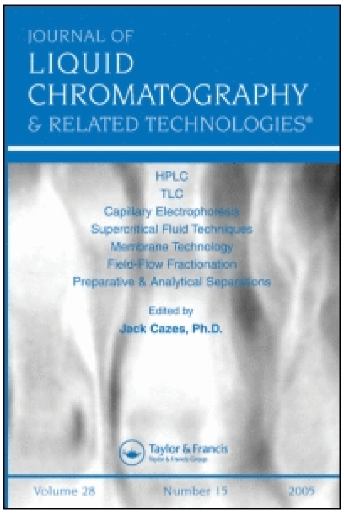

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Solute Dissociation Effects in Reversed Phase Liquid Chromatography with Mixed Eluents

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SOLUTE DISSOCIATION EFFECTS IN REVERSED PHASE LIQUID CHROMATOGRAPHY WITH MIXED ELUENTS

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ABSTRACT

Basing on the displacement model of liquid chromatography new equations are proposed for the capacity ratio of ionogenic solutes in the mixed mobile phase. Effects of different parameters on the capacity ratio of the monotropic acids are systematically studied for RPLC process.

INTRODUCTION

The great majority of biologically significant compounds contain ionogenic groups. The separation of acidic, basic and

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zwitterionic substances is a very important problem. Usually, thin-layer, ion-exchange or reversed phase chromatographic techniques are used for such separations (1-4). Numerous studies proved that dissociation of the solute strongly affects its retention (3-10). A modulation of the selectivity of chromatographic separations by using the ionization control is possible. Changes in the pH of the eluent can modify the retention of ionogenic compounds while leaving the retention of neutral substances unchanged (5). The most general theory of liquid chromatography for ionogenic substances was formulated by Horvath et al. (2,3).

Recently, the mixed organic/aqueous solvent was used for separation compounds such as aminoacids and pepticides (4,5). Different theoretical approaches to reversed phase liquid chromatography with mixed mobile phase were discussed by Melander and Horvath (11).

In this paper, new expressions for the capacity ratio of acids and basis chromatographed in mixed eluents are presented. The effects of the solute ionization in such chromatographic systems are discussed. This treatment was formulated on a basis of the quasichemical theory of liquid/solid chromatography (12,13).

THEORETICAL

The chromatographic system consists of the solute (s), aqueous/organic solvent and nonpolar stationary phase. The solute s is assumed to be a weak acid (AH) or a weak base (B).

The layer of a liquid immediately adjacent to the adsorbent covered by long hydrocarbon chains is called the surface phase. When the whole adsorbent surface is bonded by hydrocarbons we can say that it is a homogeneous one. However, a partially covered solid surface may be treated as an energetically heterogeneous adsorbent.

The chromatographic process is viewed as the exchange of molecules between the mobile and surface phases. For the ensuing discussion, we shall assume that the liquid mixture is ideal and the adsorbent is homogeneous. Let us discuss retention of the weak monotropic acid. The dissociation AH in the mobile phase is governed by the following equilibrium:

$$AH \rightleftarrows A^- + H^+ \tag{1}$$

In this case, the dissociation constant is given by

$$L = c_{H^+} \cdot c_{A^-} / c_{AH} = (x_{A^-} / x_{AH}) \cdot 10^{-pH} \tag{2}$$

where c_β is the molarity of the βth component (ion or molecule) whereas x_β denotes its mole fraction in the mobile phase. It follows from equation 2 that

$$x_A = x_{AH} \cdot 10^{pH-pL} \tag{3}$$

where pL = -log L.

The total mole fraction of the s-th solute in the mobile phase is equal to:

$$x_s = x_{A^-} + x_{AH} = x_{AH}\ (1 + 10^{pH-pL}) \tag{4}$$

In the chromatographic process under consideration, solute retention is assumed to occur because of exchange of molecules or ions between mobile and surface phases:

$$(AH) + r\ (o)^{\sigma} \rightleftarrows (AH)^{\sigma} + r\ (o) \tag{5}$$

$$(A^-) + r\ (o)^{\sigma} \rightleftarrows (A^-)^{\sigma} + r\ (o) \tag{6}$$

$$(o) + (w)^{\sigma} \rightleftarrows (o)^{\sigma} + (w) \tag{7}$$

where symbols (o) and (w) denote molecules or organic solvent and water, respectively. The superscript σ refers to the surface phase whereas absence of an index indicates the mobile phase. The stoichiometric coefficient r is defined as the ratio of molecular sizes of the solute and the organic solvent. The molecular sizes of both the solvents are assumed to be identical (12,13).

The adsorption constants for nondissociated acid (AH), dissociated acid (A^-) and the organic solvent are given by:

$$K_{AH} = (y_{AH}/x_{AH})\ (x_o/y_o)^r \tag{8}$$

$$K_{A^-} = (y_{A^-}/x_{A^-})\ (x_o/y_o)^r \tag{9}$$

$$K_{ow} = (y_o/x_o)\ (x_w/y_w) \tag{10}$$

where y_β denotes the mole fraction of the β-th component in the surface phase.

From equations 8 and 9 we have:

$$y_{AH} = K_{AH}\, x_{AH}\, (y_o/x_o)^r \tag{11}$$

$$y_{A^-} = \alpha K_{AH}\, x_{A^-} \cdot (y_o/x_o)^r \tag{12}$$

where the coefficient α is defined as:

$$\alpha = K_{A^-} \,/\, K_{AH} \tag{13}$$

and it characterizes the difference in adsorption properties of dissociated and undissociated acids.

Analogously as for the mobile phase, one can calculate the total mole fraction of the solute in the surface phase:

$$y_s = y_{AH} + y_{A^-} = x_{AH}(1 + \alpha 10^{pH-pL})\, K_{AH}\, (y_o/x_o)^r \tag{14}$$

The capacity rate of the solute is given by:

$$k'_s = q\, (y_s/x_s) = q\, F_A\, K_{AH}\, (y_o/x_o)^r \tag{15}$$

where q is the constant characteristic for a given adsorbent (14). F_A is the function describing acidic dissociation effects:

$$F_A = (1 + \alpha 10^{pH-pL}) \;/\; (1 + 10^{pH-pL}) \qquad (16)$$

Let us notice that the capacity ratio defined by equation 15 is a product of function F_A and the expression $q\ K_{AH}\ (y_o/x_o)^r$ characterizing adsorption equilibrium in the system. When a pure solvent is used, equation 15 becomes the form of equation discussed by Horvath and his coworkers (2), which is analogous to that proposed for liquid/liquid chromatography (15). Equation 15 may be rewritten in a more convenient form:

$$\ell n\ k'_s = \ell n\ qK_{AH} + \ell n\ F_A + r\ \ell n\ (y_o/x_o) \qquad (17)$$

From equation 10 describing adsorption equilibrium between solvents we have (12,13):

$$y_o/x_o = K_{ow}/[1 + (K_{ow} - 1)\ x_o] \qquad (18)$$

Combination of equations 15 and 18 allows us to express the capacity ratio as the analytical function of the mobile phase composition, i.e., $k' = k'(x_o)$.

In a real chromatographic system a lot of other parameters affect equilibria given by equations 1 and 5-7. Two paramters seem to be most important: nonideality of the solution and heterogeneity of the surface.

First we shall discuss the effect of nonideality of the mobile phase on the solute retention. It follows from many studies that the surface may be assumed to be ideal (12). Replacing concentrations by activities in equations 2 and 8-10, we obtain:

$$k' = q\, F_A^{nid}\, (y_o/a_o)^r \tag{19}$$

where a_o denotes the activity of the organic solvent in the mobile phase and F_A^{nid} is a function characterizing dissociation in the nonideal mobile phase:

$$F_A^{nid} = (\gamma_{AH} + \gamma_{A^-} \cdot \alpha 10^{pH-pL}) \; / \; (1 + 10^{pH-pL}) \tag{20}$$

where γ_β denotes the activity coefficient of the β-th component in the mobile phase, whereas L is defined by equation 2 and it is connected with the thermodynamic dissociation constant L^* (which is expressed by means of activities) in the following way:

$$L = L^* \, \gamma_{AH} / \, (\gamma_{A^-} \cdot \gamma_{H^+}) \tag{21}$$

When $\gamma_{AH} = \gamma_{A^-} = (\gamma_o)^r$ the capacity ratio may be calculated from equations 15 and 16 in which L is the function defined by equation 21.

On the basis of experimental studies reported by Bacarella <u>et al</u>. (16) the following empirical relationship may be proposed for the mole fractions x_o from the interval (0 - 0.8):

$$pL = \eta x_o + \xi \tag{22}$$

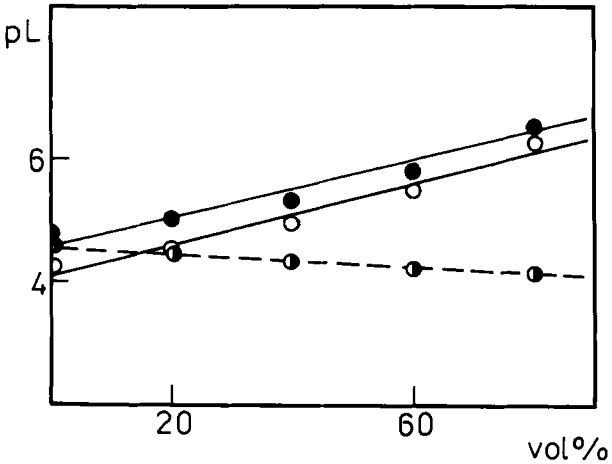

Figure 1. Approximation of the experimental data pL vs. solvent vol % for acetic acid (●), benzoic acid (o) and aniline (◑) (16) by equation 22.

where η, ξ are constants. It follows from Figure 1 that equation 22 gives a good representation of the pL vs. x_o data.

Now we shall discuss the effect of adsorbent heterogeneity in the chromatographic system under consideration. Analogously as in the theory of NPLC process the capacity ratio of the s-th solute on an entire heterogeneous surface $k'_{s,t}$ is defined as follows (14,17,18):

$$k'_{s,t} = \sum_{p=1}^{P} f_p \, k'_{s,p} \tag{23}$$

where f_p is the ratio of the number of adsorption sites of the p-th type to the total number of adsorption sites. The capacity ratio $k'_{s,p}$ is given by an equation analogous to equation 15:

$$k'_{s,p} = q\ F_A\ K_{AH,p}\ (y_{o,p}\ /\ x_o)^r \tag{24}$$

Combination of equations 23 and 24 leads to:

$$k'_{s,t} = (q\ F_A/x_o^{\ r}) \sum_{p=1}^{P} f_p\ K_{AH,p}\ (y_{o,p})^r \tag{25}$$

It has been shown by computer simulations that the following approximation is justified (19):

$$\sum_{p=1}^{P} f_p\ K_{AH,p}\ (y_{o,p})^r \approx \overline{K_{AH}}\ \ (y_{o,t})^{r/m} \tag{26}$$

where $\overline{K_{AH}}$ is an average from the $K_{AH,p}$ values, m denotes the heterogeneity parameter, whereas $y_{o,t}$ is the total mole fraction of the organic solvent on a heterogeneous surface; it is given by the adsorption isotherm corresponding to adsorbents characterized by quasi-Gaussian energy distribution (12):

$$y_{o,t} = (\bar{K}_{ow}\ x_{ow})^m\ /\ [1 + (\bar{K}_{ow}\ x_{ow})^m] \tag{27}$$

where $x_{ow} = x_o\ /\ x_w$, and $\overline{K_{ow}}$ is an average from $K_{ow,p}$ values. When r=1 from equations 25-27 we obtain:

$$k'_{s,t} = q\ F_A\ \overline{K_{AH}}\ \overline{K_{ow}}\ [(1 - x_o)^m + (x_o\ \overline{K_{ow}})^m]^{-1/m} \tag{28}$$

If the whole adsorbent surface is chemically bonded (m=1), then equation 28 becomes an equation which may be obtained from equations 15 and 18 for r=1.

Analogous formulas may be obtained for basis. The ionization of a weak base B in the mobile phase takes place according to the following equilibrium:

$$BH^{+} \rightleftarrows B + H^{+} \tag{29}$$

where BH^{+} is the protonated base. The equilibrium given by equation 29 is usually characterized by dissociation constant of the acid BH^{+} conjugated with base B which for ideal solution may be written as:

$$L = (x_B \;/\; x_{BH^+}) \cdot 10^{-pH} \tag{30}$$

In this case, the capacity ratio may be expressed by equations 15 or 28 in which the acidic dissociation function F_A is replaced by the function for basic solute:

$$F_B = (1 + \alpha 10^{pL-pH}) \;/\; (1 + 10^{pL-pH}) \tag{31}$$

and constants K_{AH} or $\overline{K_{AH}}$ by analogous parameters K_B or $\overline{K_B}$.

Following the above procedure the expressions for capacity ratios of polytropic electrolytes may be obtained. The capacity ratio is always a product of the adsorption term and the dissociation function which is the same as that proposed for liquid/liquid chromatography (15).

RESULTS AND DISCUSSION

Extensive model calculations connected with acidic dissociation effects have been performed according to equations 15, 16 and 28. It has been assumed that energies of interactions between molecules and the surface (E) for the solute and solvent satisfy the following inequality $E_s > E_o > E_w$. The capacity ratio depends upon parameters connected with the solute (r, K_{AH}, α, L), constant K_{ow} characterizing adsorption effects in the mixed solvent and pH which may be controlled by using suitable buffers. The calculations have been made for $qK_{AH} = 1.25$ and $r=2$ (only for heterogeneous surface $r=1$). Two types of curves are discussed: k' vs. pH for x_o = const and dependences of the capacity ratio upon mobile phase composition k' vs. x_o.

First we consider chromatographic process for ideal mobile phase (i.e. for $L=L^*$). Let us notice that for a given composition "*the adsorption term*" $qK_{AH}\ (y_o\ /\ x_o)^r$ = const. In this case, the capacity ratio defined by equations 15 and 16 is proportional to that proposed by Horvath et al. (2) for a pure solvent. It means that curves k' vs. pH for a mixed eluent have the same shape as those for pure mobile phases. Figures 2 - 4a show k' vs. pH curves calculated for $x_o = 0.25$ and different values of parameters K_{ow}, α and L. When $\alpha = 0$ curves under consideration have the same course as these describing dependences of the extraction coefficients upon pH in theory of liquid/liquid chromatography (15,20,21). The conclusions concerning the essential features of the curves are summarized by Soczewiński in the review (15). They are sigmoid in shape with inflection point at pH = pL.

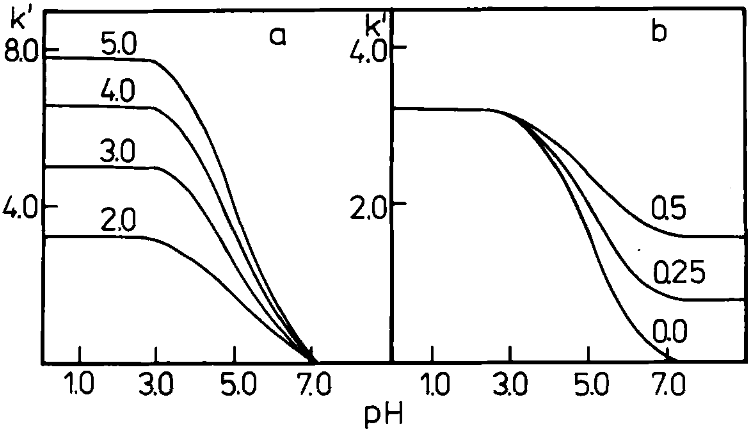

Figure 2. Effect of adsorption parameters K_{ow} and α on k' vs. pH curves. Parameters: r=2.0, L=5.0, x_o=0.25; (a) α=0 and K_{ow}= 2.0, 3.0, 4.0, 5.0; (b) K_{ow}=2.0 and α=0, 0.25, 0.5.

An influence of adsorption parameters K_{ow} and α on the k' vs. pH curves is presented in Figures 2a-b. The strong adsorption of the organic solvent (high value of K_{ow}) causes considerable increase of the capacity ratio for low values of pH. If pH is bigger than pL then the capacity ratio is very small for any values of the adsorption constant K_{ow}. However, an opposite effect is observed when coefficient α is varied. It considerably affects the retention for pH > pL. An increase of the difference in adsorption properties of dissociated and non-dissociated acid molecules (a decrease of α) brings about a decrease in the capacity ratio.

An effect of ionization constant on the position of k' vs. pH curves is presented in Figure 3. It follows from the figure that dissociation of chromatographed acid causes a decrease of its capacity ratio.

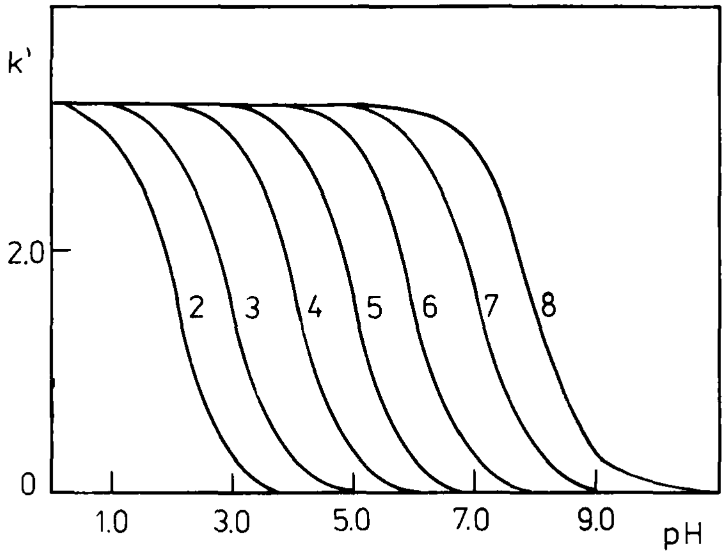

Figure 3. Effect of acidic ionization constant L on the position of k' vs. pH curves. Parameters: K_{ow}=2.0, r=2.0, α=0, x_o=0.25 and pL=2,3,4,5,6,7,8.

An important question is how the composition of the mixed mobile phase affects the retention of a given solute. In Figure 4a k' vs. pH curve is plotted for different concentrations of the organic solvent. An analysis of the results shows that for L = L^* an increase of x_o always brings about a decrease of the capacity ratio. This effect is important only for pH < pL or pH $\simeq$ pL (compare Figures 4a and 4b).

Melander and Horvath (4) reported the retention data of homovanilic acid in aqueous solutions of methanol. The percentage of CH_3OH was varied from 0% to 40%. The change of pL is comparatively small in this concentration region (pL varies from $\sim$ 4.2 in pure water to $\sim$ 4.6 in 40:60 CH_3OH/H_2O). The conclusions which one can

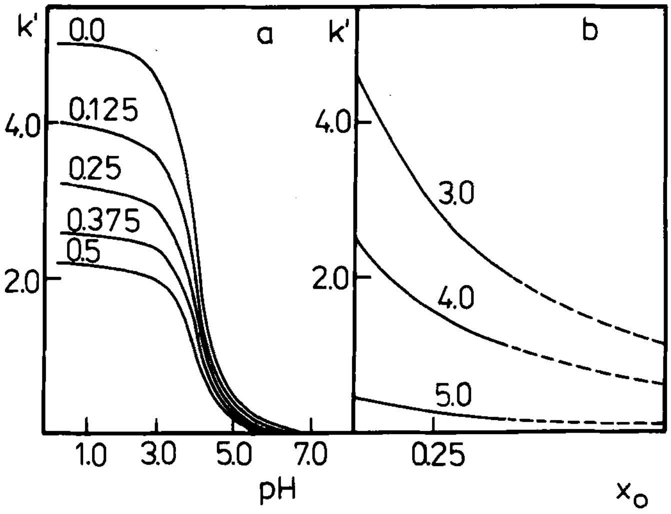

Figure 4. Effect of the ideal mobile phase composition on k' vs. pH and k' vs. x_o curves. Parameters: K_{ow}= 2.0, r=2.0, α=0, L=4.0; (a) x_o=0, 0.125, 0.25, 0.375, 0.5 (b) pH=3.0, 4.0, 5.0.

draw from these studies are consistent with those following from theoretical treatment discussed here.

Figure 4b shows an example of k' vs. x_o curves. The mathematical analysis shows that for $L = L^* =$ const the function $k' = k'(x_o)$ monotonically decreases for any values of paramters K_{ow}, α, L and pH. In a real chromatographic system the assumption $L = L^* =$ const may be sufficiently justified for $x_o < 0.5$ only (16). Therefore, the fragments of k' vs. x_o curves for $x_o > 0.5$ are represented by dashed lines. In Figure 5, curves ℓn k' vs. ℓn x_o calculated according to equation 17 are presented for different values of constants K_{ow} and L.

The simultaneous influence of the solute dissociation and heterogeneity of the adsorbent on retention was also studied. As

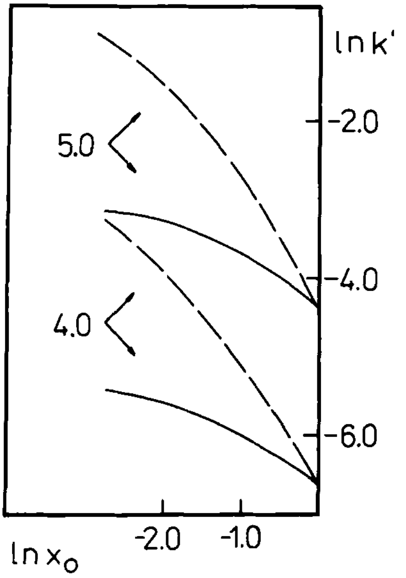

Figure 5. Theoretical curves ℓn k' vs. ℓn x_0 calculated for r=2.0, α=0, pH=7.0, L=4.0 or 5.0 and K_{OW}=2.0 (the solid lines) or 8.0 (the dashed lines).

follows from Figure 6 for considerably heterogeneous surfaces (low value of the heterogeneity parameter m), a deep minimum on k' vs. x_o curve may be observed.

Generally, for the ideal mobile phase an increase of the capacity ratio may be caused by an increase of the adsorption parameters k_{ow}, α and m or by a decrease of the acidic dissociation constant L and pH.

Similar calculations were carried out on a basis of equations 19, 20 and 22. In this case, a dependence of the capacity ratio upon mobile phase composition is more complicated. Figure 7 shows k' vs. pH curves for different values of x_o and

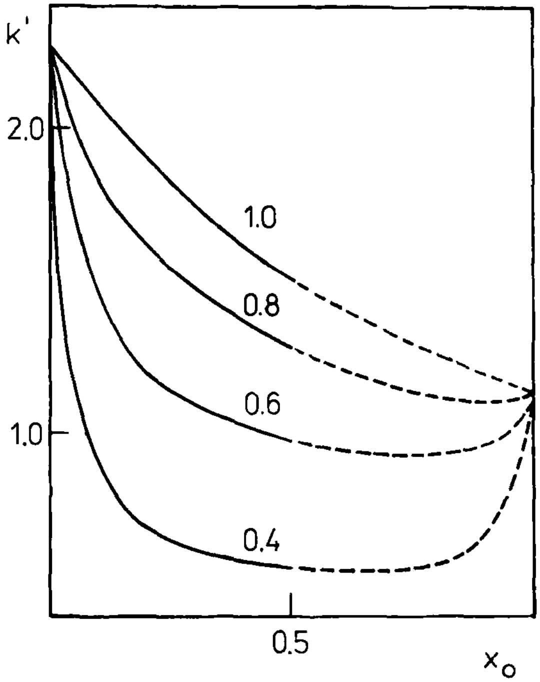

Figure 6. Effect of surface heterogeneity on k' vs. x_o curves. Parameters: r=1.0, K_{OW}=2.0, α=0, L=4.0, pH=3.0 and m=1.0, 0.8, 0.6, 0.4.

examples of suitable curves k' vs. x_o. In calculations the parameters η and ξ from the linear dependence given by equation 22 were chosen after an analysis of experimental values of L for benzoic acid in methanol water solutions (16). Equation 22 approximated experimental data for $0 < x_o < 0.8$. When $x > 0.8$ pL dramatically increases. As follows from Figure 7a, the k' vs. pH curves corresponding to different values of x_o can intersect. It means that extrema may be observed on curves k' vs. x_o (Figure 7b).

An influence of a type of functional relationship pL = pL(x_o) on the capacity ratio may be shown by comparison of Figures 4b

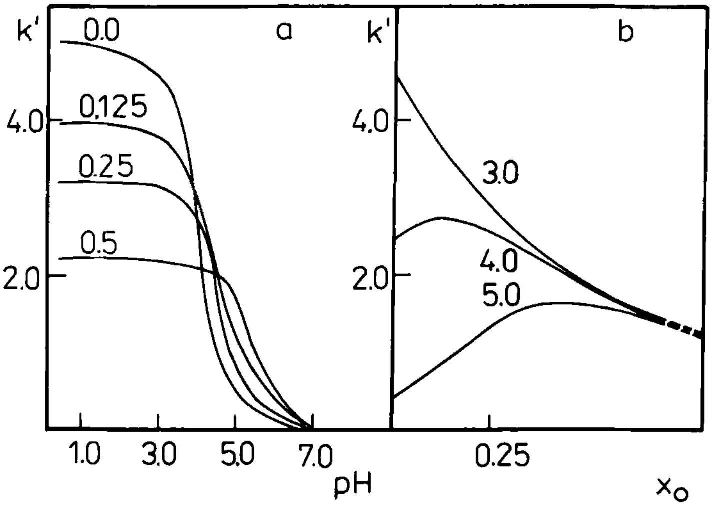

Figure 7. Effect of nonideal mobile phase composition on k' vs. pH and k' vs. x_o curves. Parameters: $r=2.0$, $K_{ow}=2.0$, $\alpha=0$, $\eta=3.0$, $\xi=4.0$ (a) $x_o=0$, 0.125, 0.25, 0.5 (b) pH=3.0, 4.0, 5.0.

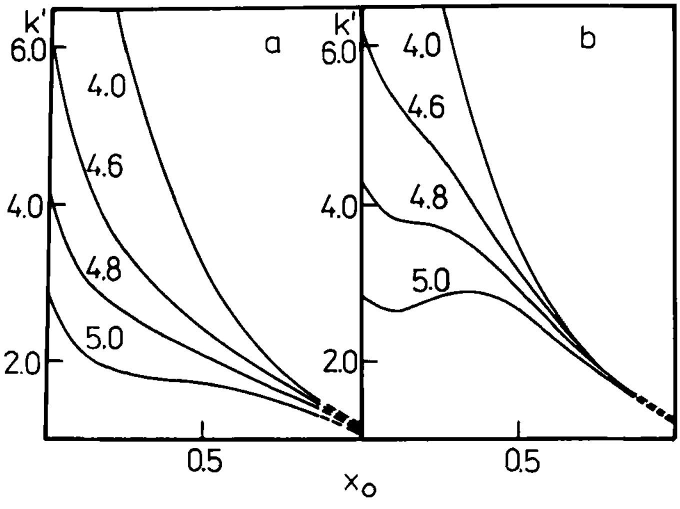

Figure 8. Theoretical curves k' vs. x_o calculated for $r=2.0$, $K_{ow}=2.0$, $\alpha=0$, pH=4.0, 4.8, 5.0 and (a) $\eta=2.0$, $\xi=4.0$ (b) $\eta=3.0$, $\xi=4.0$.

($\eta = 0$, $\xi = 4$) and 6b or Figures 8a and 8b. A change of parameters η and ξ brings about considerable differences in a course of k' vs. x_o curves.

Analogous calculations may be performed for a basic dissociation of the solute. The fundamental conclusions may be foreseen on a basis of the above results and an analysis of equation 31. It is easy to check that the capacity ratio of a basis increases as all parameters K_{ow}, α, m, L and pH increase.

Concluding, the above approach facilitates the theoretical description of the solute dissociation effects in RPLC with mixed mobile phase in light of quasichemical theory of liquid chromatography.

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REFERENCES

1. Walton, H.F., Anal. Chem. 48, 1976, 52R.
2. Horvath, C., Melander, W.R. and Molnar, I., Anal. Chem. 49, 1977, 142.
3. Melander, W.R. and Horvath, C., High Performance Liquid Chromatography, vol. 2, Academic Press, 1980.
4. Karger, B.L., LePage, J.N. and Tanaka, N., High Performance Liquid Chromatography, vol. 1, Academic Press, 1980.
5. Koroeff, E.P. and Pietrzyk, D.J., Anal. Chem. 50, 1978, 502.

6. Krummen, K. and Frei, R.W., J. Chromatogr., 132, 1977, 27.

7. Krummen, K. and Frei, R.W., J. Chromatogr. 132, 1977, 429.

8. Chu, Ch. H. and Pietrzyk, D.J., Anal. Chem. 46, 1974, 330.

9. Pietrzyk, D.J. and Chu, Ch. H., Anal. Chem., 49, 1977, 757, 860.

10. Pietrzyk, D.J., Kroeff, E.P. and Rotsch, T.D., Anal. Chem., 50, 1978, 497.

11. Horvath, C. and Melander, W.R., Chromatographia, 18, 1984, 353.

12. Borówko and Jaroniec, M., Advances in Colloid Interface Sci., 19, 1983, 137.

13. Jaroniec, M., Martire, D.E. and Borówko, M., Advances in Colloid Interface Sci., 22, 1985, 177.

14. Snyder, L.R., Principles of Adsorption Chromatography, M. Dekker, 1968, New York.

15. Soczewiński, Advances in Chromatography (J.C. Giddings and R.A. Keller, eds.), 5, 1968, 3.

16. Bacarella, A.L., Grunwald, E., Marshall, H.P. and Porlee, E.L., J. Org. Chem., 20, 1955, 747.

17. Jaroniec, M. and Ościk, J., Journal HRC & CC, 5, 1982, 3.

18. Jaroniec, M., Różyło, J.K. and Gołkiewicz, W., J. Chromatogr., 178, 1979, 27.

19. Dąbrowski, A. and Jaroniec, M., Materials Chem. Phys., 12, 1985, 339.

20. Waksmundzki, A. and Soczewiński, E., in Stationary Phase in Paper and Thin Layer Chromatography (K. Macek and I.M. Hais, eds.), Elsevier, Amsterdam, 1965, p. 300.

21. Waksmundzki, A., Soczewiński, E. and Gross, J., J. Pol. Chem., 35, 1961, 639.