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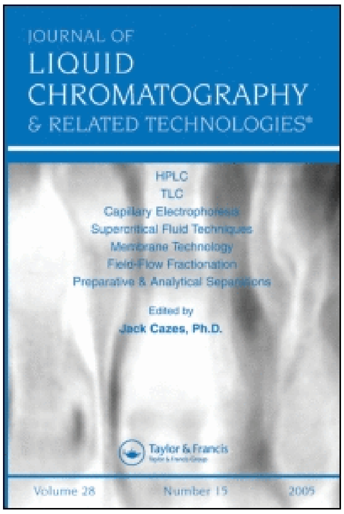

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REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH MIXED ELUENTS: PARTITION MODEL OF RETENTION FOR IONOGENIC SOLUTES

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ABSTRACT

Basing on the pure partition model of solute retention new theoretical equations for ionogenic solutes are discussed. These equations define dependence of the capacity ratio on the mobile phase composition, which is characteristic for the reversed-phase liquid chromatography.

INTRODUCTION

The reversed-phase liquid chromatography, RPLC, becomes one of the most popular methods of analysis of biologically active compounds. Many of these compounds have ionogenic character. Their retention mechanism is still incompletely described. Experimental studies of RPLC were focused on the system with aqueous buffers used as the mobile phase /1-7/. Recently, the mixed organic/aqueous eluents are more and more used for separation of these compounds /1,8/.

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Theoretical approaches to RPLC were critically discussed by Horvath and Melander /9/. However, Jaroniec and Martire /10/ proposed a simple unified theory of liquid chromatography, LC, with mixed mobile phases. Their treatment is formulated on the basis of the quasichemical approach to LC /11,12/. They proposed general expressions for the distribution coefficient of a solute involving concurrent adsorption and partition effects.

In our previous paper /13/ the adsorption model of RPLC with the mixed mobile phase was applied for describing the retention of ionogenic solutes. However, in this paper the solute dissociation effects are discussed for RPLC systems, in which the partition mechanism plays a predominant role in the solute retention.

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THEORETICAL

Let us consider a chromatographic system consisting of the s-th solute, aqueous/organic eluent and adsorbent with chemically bonded phase or covered by a nonpolar stationary liquid; s-th solute may be a weak acid or a weak base dissociating as follows:

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

$$BH^+ \rightleftarrows B + H^+ \tag{2}$$

where BH^+ is the protonated base.
The dissociation equilibria are usually characterized by the acidic ionization constant L^*:

$$L^* = \begin{cases} a_1 a_{H^+}/a_0 & /\text{acid}/ \\ a_0 a_{H^+}/a_1 & /\text{base}/ \end{cases} \tag{3}$$

where a_{H^+} and a_α $/\alpha=0,1/$ are the activities of ion H^+ and the α-th form of the s-th solute in the mobile phase, respectively; $\alpha=0$ refers to the neutral molecules /AH or B/, whereas $\alpha=1$ denotes ions /A^- or BH^+/. After simple transformations we obtain /13/:

$$x_s^{(1)} = x_s^{(0)} F^{(1)} \tag{4}$$

where $x_s^{(\alpha)}$ is the mole fraction of the α-th form of the s-th solute in the mobile phase; the function $F^{(1)}$ is defined as follows:

$$F^{(1)} = 10^{\pm(pH-pL)} \tag{5}$$

where

$$pL = -\log L \qquad \text{for} \quad L=L^*\left(f_s^{(0)} / f_s^{(1)}\right)^{\pm 1}/f_{H^+} \tag{6}$$

and f_{H^+}, $f_s^{(\alpha)}$ denote the suitable activity coefficients in the mobile phase. In several equations a double sign appears; then the upper sign refers to the acid whereas the lower sign is connected with the base.

The capacity factor of the s-th solute is given by /13/:

$$k_s = q\left(y_s^{(0)}+y_s^{(1)}\right)/\left(x_s^{(0)}+x_s^{(1)}\right) \tag{7}$$

where $y_s^{(\alpha)}$ is the mole fraction of the α-th form of the s-th solute in the stationary phase, and q is a constant characteristic for the chromatographic bed /14/. Let us define the capacity ratio of the α-th form of the s-th solute as follows:

$$k_s^{(\alpha)} = q\left(y_s^{(\alpha)}/x_s^{(\alpha)}\right) \tag{8}$$

Combination of equations 4-8 gives:

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$$k_s = \left(k_s^{(o)} + k_s^{(1)} F^{(1)}\right) / \left(1 + F^{(1)}\right) \tag{9}$$

Equation 9 may be rewritten in the following form:

$$k_s = k_s^{(o)} + \left(k_s^{(1)} - k_s\right) \left\{L/[H^+]\right\}^{\pm 1} \tag{10}$$

In a general case L is a function of the solvent composition and pH of the buffered mobile phase because the activity coefficients depend upon these quantities. The majority of theoretical approaches to the retention of ionogenic solutes neglect the dependence of L upon pH. Then, L is assumed to be constant for a given concentration of organic solvent. In this case the curves k_s vs. $(k_s^{(1)} - k_s)/[H^+]$ /for acid/ and k_s vs. $(k_s^{(1)} - k_s)[H^+]$ /for base/ have linear courses.

Equation analogous to 10 has been derived by Horvath et al./15/ for the acidic solute and buffered aqueous mobile phase. They proposed the chromatographic method for determining the dissociation constant L^* /in their treatment $f_{H^+} = f_s^{(\alpha)} = 1$ for $\alpha = 0,1$, so $L^* = L$/. However, equation 10 allows us to determine the function $L = L(x_o)$; x_o is the mole fraction of the organic solvent in the mobile phase.

It follows from experimental studies /15/ that the capacity ratio of ionized form /$k_s^{(1)}$/ is considerably smaller than the capacity ratio of the unionized one. Frequently the assumption $k_s^{(1)} = 0$ is sufficiently justified. In this case we have:

$$k_s = k_s^{(o)} F^* \tag{11}$$

where

$$F^* = F^*(x_o) = \left(1 + F^{(1)}\right)^{-1} \tag{12}$$

Equations 9 and 10 may be generalized for polytropic electrolytes.
The dissociation of the acid AH_n may be described by the following reactions:

$$\begin{aligned} &AH_n \rightarrow AH_{n-1}^{-1} + H^+ \quad ; && \Gamma^{(1)} \\ &AH_{n-1}^{-1} \rightarrow AH_{n-2}^{-2} + H^+ \quad ; && \Gamma^{(2)} \\ &\vdots \\ &AH^{-(n-1)} \rightarrow A^{-n} + H^+ \quad ; && \Gamma^{(n)} \end{aligned} \tag{13}$$

characterized by the stepwise ionization constants $\Gamma^{(n)}$.
One can write analogous reactions for the protonated base $B(H^+)_n$.

Simplifying the problem by assuming ideality of the solution, we obtain the following formula for the capacity ratio of the polytropic acid or base:

$$k_s = \sum_{i=0}^{n} k_s^{(i)} F^{(i)} / \sum_{i=0}^{n} F^{(i)} \tag{14}$$

for

$$F^{(i)} = 10^{\pm(ipH-pL^{(i)})} \tag{15}$$

where

$$pL^{(i)} = -\log\left(\prod_{j=0}^{i} \Gamma^{(j)}\right) ; \quad \Gamma^{(o)} = 1 \tag{16}$$

and $k_s^{(i)}$ is the capacity ratio of the ion with the charge equal to i.
When $k_s^{(i)} = 0$ $(i=1,2,..n)$ equation 14 becomes the form 11 with the function F^* given by:

$$F^* = F^*(x_o) = \left(\sum_{i=0}^{n} F^{(i)}\right)^{-1} \tag{17}$$

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The equations for monotropic electrolytes are the limiting case of the above expressions for $\Gamma^{(i)} \rightarrow 0$ /acid/ or $\Gamma^{(i)} \rightarrow \infty$ /base/; $i \geqslant 2$.
The problem of an analysis of polytropic electrolytes by using liquid-liquid chromatography was discussed in the paper by Soczewiński /16/.

All above discussed equations are true for any mechanism of the solute retention. However, a dependence of the capacity ratio upon the mobile phase composition $k_s = k_s(x_o)$ may be obtained only for chosen model of the solute distribution between the mobile and stationary phases. Using the method proposed in the paper /13/ one can calculate the function $k_s = k_s(x_o)$ for the chromatographic systems in which the competitive adsorption play a considerable role.

When the pure partition model /10/ is a more adequate one the capacity ratio may be calculated from equations 9 or 14 by using the following expressions for the capacity ratios of the ionized and neutral forms of the s-th solute:

$$k_s^{(\alpha)} = q\left(f_s^{(\alpha)} / g_s^{(\alpha)}\right) \tag{18}$$

where $g_s^{(\alpha)}$ is the activity coefficient of the α-th form in the stationary phase.
According to the paper /10/ we can write:

$$\log k_s^{(\alpha)} = x_o \log k_{s(o/c)} + x_w \log k_{s(w/c)}^{(\alpha)} - 0.434\, r_s\, \chi_{ow} x_o x_w \tag{19}$$

where x_w denotes the mole fraction of the water in the mobile phase, r_s is the ratio of molecular sizes of the solute and solvents /molecular sizes of the solvents are assumed to be equal/, χ_{ow} is the interaction parameter characterizing the mixed eluent, whereas $k_{s(j/c)}^{(\alpha)}$ /j=o,w/

represents partition of the α-th form of the s-th solute between the j-th pure solvent and a stationary phase containing only the immiscible with this solvent component c.

Because a dielectric constant of the component c chemically bonded with the surface is rather small the concentration of ionic forms should be low. Thus, we can assume that $k_s^{(\alpha)}=0$ for $\alpha=1$ and use equation 11 for calculating capacity ratio k_s.

Combination of equations 11 and 19 leads to the following expression:

$$\log k_s = Ax_o^2 + Bx_o + C \tag{20}$$

where

$$A = 0.434\, r_s\, \chi_{ow}$$

$$B = \log\left(k^{(o)}_{s\,(o/c)} / k^{(o)}_{s\,(w/c)}\right) - A \tag{21}$$

$$C = \log k^{(o)}_{s\,(w/c)} + \log F^*(x_o)$$

Equations 20,21 and 12 or 17 allow us to predict the functional dependence of the capacity ratio upon the mixed solvent composition for the ionogenic solutes. When dissociation effects are not observed $\log F^*=0$ and equation 20 is analogous to that derived by Schoenmakers et al. /17,18/.

RESULTS AND DISCUSSION

The mathematical analysis of the equations 20 and 21 leads to the following conclusions concerning the essential features of the dependence $\log k_s$ vs. x_o for the weak acid or base:

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1^o When dissociation effects may be neglected /low values of pH for acid and high values of pH for base/ the dependence log k_s vs. x_o is a parabola.

2^o In the range of almost completely dissociated electrolyte /pH>pL+1 for acid or pH<pL−1 for base/ unity in the function F^* may be neglected, and:

$$\log F^* = \mp(\mathrm{pH}-\mathrm{pL}) \tag{22}$$

If we assume the following dependence for pL /13/:

$$\mathrm{pL} = \eta x_o + \xi \tag{23}$$

then we also obtain the parabola:

$$\log k_s = A' x_o^2 + B' x_o + C' \tag{24}$$

where A', B', and C' are the constants equal to:

$$\begin{aligned} A' &= A \\ B' &= B \pm \eta \\ C' &= \pm(\xi - \mathrm{pH}) + \log k_{s(w/c)}^{(o)} \end{aligned} \tag{25}$$

It is easily to show that the parabola 24 reaches a minimum at point x_o^{min} :

$$x_o^{min} = -B'/2A' \tag{26}$$

3^o Sometimes pL is practically independent on the mole fraction in a wide concentration range / η=0/, /13,19/. In this case log $F(x_o)$ =const and the curve log k_s vs. x_o calculated from the general equation 20 is a parabola for any value of pH.

4^o Deviations from parabolic course of the dependence given by equation 20 may be observed when pL is not a simple linear function of the organic solvent mole fraction. Equation 23 approximates experimental data only for $x_o<0.8$. When $x_o>0.8$ pL dramatically increases /19/.

Illustrative model calculations were performed for a monotropic acid according to equations 20 and 24. Figure 1a shows curves log k_s vs. x_o plotted according to equation 20 for pL=const and different values of pH. An increase in the pH-value causes a translation of the parabola toward lower values of the capacity ratio. The opposite effect was observed for basis. El Tayar et al. /20/ reported experimental parabolic dependences log k_s vs. x_o for several protonated basic compounds measured for the various values of pH of the methanol-water mobile phase.

In Figure 1b curves log k_s vs. x_o calculated from equation 24 are presented. An influence of type of the functional relationship $pL=pL(x_o)$ on the capacity ratio is shown here. When the parameter η increases /e.g. when pL for a given solvent composition increases/ the capacity ratio also increases and the minimum moves in direction of lower concentrations of the organic solvent.

Let us notice that equation 26 may be rewritten as follows:

$$x_o^{min} = \bar{a} \log k_{s(w/c)}^{(o)} - \bar{b} \tag{27}$$

or

$$x_o^{min} = \bar{a} \log k_{s(w/c)} - \bar{b}_1 \tag{28}$$

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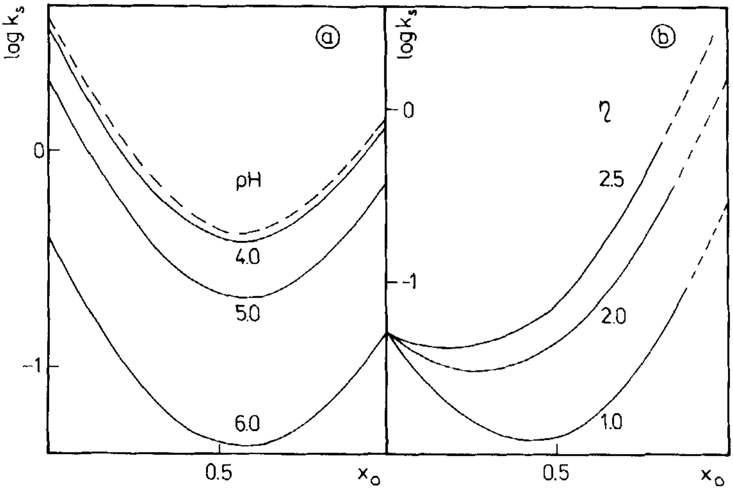

Figure 1. Theoretical dependences log k_s vs. x_o calculated for log $k^{(o)}_{s(w/c)}=0.6$, log $k^{(o)}_{s(o/c)}=0.2$ $A=3.0$ according to:

(a) equation 20 when pL=5.0 for different values of pH=6.0, 5.0, 4.0; the dashed line refers to non-dissociating solute /F=1/

(b) equation 24 when pH=7.0, $\xi=5.0$ for different values of the parameter $\eta=1.0$, 2.0, 2.5

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where

$$\bar{a} = (2A)^{-1} = (2r_s \chi_{ow})^{-1}$$

$$\bar{b} = (\log k^{(o)}_{s(o/c)} \pm \eta)/(2A) - 0.5$$

$$\bar{b}_1 = \log k_{s(o/c)}/(2A) - 0.5$$

For the series of solutes having the similar molecular sizes $/r_1=r_2=\ldots r_s/$, similar capacity ratios in a pure organic solvent and parallel linear dependences 23

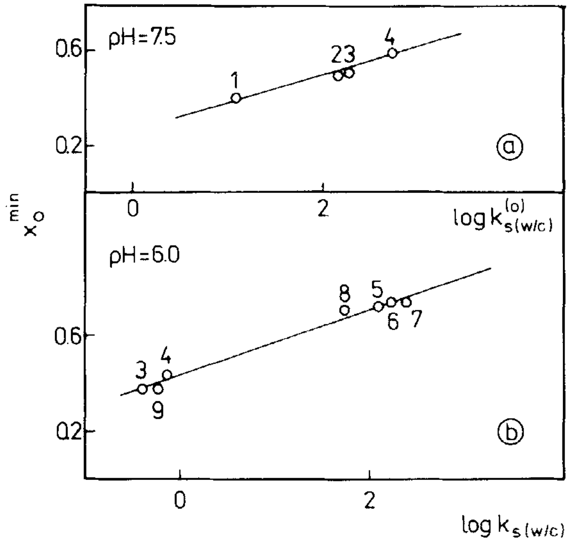

Figure 2. The example of experimental linear dependences: 27 for pH=7.5 /2a/ and 28 for pH=6.5 /2b/, and the following substances: tiapride /1/, prosulpride /2/, sulpride /3/, sultopride /4/, spiperone /5/, clebopride /6/, benperidol /7/, mezilamine /8/, sulmepride /9/; /20,21/.

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$/\eta_1 = \eta_2 = \dots \eta/$, the parameters $\bar{a}$, $\bar{b}$ or $\bar{b}_1$ are constant. It means that relationships 26 and 27 are linear ones. Figure 2a presents the example of dependence 27 for protonated basic compounds plotted for the experimental data taken from literature /20,21/. However, in Figure 2b the linear dependence 28 is shown for several solutes chromatographed in the water-mathanol mobile phase /19/. El Tayar et al./21/ reported experimental linear dependence of the type 28 for quite arbitrary compounds. The above approach allows us to predict theoretically parameters $\bar{a}$, $\bar{b}$, $\bar{b}_1$ and choose the class of the solute for which the linear dependences under consideration are rigorously satisfied.

The treatment presented in this paper facilitates the description of solute dissociation effects in the RPLC with mixed eluents and forms a background for further studies in this field.

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