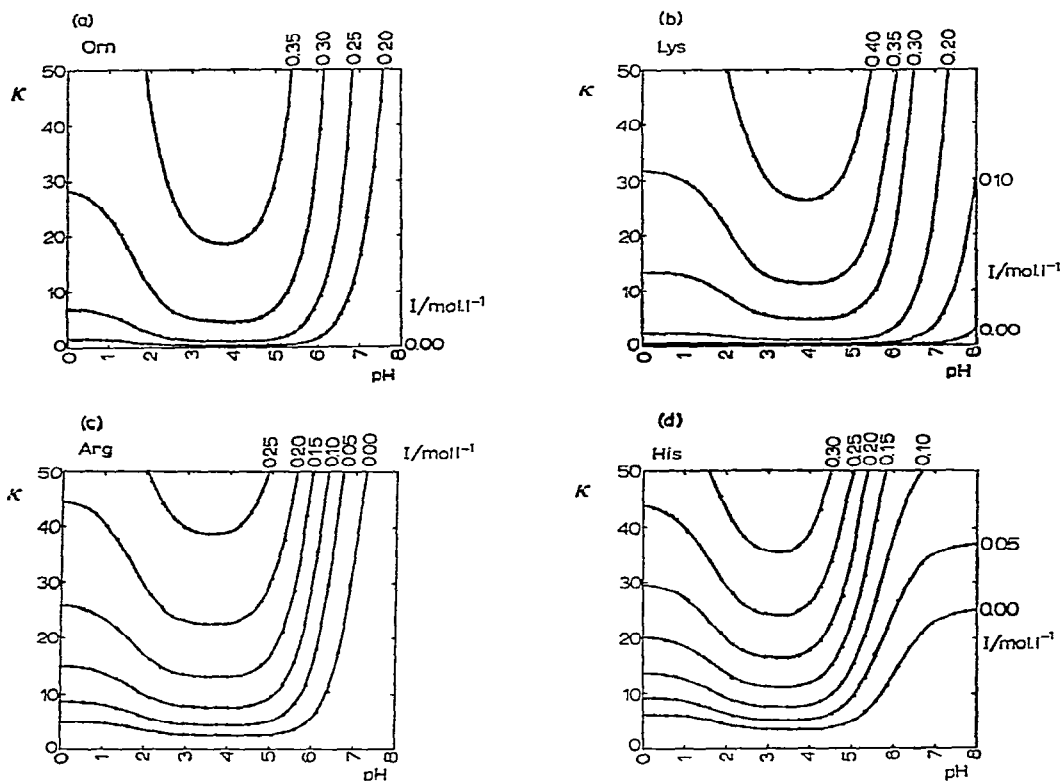


Fig. 1. The effect of the eluent pH on the capacity ratios of different ampholytes at the constant ionic strengths. (a) Ornithine; (b) lysine; (c) arginine; (d) histidine.

TABLE II

SLOPE, a , AND NATURAL LOGARITHM OF THE LIMITING CAPACITY RATIOS, b_1 , AT $I = 0$ (EQN. 3)

Amino acid	a	b_1	b_2	b_3
Orn	28.399	-5.155	-7.084	0.0178
Lys	17.061	-2.511	-3.599	3.298
Arg	10.949	1.616	0.896	8.05
His	7.773	1.843	1.191	3.228

in which U is less than 0.1. In these regions, considering the total analysis time, are found the conditions for separation. The retention time of the last peak in the mixture is denoted by a dashed line. The validity of this map was verified on the chromatogram of a mixture of the four substances (Fig. 3).

On increasing the column efficiency the valleys become wider and the ridges narrower. The overall character of the map changes not only in places where there are barely perceptible high plateaux, but new valleys are also formed at higher efficiencies (at *ca.* 10 times higher efficiency a valley is formed in the top left corner of the map; the total analysis time would be here 4–5 min).

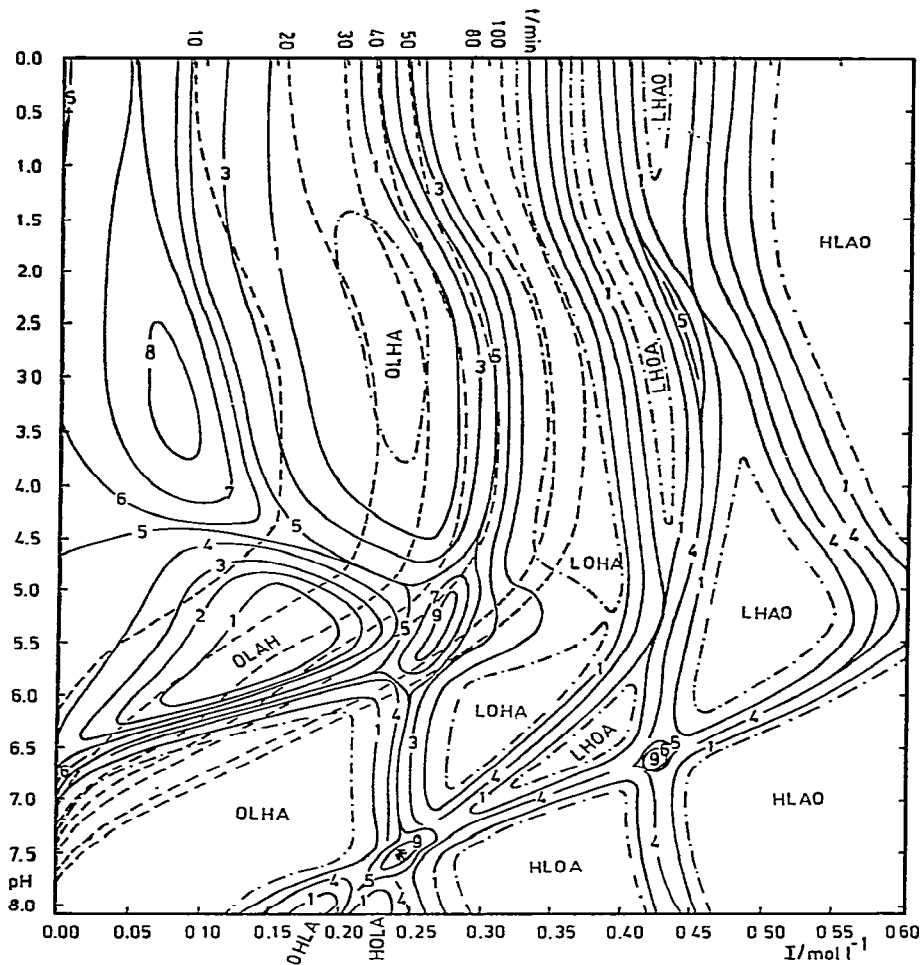


Fig. 2. Map of the influence of the eluent pH and ionic strength on the mean value of the overlap of four studied ampholytes. A = Arginine; H = histidine; L = lysine; O = ornithine.

In view of the many problems with gradient elution it is often advantageous to find isocratic separation conditions. It is possible to introduce further variables (efficiency, temperature, mixed media, etc.) and to assemble a collection of maps from which either suitable conditions can be directly found, or at least an initial point can be obtained for further computer optimization.

CONCLUSION

For practical use of this procedure, a library of data would have to be assembled for a greater number of weak electrolytes, using an adsorbent that is sufficiently widely used and is readily available. As the retention is determined by the size of the non-polar part of the molecule, the content of various elements, the polarization of some bonds, the steric conditions, etc., further work should be directed toward estimation of the absolute values of the limiting capacity ratios.

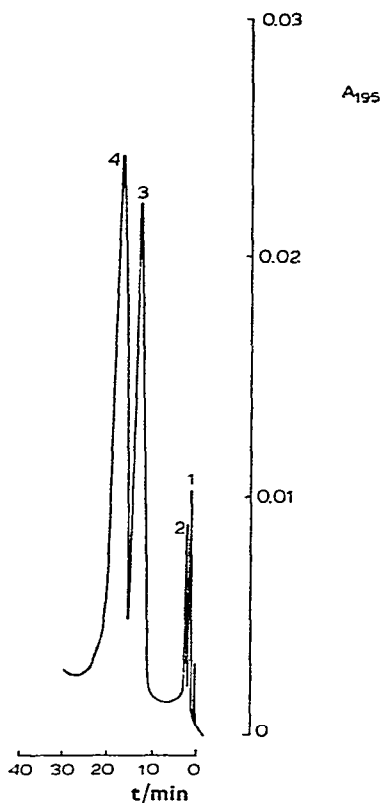


Fig. 3. Chromatogram of four basic amino acids. Operating conditions: eluent, 0.11 M phosphate buffer; pH 2.0; $I = 0.2 \text{ mol l}^{-1}$; flow-rate; 1 ml min^{-1} ; detector, UV. Peaks: 1 = Orn; 2 = Lys; 3 = His; 4 = Arg.

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REFERENCES

- 1 H.-W. Hsu and I.-J. Chung, *J. Chromatogr.*, 138 (1977) 267.
- 2 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
- 3 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.
- 4 Cs. Horváth, W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
- 5 J. L. M. van de Venne, *Thesis*, Technical University, Eindhoven, The Netherlands, 1979.
- 6 C.-H. Chu and D. J. Pietrzyk, *Anal. Chem.*, 46 (1974) 330.
- 7 D. J. Pietrzyk and C.-H. Chu, *Anal. Chem.*, 49 (1977) 757.
- 8 D. J. Pietrzyk, E. P. Kroeff and T. D. Rotsch, *Anal. Chem.*, 50 (1978) 497.
- 9 E. P. Kroeff and D. J. Pietrzyk, *Anal. Chem.*, 50 (1978) 502.
- 10 E. Smolková, J. Zima, F. P. Dousek, J. Jansta and Z. Plzák, *J. Chromatogr.*, 191 (1980) 61.
- 11 C. W. Davies, *J. Chem. Soc.*, (1938) 2093.
- 12 D. D. Perrin, *Dissociation constants of organic bases in aqueous solution*, Butterworths, London, 1965.
- 13 D. D. Perrin and B. Dempsey, *Buffers for pH and metal ion control*, Chapman & Hall, London, 1974.