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Journal of Chromatography A, 859 (1999) 1–12

JOURNAL OF CHROMATOGRAPHY A

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Reversed-phase high-performance liquid chromatography of ionogenic compounds: comparison of retention models

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Received 21 June 1999; received in revised form 2 August 1999; accepted 3 August 1999

Abstract

Two kinds of retention models describing a behaviour of ionogenic substances in reversed-phase chromatographic systems were compared. Model A utilises a concept of limiting retention factors and is especially suitable for the prediction of retention of compounds co-existing in several forms in mobile phase. An effect of the concentration of organic modifier (e.g., methanol) on the magnitudes of the limiting retention factors and equilibrium constants (dissociation constants of the separated substances) can be expressed with the aid of various, more or less sophisticated, relationships. A stoichiometric displacement model (model B) in its original form simply relates the analyte retention to the content of organic modifier in the mobile phase. In this work, it was modified to also express an effect of the mobile phase pH introducing side equilibria (acid–base) into the model. Both models predict a sigmoidal dependence of the analyte retention factor on the mobile phase pH in accordance with experimental data, and allow, among others, to estimate dissociation constants from those data. Experimental dependencies between the analyte retention and the concentration of methanol in the mobile phase comply well with model A, whereas the stoichiometric displacement model could be used only in a limited range of the methanol concentrations.

Keywords: Retention models; Stoichiometric displacement model; Limiting retention factors; Ionogenic compounds; Organic acids

1. Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) belongs to the most frequently used analytical separation techniques. A characteristic feature of RP-HPLC is a lower polarity of stationary phase (typically alkyl phases chemically bonded on a silica gel matrix) in comparison with mobile phase (typically aqueous solutions with organic modifier). These chromatographic systems are especially suitable for separations and determinations of low-polar solutes, relatively strongly retained on the non-polar stationary phases. Because of a great practical importance of this kind of separations, RP-HPLC systems were extensively studied and a number of retention models were suggested to explain retention and separation mechanisms, as reviewed in Refs. [1–5]. Most of the models describe the retention of organic, more or less non-polar, solutes in dependence on the mobile phase com-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00837-7

position (on the organic modifier concentration in the first place). It is well known that changes in the content of the organic modifier often result in significant changes of the analyte retention.

It is supposed in the case of typical organic solutes that their chemical nature do not change significantly with the mobile phase composition. On the other hand, there is a large group of compounds, which can co-exist in several forms, one or more of them having ionic nature; these compounds are called ionisable or ionogenic. Behaviour of these compounds in solutions and subsequently their retention in RP-HPLC can be affected employing side chemical equilibria, such as acid–base (most often), ion-pairing, complex-forming, etc. A general theory of side equilibria in HPLC was developed by Foley and May [6], but a similar approach was used by Horváth et al. [7] years before, and then by many other authors [8–12]. Mechanisms of the separation of the ionogenic substances in RP-HPLC were investigated extensively by Schoenmakers and co-workers [4,10,13], more recently by Rosés and co-workers [14–18] and Barbosa [19,20].

In the present work, two kinds of retention models describing behaviour of ionogenic solutes in the RP-HPLC systems are compared. Weak organic acids, which can co-exist simultaneously in dissociated (ionised) and non-dissociated forms during the separation, were chosen as examples of the ionogenic analytes. The discussion will be focused mainly on simple monoprotic acids, but an extension and generalisation on polyprotic acids or bases is not difficult [21,22].

2. Retention models

2.1. Model A: concept of limiting retention factors

As stated above, the characteristic feature of the ionogenic substances is their ability to participate in side equilibria in the mobile phase during the separation process. For example, a monoprotic acid, HA, undergoes dissociation/protonation (acid–base equilibrium) according to the equation

$$HA \Leftrightarrow H^+ + A^-; \quad K_a = \frac{[H^+][A^-]}{[HA]} \tag{1}$$

As a result of that equilibrium, the separated substance is present in various forms in the chromatographic system (here as HA and A^- species). It is assumed that the chemical equilibrium is sufficiently fast in comparison with the chromatographic process–equilibrium model. (In the opposite case, the both species behave as chemical individuals and can be mutually separated during the chromatographic process).

A retention factor of the compound co-existing in several forms can be expressed as a weighted average of the retention factors of individual species:

$$k = \sum k_i x_i \tag{2}$$

where k is the observed retention factor, k_i are the limiting retention factors and x_i are the respective mole fractions. The mole fractions can be expressed using equations for the side equilibria; hence, introducing Eq. (1) into Eq. (2), we can write for the retention factor of the HA acid after rearrangement

$$k = \frac{k_{HA}[H^+] + k_{A^-}K_a}{[H^+] + K_a} \tag{3}$$

where the concentration $[H^+]$ can be further expressed with the aid of a pH value. The limiting retention factors k_i (k_{HA}, k_{A^-}) are characteristic for the given species and stationary phase, and depend primarily on the mobile phase composition. A number of relationships were suggested to describe the dependence of the retention factors on the concentration of organic modifier in the mobile phase [3]. The simplest equation expresses a linear dependence between a logarithm of the retention factor and a concentration of organic modifier – we can write for k_{HA} and k_{A^-}:

$$\log k_{HA} = \log k^0_{HA} + S_{HA}\varphi \tag{4}$$

$$\log k_{A^-} = \log k^0_{A^-} + S_{A^-}\varphi \tag{5}$$

where k^0_{HA} and $k^0_{A^-}$ are retention factors in pure water (aqueous phase), S_{HA} and S_{A^-} are constants for given solutes and stationary phase and φ is a volume fraction of the organic modifier in the mobile phase. From a solubility parameter theory [23], a more complex relationship containing also a quadratic term was derived. In a solvatochromic model, mobile phase properties are described using a so-called

$E_T(30)$ parameter (Dimroth–Reichardt polarity parameter) [24] and a linear dependence between log k and $E_T(30)$ is expected; for many chromatographic systems experimental data comply well with this model [3]. The solvatochromic retention model was modified by Rosés and co-workers [25,26] introducing a new solvent parameter and a single solute parameter. Suggested general linear equations described chromatographic retention over the full range of mobile phase compositions.

The equations expressing a direct relation of the analyte retention on the content of organic modifier in the mobile phase are more practicable for most chromatographers. Simple equations such as Eqs. (4) and (5) provide a quite satisfactory agreement with experimental data over a limited range of φ, especially for methanol as organic modifier.

The content of organic modifier in the mobile phase affects also a dissociation constant value (K_a). This dependence can be expressed by an empirical equation [4]:

$$\log K_a = \log K_a^0 + Q_1\varphi + Q_2\varphi^2 \quad (6)$$

where K_a^0 is a dissociation constant in water, Q_1 and Q_2 are constants. More recently, Rosés and coworkers [14,15] derived a more sophisticated relationship for pK_a (p$K_a = -\log K_a$):

$$pK_a = pK_a^0 + \frac{a\varphi}{1 + b\varphi} \quad (7)$$

where a and b are constants. As follows from the published plots [14,15] and magnitudes of the a and b constants, the log K_a (or pK_a) vs. φ dependencies are nearly linear for low and medium contents of methanol in the mobile phase; more pronounced curvatures of the dependencies were observed only for the methanol contents above ca. 80%. A nearly linear increase of the pK_a values with increasing concentration of organic modifier in the range of ca. 0 to 80% (v/v) was found also by Sarmini and Kenndler [27–29] for lower alcohols and acetonitrile.

Introducing Eqs. (4), (5) and (6) or (7) (or some of their more sophisticated forms) into Eq. (3) or (2) we can describe the dependence of the analyte retention factor on the principal parameters of the mobile phase affecting the retention of the ionogenic compounds in RP-HPLC – the concentration of organic modifier and pH. Lopes Marques and Schoenmakers [13] have studied a number of these models in detail.

For constant concentrations of the organic modifier (k_{HA}, k_{A^-}, K_a = const.), Eq. (3) express a typical sigmoidal dependence of the retention factor on the pH value, which can be interpreted as follows: at low pH values (high concentrations of H^+ ions), the terms with K_a may by neglected in comparison with the terms with $[H^+]$, and then $k = k_{HA}$. At high pH values and low concentrations of H^+ ions, on the other hand, the terms with $[H^+]$ may be neglected in comparison with the terms with K_a, and $k = k_{A^-}$. It is assumed that undissociated forms of analytes are retained more strongly then dissociated (ionized) ones on non-polar stationary phases, hence $k_{HA} > k_{A^-}$. If the k_{A^-} may be neglected under certain conditions, Eq. (3) can be further simplified and rearranged into the form

$$\frac{1}{k} = \frac{K_a}{k_{HA}[H^+]} + \frac{1}{k_{HA}} \quad (8)$$

expressing a linear dependence between a reciprocal value of the retention factor and a reciprocal value of the H^+ ions concentration.

In RP-HPLC, the dependencies of the retention factors on the concentration of organic modifier were studied most frequently; a great amount of experimental data is summarised in the study [30]. Linear log k vs. log φ plots were usually found for aqueous–methanol mobile phases and non-polar analytes. As follows from Eq. (1), the linear plot (straight line) can be hardly expected for analytes co-existing in several forms, even if the plots for the limiting retention factors of individual species are linear do. This is demonstrated on the model example in Fig. 1 – the plots for the limiting retention factors k_{HA} and k_{A^-} are linear (calculated from Eqs. (4) and (5)), whereas the dependence of the observed (overall) retention factor on the methanol content calculated from Eq. (2) for $x_{HA} = x_{A^-} = 0.5$ (bold line) is markedly curved. Even a more complex dependence is obtained in the case when the values of mole fractions must not be considered constant. Even if the dependence of log k vs. log φ is measured at the constant pH value, the change of the

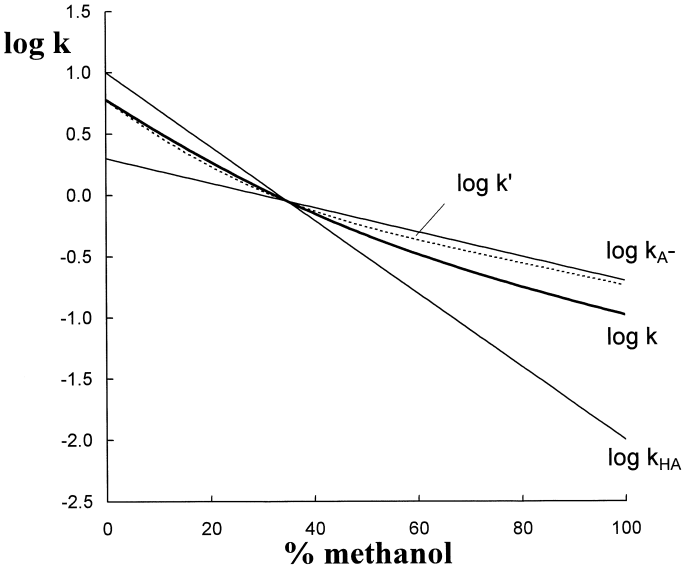

Fig. 1. Dependence of the retention factor on the methanol concentration. Model A: limiting retention factors k_{HA} and k_{A^-} calculated from Eqs. (4) and (5), retention factor k (bold line) calculated from Eq. (2) assuming that the mole fractions do not vary ($x_{HA}=x_{A^-}=0.5$), retention factor k' (dashed line) calculated from Eq. (2) on the assumption that the K_a value and subsequently the mole fractions vary with the methanol concentration [pH = const. $\approx pK_a^0$, pK_a changes linearly with the methanol concentration with the slope $\partial(pK_a)/\partial(\%\ \text{methanol}) = 0.01$].

pK_a value with the change of the methanol content can manifest itself (especially at pH values close to pK_a) causing the change of the analyte dissociation (x_{HA}/x_{A^-} ratio) and subsequently the curvature of the log k vs. log φ dependence (dashed line in Fig. 1). It is evident that for ionogenic analytes the simple dependencies described by equations such as Eqs. (4) and (5) are valid only under such conditions, when the only one form of analyte is prevailing.

2.2. Model B: stoichiometric displacement model

A stoichiometric displacement model for RP-HPLC was suggested by Cheng and Regnier to describe the retention of proteins [31,32] and later it was extended also to low-molecular-mass solutes [33]. In this model, it is assumed that analyte molecule, X, displaces certain number z of previously retained molecules of organic solvent S at the interface between the solvated analyte and the solvated stationary phase:

$$X + zS_s \Leftrightarrow X_s + zS \quad (9)$$

where subscript s refers to the molecules in stationary phase. Using a procedure commonly employed in adsorption chromatography or ion-exchange chromatography [34,35], a relationship between the analyte retention factor and the concentration of the displacing agent (organic modifier, e.g., methanol) can be readily derived [3],

$$\log k = \log I - z\log[S] \quad (10)$$

where I is a constant for a given solute and reversed-phase system, and [S] is the organic modifier concentration in the mobile phase (in mol/l).

For ionogenic solutes taking part in the retention process in the forms of various species (as HA and A^- in the case of a weak organic acid), an equation similar to Eq. (9) has to be written for each of the species, e.g., for HA and A^-, as follows:

$$HA + z_1S_s \Leftrightarrow HA_s + z_1S; \quad p_1 \quad (11)$$

$$A^- + z_2S_s \Leftrightarrow A_s^- + z_2S; \quad p_2 \quad (12)$$

where p_1 and p_2 express the contribution of the respective partial reaction to the retention process

(see the concept of Mongay et al. [36]). Evidently, p_1 and p_2 are related to the mole fractions x_1 and x_2, and $p_1+p_2=1$. The global equilibrium is

$$p_1\mathrm{HA} + p_2\mathrm{A}^- + (p_1z_1 + p_2z_2)\mathrm{S}_s \Leftrightarrow$$
$$p_1\mathrm{HA}_s + p_2\mathrm{A}_s^- + (p_1z_1 + p_2z_2)\mathrm{S} \quad (13)$$

with the global equilibrium constant

$$K = \frac{[\mathrm{HA}]_s^{p_1}[\mathrm{A}^-]_s^{p_2}[\mathrm{S}]^{p_1z_1+p_2z_2}}{[\mathrm{HA}]^{p_1}[\mathrm{A}^-]^{p_2}[\mathrm{S}]_s^{p_1z_1+p_2z_2}} \quad (14)$$

The analyte retention factor is given as the ratio of the amounts of the analyte in the stationary phase to that in the mobile phase:

$$k = \frac{w}{V_m}\frac{[\mathrm{HA}]_s + [\mathrm{A}^-]_s}{[\mathrm{HA}] + [\mathrm{A}^-]} \quad (15)$$

Neglecting the amount of the analyte retained on the stationary phase in comparison with the amount of the solvent S_s, the column (loading) capacity can be expressed as follows:

$$Q = [\mathrm{S}]_s \quad (16)$$

In order to solve this set of equations we need to express a relative intensity of the retention of the HA and A^- species. Here, we will suppose that the undissociated form HA is *a*-times more strongly retained than the ionized A^- form. As the concentration of each species in the stationary phase is proportional to its concentration in the mobile phase, the following holds true [36,37]:

$$\frac{[\mathrm{HA}]_s}{[\mathrm{A}^-]_s} = \frac{a[\mathrm{HA}]}{[\mathrm{A}^-]} \quad (17)$$

On combining Eqs. (14), (16) and (17), and substituting in Eq. (15) we obtain

$$k = \frac{Ca^{-p_1}}{[\mathrm{S}]^{p_1z_1+p_2z_2}}\left(\frac{a[\mathrm{H}^+] + K_a}{[\mathrm{H}^+] + K_a}\right) \quad (18)$$

where the constant *C* incorporates the column capacity, the equilibrium constant *K* and the phase ratio w/V_m.

With the aid of Eq. (18), one can describe the analyte retention as a function of the mobile phase pH and the concentration of organic modifier. The dependence of the dissociation constant on the organic modifier concentration according to Eqs. (6) or (7) should be introduced into the retention model again.

For the constant pH value, Eq. (18) can be simplified and rearranged into the form

$$\log k = \log C' - (p_1z_1 + p_2z_2)\log[\mathrm{S}] \quad (19)$$

which expresses a linear dependence between the logarithm of retention factor and the logarithm of the concentration of organic modifier in the mobile phase, provided that p_1 and p_2 do not vary (Fig. 2). However, the K_a value changes with the change of the organic modifier concentration causing, under certain conditions, variations of the mole fractions x_{HA} and x_{A^-} as well as of the p_1 and p_2 values, even if the pH value is kept constant. This will manifest itself especially in the vicinity of the pK_a value and could cause a curvature of the log *k* vs. log [S] dependence (see dashed line in Fig. 2).

For the constant concentration of the organic modifier S, Eq. (18) represents a sigmoidal dependence of the retention factor on the mobile phase pH, analogously to Eq. (3) in model A. The term *a* has a similar meaning as the ratio k_{HA}/k_{A^-} in model A.

3. Experimental

The liquid chromatograph consisted of an HPP 5001 high-pressure pump, an LCI 30 injection valve with a 20-μl sampling loop (Laboratorni Pristroje, Prague, Czech Republic), an UV–Vis photometric detector Model 732870 (Knauer, Berlin, Germany) or LCD 2563 (Laboratorni Pristroje) operating at a wavelength of 254 nm, and a TZ 4621 chart recorder (Laboratorni Pristroje). Separations were carried out on a glass column 150×3 mm packed with octadecyl-bonded silica Separon SGX C_{18}, 5 μm (Tessek, Prague, Czech Republic).

The following stock solutions were prepared: 1 mol/l acetic acid, 1 mol/l sodium acetate. Mobile phases were prepared by mixing the stock solutions with methanol in appropriate ratios in order to keep the acetate concentration in final solution constant (0.1 mol/l). The mobile phase pH values were finely adjusted with acetic acid or sodium hydroxide after mixing the aqueous and methanol portions. In the

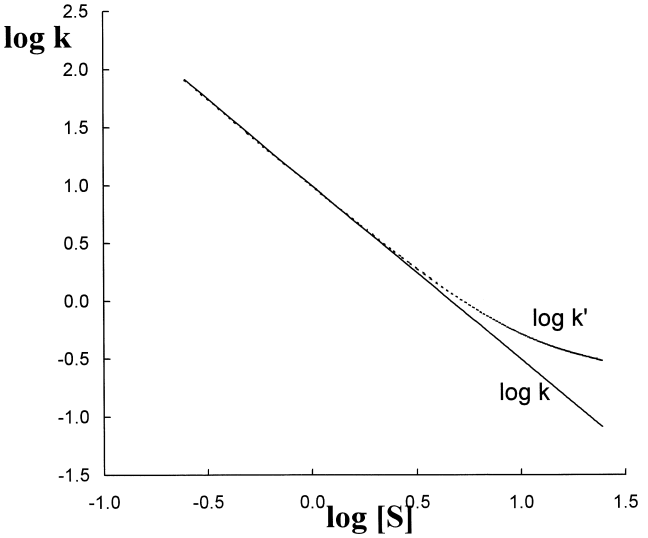

Fig. 2. Dependence of the retention factor on the methanol concentration. Model B: a solid line represents a simple dependence according to Eq. (10) ([S] is the methanol concentration in mol/l), a dashed line calculated from Eq. (19) on the assumption that p_1 and p_2 are related to x_{HA} and x_{A^-}, which change with the methanol content in a similar way as in Fig. 1.

case of mobile phases with the lowest pH values, 1–2 drops of diluted hydrochloric acid were added to adjust a desired pH value. The pH values of the aqueous–methanol mobile phases were measured by an OP 211/1 pH meter (Radelkis, Budapest, Hungary) with a combined glass electrode Ross 81-02 (Orion, USA) previously calibrated using mixed water–methanol solutions of hydrogen phthalate (0.05 mol/kg) as a reference value standard [38–41]. Analyte stock solutions were prepared by dissolving in water and diluting with the respective mobile phase to the concentration of 1% before measurements. Bi-distilled water and HPLC-grade methanol (Fluka, Buchs, Switzerland) were used for preparing the solutions and mobile phases. All the chemicals used were of reagent-grade purity, obtained from Lachema, Brno, Czech Republic.

The mobile phases were deaerated in an ultrasonic bath before measurements. The mobile phase flow-rate was 0.3 ml/min. Measurements were carried out at laboratory temperature 22±1°C.

Retention times of analytes were determined as an average from at least triplicate injections of individual compounds and retention factors were calculated by the usual method. The column dead volume (time) was assessed from disturbances on chromatograms caused by the injection of sample, water or pure methanol.

ADSTAT (TriloByte, Pardubice, Czech Republic) and Origin 5.0 (Microcal Software, Northampton, USA) statistical software was used for a statistical evaluation of the experimental data. TableCurve 3D software (Jandel Scientific, San Rafael, USA) was used for a visualisation of the three-dimensional surface plots.

4. Results and discussion

Dependencies of the retention of selected organic acids (benzoic, salicylic, anthranilic and phthalic) on the content of methanol in mobile phase were measured at two constant pH values (low pH value 2.5 and relatively high pH value 6.5) and at a nearly constant ionic strength (ca. 0.1 mol/l acetate). It is supposed that at low pH values the analytes are present predominantly in their undissociated forms (this is not entirely valid for salicylic acid having low pK_a about 3), whereas the respective acids are almost completely dissociated at pH 6.5 (phthalic acid virtually to the second degree). The experimental dependencies in the log k vs. φ coordinates

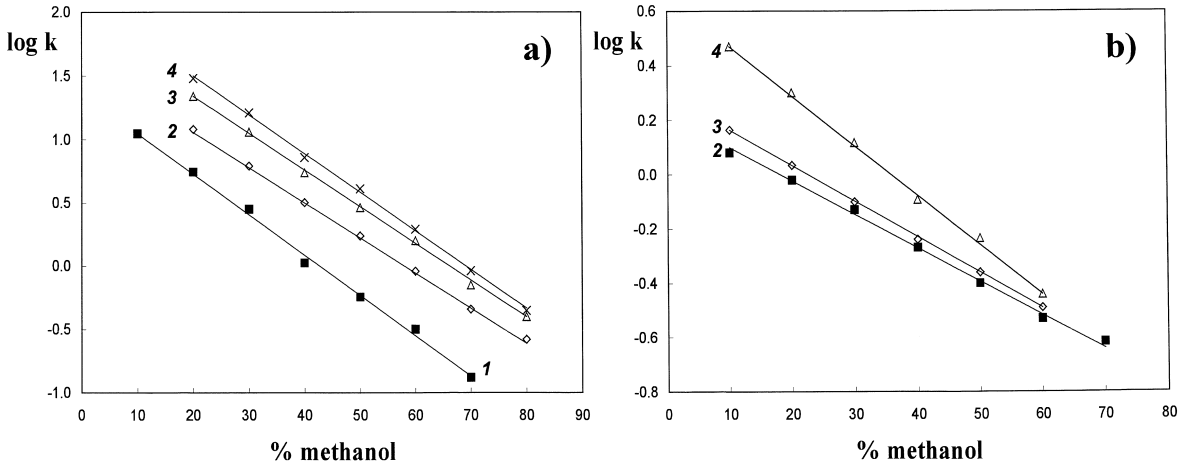

Fig. 3. Experimental dependencies of the retention factors on the methanol concentration at constant pH, log k vs. % methanol (model A). (a) pH 2.5, (b) pH 6.5. 1=Phthalic acid, 2=anthranilic acid, 3=benzoic acid, 4=salicylic acid.

(model A) are presented in Fig. 3. As can be seen, the dependencies are linear in the investigated concentration range. Slopes of the dependencies and correlation coefficients are summarised in Table 1. It is worth noticing that for the selected group of acids, which do not differ significantly in their molecular size and structure, the slopes at constant pH are rather close each other (with an exception for anthranilic acid at pH 6.5; at this pH, however, the retentions of all analytes are very low and hence their separation is almost impossible). It follows from this fact that a mutual separation of this substances can be affected by the change of the methanol concentration only to a limited extend.

The dependencies of the analyte retention on the methanol content are presented in Fig. 4 in the log k vs. log [S] coordinates (model B). It is evident that dependencies are markedly non-linear, especially for low methanol contents. According to Cheng and Regnier [33], the non-linearity is caused by variations in activity coefficients or (more probably) by variations in amounts of the pre-adsorbed organic displacing agent on the stationary phase (for more detailed discussion see Ref. [33]). Linear dependencies are expected only for higher concentrations of methanol above 30% (v/v).

For benzoic and salicylic acids, the dependencies of the retention factors on pH were measured at various concentrations of methanol in the mobile phase (Fig. 5). From the experimental data, pK_a values and limiting retention factors (parameters of model A) were estimated with the aid of a numerical curve-fitting procedure (sigmoidal curve fitting). For the low-pH parts of the dependencies, the $1/k$ vs. $1/[H^+]$ plots are linear (Fig. 6), which allows to estimate the pK_a and k_{HA} values graphically using Eq. (8). Both estimations are compared in Table 2. Values of the limiting retention factors k_{HA} and k_{A^-} estimated from the experimental curves in Fig. 5 are plotted against the methanol concentration in the log

Table 1
Slopes of the experimental log k vs. log φ dependencies

Analyte	pH 2.5		pH 6.5	
	Slope	Correlation coefficient	Slope	Correlation coefficient
Benzoic acid	−0.0292	0.9995	−0.0131	0.9998
Salicylic acid	−0.0306	0.9994	−0.0182	0.9991
Anthranilic acid	−0.0278	0.9998	−0.0121	0.9984
Phthalic acid	−0.0320	0.9984		

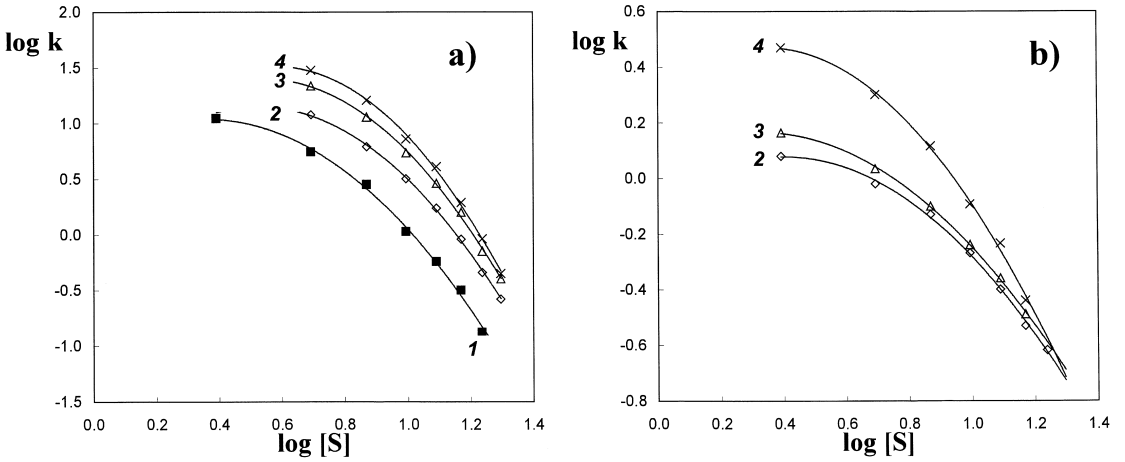

Fig. 4. Experimental dependencies of the retention factors on the methanol concentration at constant pH, log k vs. log [S] (model B). (a) pH 2.5, (b) pH 6.5. 1=Phthalic acid, 2=anthranilic acid, 3=benzoic acid, 4=salicylic acid.

coordinates (Fig. 7) for benzoic and salicylic acids. The slopes of the log dependencies for the k_{HA} limiting retention factors are −0.0285 and −0.0330 for benzoic and salicylic acids, respectively, which is in agreement with the slopes of experimental dependencies measured at pH 2.5 (Table 1). The slopes of the dependencies for the k_{A^-} limiting retention factors are −0.0153 and −0.0186 for benzoic and salicylic acids, respectively, which is in agreement with the slopes of experimental dependencies at pH 6.5 (Table 1).

As shown above, the dissociation constants of the separated acids could be determined from the k–pH dependencies. This possibility, together with presumptions and limitations, are discussed in [4]. Szokoli et al. [21] and more recently Hardcastle and co-workers [22,42] calculated dissociation constants from chromatographic data. Although the pK_a values obtained in this way may be hardly considered ''true'' values [4], they are useful for prediction of the chromatographic behaviour. As can be seen from Fig. 8, the dependencies between pK_a and methanol concentration are straight lines for benzoic and salicylic acids in the examined range, which is not in

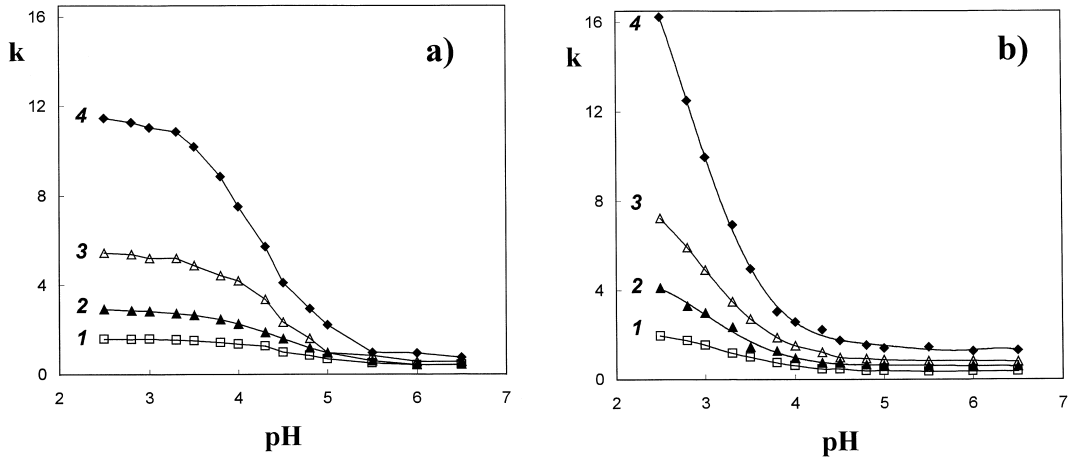

Fig. 5. Experimental dependencies of the retention factors on pH at various concentrations of methanol: (a) benzoic acid, (b) salicylic acid. 1=60% Methanol, 2=50% methanol, 3=40% methanol, 4=30% methanol.

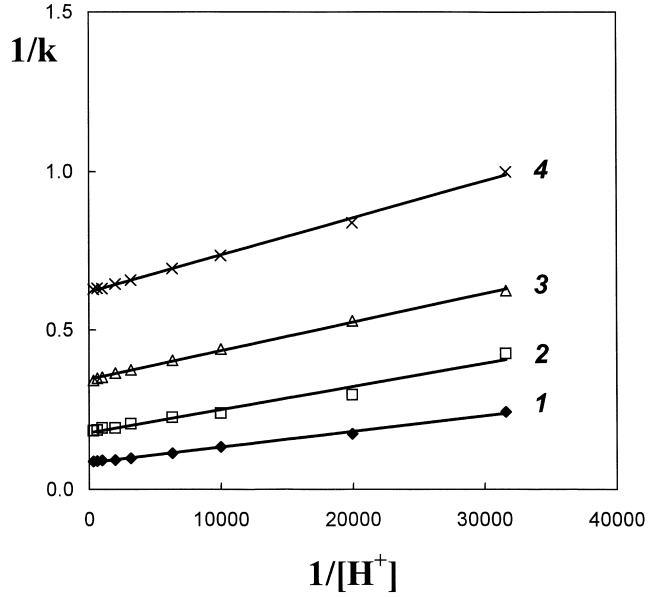

Fig. 6. Dependencies of $1/k$ vs. $1/[H^+]$ for benzoic acid. 1=30% Methanol, 2=40% methanol, 3=50% methanol, 4=60% methanol.

contradiction with published relationships [4,14,15] and agrees with the measurements of Sarmini and Kenndler [27–29]. For comparison, also the pK_a values calculated from literature data [10] are presented in Fig. 8.

5. Conclusions

It is evident that model A employing a concept of limiting retention factors is able to successfully describe chromatographic behaviour of ionogenic compounds in RP-HPLC systems. Relatively simple equations can be used to express the dependencies of limiting retention factors and dissociation constants on the mobile phase composition. When necessary, more sophisticated equations can be introduced into the model without principal problems. (However, more experimental data are needed to establish the model parameters, such as constants in the equations for the limiting retention factors and pK_a.) The model allows to predict the dependence of the analyte retention factor on the mobile phase pH and methanol concentration (Fig. 9a). A separation factor as the simplest separation characteristic (separation factor $=k_2/k_1$) can be estimated from the retention model, too. As can be seen from Fig. 9b, the separation factor depends strongly on the pH value [provided that p$K_a(1) \neq$ p$K_a(2)$]. Procedures developed for an optimisation of electrophoretic separations [43,44] can be used to find the optimum pH of the HPLC separation, because the concept of limiting retention factors is formally identical with the concept of effective mobilities commonly used in electromigration separation methods. It was shown that under certain conditions the optimum pH is between p$K_a(1)$ and p$K_a(2)$ [44] (compare with Fig. 9b). The change in the methanol content, on the other hand, although very effective in governing the

Table 2
Determination of the limiting retention factors and pK_a values from the k–pH curves for benzoic acid (Fig. 5a)

% Methanol	Graphical approach (Fig. 6)		Sigmoidal curve-fitting		
	k_{HA}	pK_a	k_{HA}	k_{A^-}	pK_a
30	11.96	4.14	11.63	0.77	4.22
40	5.62	4.41	5.37	0.56	4.34
50	2.90	4.58	2.94	0.38	4.46
60	1.61	4.79	1.59	0.41	4.57

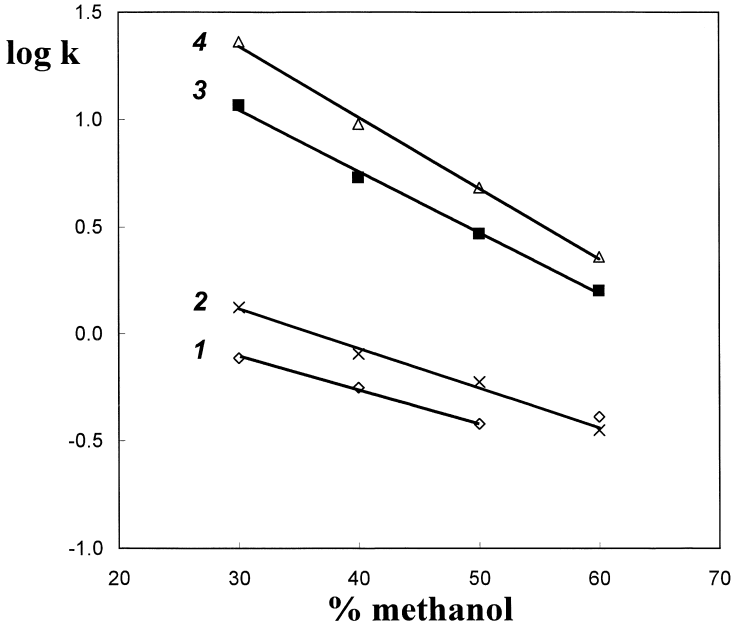

Fig. 7. Dependencies of limiting retention factors on the methanol concentration. The limiting retention factors were calculated by the numerical curve-fitting method from the dependencies in Fig. 5. $1=k_{A^-}$ for benzoic acid, $2=k_{A^-}$ for salicylic acid, $3=k_{HA}$ for benzoic acid, $4=k_{HA}$ for salicylic acid.

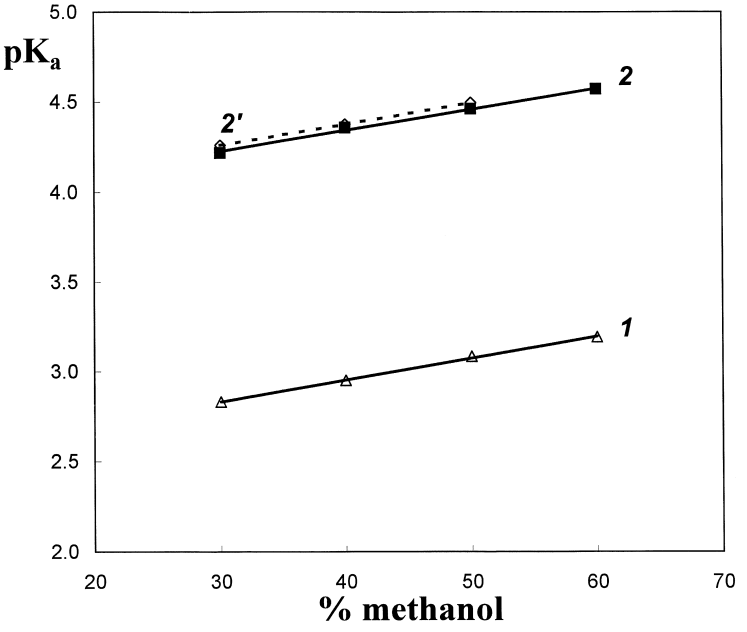

Fig. 8. Dependencies of pK_a on the methanol concentration. 1=Salicylic acid, 2=benzoic acid, 2′=benzoic acid, data from Ref. [10].

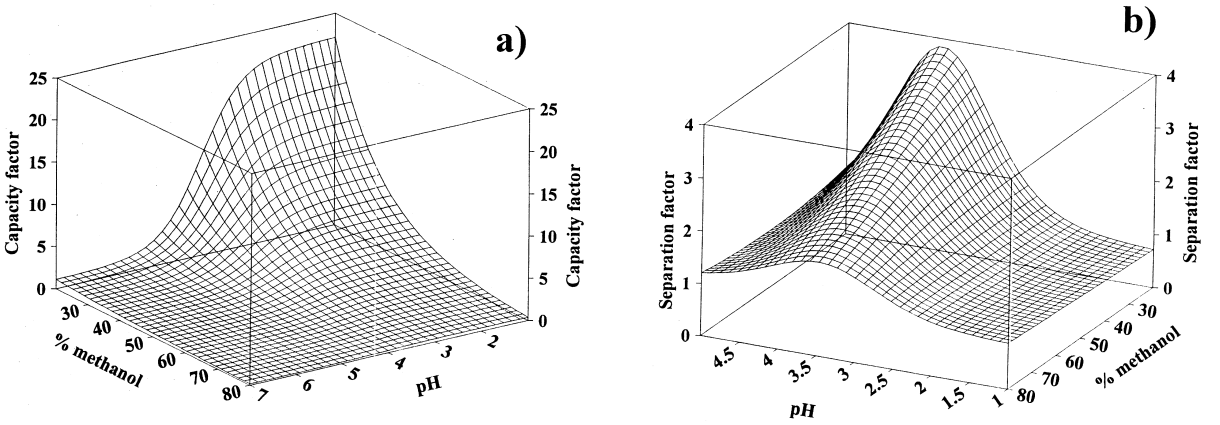

Fig. 9. Dependencies of retention factor of benzoic acid (a) and separation factor for the benzoic acid/salicylic acid pair (b) on pH and methanol content.

analyte retention, seems to be less effective in affecting the separation.

The stoichiometric displacement model (model B) can be modified to describe a chromatographic behaviour of ionogenic analytes, as shown in Section 2.2. The derived equations express the dependence of retention factor on the mobile phase pH value in accordance with experimental data. However, the experimental dependencies of the retention factors on the methanol content do not agree with those predicted from the model. The stoichiometric displacement model could be used only in a limited range of methanol concentrations (above 30%, v/v).

Acknowledgements

This work was supported by an internal grant from the Pedagogical Faculty, University of Jan Evangelista Purkyne, Ústí nad Labem, Czech Republic.

References

[1] C.H. Lochmüller, C. Reese, A.J. Aschman, S.J. Breiner, J. Chromatogr. A 656 (1993) 3.
[2] P. Jandera, J. Chromatogr. A 656 (1993) 437.
[3] K. Valkó, L.R. Snyder, J.L. Glajch, J. Chromatogr. A 656 (1993) 501.
[4] P.J. Schoenmakers, R. Tijssen, J. Chromatogr. A 656 (1993) 577.
[5] J.M. LePree, M.E. Cancino, J. Chromatogr. A 829 (1998) 41.
[6] J.P. Foley, W.E. May, Anal. Chem. 59 (1987) 102.
[7] C. Horváth, W.R. Melander, I. Molnár, Anal. Chem. 49 (1977) 142.
[8] B. Rittich, M. Pirochtová, J. Chromatogr. 523 (1990) 227.
[9] J.W. Dolan, D.C. Lommen, L.R. Snyder, J. Chromatogr. 535 (1990) 55.
[10] P.J. Schoenmakers, S. van Molle, C.M.G. Hayer, L.G.M. Uunk, Anal. Chim. Acta 250 (1991) 1.
[11] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, L.R. Snyder, I. Molnár, J. Chromatogr. 592 (1992) 183.
[12] D. Sýkora, E. Tesařová, M. Popl, J. Chromatogr A 758 (1997) 37.
[13] R.M. Lopes Marques, P.J. Schoenmakers, J. Chromatogr. 592 (1992) 157.
[14] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[15] M. Rosés, I. Canals, H. Allemann, K. Siigur, E. Bosch, Anal. Chem. 68 (1996) 4094.
[16] D. Bolliet, C.F. Poole, M. Rosés, Anal. Chim. Acta 368 (1998) 129.
[17] E. Bosch, S. Espinosa, M. Rosés, J. Chromatogr. A 824 (1998) 137.
[18] M. Rosés, D. Bolliet, C.F. Poole, J. Chromatogr. A 829 (1998) 29.
[19] J. Barbosa, Anal. Chim. Acta 389 (1999) 31.
[20] J. Barbosa, Anal. Chim. Acta 389 (1999) 43.
[21] F. Szokoli, Z. Németh, J. Inczédy, Chromatographia 29 (1990) 265.
[22] I. Jano, J.E. Hardcastle, K. Zhao, R. Vermillio-Salsbury, J. Chromatogr. A 762 (1997) 63.
[23] P.J. Schoenmakers, H.A.H. Billiet, L. de Galan, J. Chromatogr. 282 (1983) 107.
[24] J.J. Michels, J.G. Dorsey, J. Chromatogr. 457 (1988) 85.
[25] M. Rosés, E. Bosch, Anal. Chim. Acta 274 (1993) 147.
[26] E. Bosch, P. Bou, M. Rosés, Anal. Chim. Acta 299 (1994) 219.

[27] K. Sarmini, E. Kenndler, J. Chromatogr. A 811 (1998) 201.
[28] K. Sarmini, E. Kenndler, J. Chromatogr. A 818 (1998) 209.
[29] K. Sarmini, E. Kenndler, J. Chromatogr. A 833 (1999) 245.
[30] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, Anal. Chem. 58 (1986) 2354.
[31] X. Cheng, F.E. Regnier, Proc. Am. Pept. Chem. (1984) 727.
[32] X. Cheng, F.E. Regnier, J. Chromatogr. 296 (1984) 15.
[33] X. Cheng, F.E. Regnier, J. Chromatogr. 332 (1985) 147.
[34] L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968.
[35] P.R. Haddad, P.E. Jackson, Ion Chromatography – Principles and Applications, Elsevier, Amsterdam, 1990.
[36] C. Mongay, C. Olmos, A. Pastor, J. Chromatogr. A 683 (1994) 355.
[37] P. Janoš, P. Aczel, J. Chromatogr. A 749 (1996) 115.
[38] T. Mussini, A.K. Covington, P. Longhi, S. Rondinini, Pure Appl. Chem. 57 (1985) 865.
[39] S. Rondinini, P.R. Mussini, T. Mussini, Pure Appl. Chem. 59 (1987) 1549.
[40] T. Mussini, A.K. Covington, F. Dal Pozzo, P. Longhi, S. Rondinini, Z.Y. Zou, Electrochim. Acta 28 (1983) 1593.
[41] K. Izutsu, H. Yamamoto, Talanta 47 (1998) 1157.
[42] J.E. Hardcastle, I. Jano, J. Chromatogr. B 717 (1998) 39.
[43] V. Jokl, Českoslov. Farm. 6 (1957) 432.
[44] F. Foret, L. Křivánková, P. Boček, in: Capillary Zone Electrophoresis, VCH, Weinheim, 1993, p. 99.