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Retention modelling of electrostatic and adsorption effects of aliphatic and aromatic carboxylic acids in ion-exclusion chromatography
II. Calculations of adsorption coefficients in unbuffered eluents

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Abstract

Previous models for the retention behaviour of carboxylic acids in ion-exclusion chromatography are applicable only when the degree of ionisation of the analyte is constant over the entire chromatographic peak. When solutions of sulfuric acid are used as eluents, this condition applies only when the eluent concentration is considerably higher than that of the analyte. Since it is common for dilute solutions of sulfuric acid to be used as eluents, a retention model which accounts for unbuffered eluents has been developed. This model also considers the effects on retention of hydrophobic adsorption of the undissociated and dissociated forms of the analyte onto the stationary phase substrate, as well as the effects of organic solvents added to the eluent. The derivation of this model is presented and it has been evaluated using a comprehensive set of retention data obtained using three different sulfonated stationary phases over a range of eluent conditions. The adsorption coefficients calculated from the model are in accordance with expected trends and showed that both the undissociated and dissociated forms of the analyte acids were retained by hydrophobic adsorption effects, although this adsorption was much stronger for the undissociated analytes.

Keywords: Retention models; Mathematical modelling; Ion-exclusion chromatography; Adsorption; Carboxylic acids

1. Introduction

Ion-exclusion chromatography finds application in the separation of a wide range of small, neutral or partially ionised molecules [1]. A characteristic feature of the technique is that the polarity of the charge of the functional groups on the ion-exchange resin used to effect the separation is the same sign as that of the analytes, that is, negatively charged analytes such as carboxylic acids are separated on cation-exchange resins with anionic sulfonic acid functional groups. Similarly, positively charged analytes are separated on anionic-exchange resins typically containing cationic tetraalkylammonium functional groups. The columns used in ion-exclusion chromatography have high ion-exchange capacities, are composed of strong ion-exchange groups and are typically larger in dimensions than conventional ion chromatography columns.

The chromatographic system in ion-exclusion chromatography can be considered to consist of three phases. When the column is equilibrated with an

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aqueous eluent, water molecules accumulate as hydration spheres around the dissociated functional groups of the resin, and eluent becomes trapped in the pores of the resin. This immobilised eluent and the water in the hydration spheres forms the stationary phase and we can assume that the resin functional group counter-ions, together with the eluent ions, are dissolved in this stationary phase. Therefore, there are two liquid phases, namely the occluded stationary phase within the resin network and the interstitial eluent moving between the resin particles. These phases can be considered to be separated by the functional groups on the resin which behave as a semi-permeable Donnan membrane. This retention mechanism has been described by analytical equations and by results obtained from computer simulations of column performance (using global thermodynamic and chromatographic equations or the Craig method) [1].

In the case of ion-exclusion chromatography of carboxylic acids, separation can be achieved on either silica- or polymer-based cation-exchange stationary phases with chemically bound sulfonate or carboxylate functional groups. In accordance with the Donnan exclusion effect, completely dissociated strong acids are excluded from the stationary phase and are eluted at the void volume, which corresponds to the volume of the mobile phase in the column (V_m). On the other hand, small, neutral species are able to enter the resin network and are eluted together at a volume corresponding to the sum of the void volume and the occluded liquid volume, thereby allowing determination of the occluded liquid volume [3]. However, partially ionised species like weak carboxylic acids (pK_a=2.5–6.5) permeate selectively into the stationary phase volume (V_s) and retention is governed primarily by electrostatic repulsion and hydrophobic adsorption [2,3]. Therefore, carboxylic acids with higher pK_a values will have higher retention volumes and a good correlation between pK_a and retention volume has been demonstrated by Tanaka et al. [3].

Like other chromatographic techniques, ion-exclusion chromatography is named to reflect the primary retention mechanism operating, but the ion-exclusion process is seldom the sole retention mechanism. A mixed retention mechanism involving both electrostatic repulsion and hydrophobic adsorption has been observed for weak carboxylic acids and aromatic compounds [6]. Aromatic compounds are found to be retained almost solely by $\pi-\pi$ electron interaction with the styrene aromatic rings on the unfunctionalised regions of the polymeric resin network. In addition, other mechanisms which affect ion-exclusion chromatography include size-exclusion, the effect of functional group screening in the analysed sample, normal-phase retention, and Van der Waals and polar interactions of the sample compound with the resin [7]. Accurate mathematical modelling of retention in ion-exclusion chromatography is therefore a difficult task.

The retention mechanism in ion-exclusion chromatography has been previously described in a number of models [2–4]. Considering the most general case of a buffered mobile phase and assuming that solute retention is influenced by changes in the activity coefficients within the stationary phase, changes in the dielectric constant in the stationary phase and the buffer, and changes in the degree of dissociation of the stationary phase functional groups, the following equation can be derived [8]:

$$K_d = \frac{1 + \dfrac{2K_a^S \gamma_f^S}{\sqrt{K_f^2 + 4K_f c_f \gamma_f^S \gamma_{H^+}^S} - K_f}}{1 + \dfrac{2K_a^M}{\sqrt{K_b^2 + 4K_b c_b} - K_b}} \cdot K_p \qquad (1)$$

where K_d denotes the overall distribution coefficient of the solute between the mobile and stationary phases, K_a^S and K_a^M are the acid dissociation constants in the stationary and mobile phases, respectively, γ_f is the activity coefficient of the functional groups, c_f is the concentration of functional groups on the stationary phase, $\gamma_{H^+}^S$ is the activity coefficient of hydrogen ions in the stationary phase, K_p is the partition coefficient of the neutral solute between the mobile and stationary phases, K_f is the dissociation constant of the functional group on the stationary phase, c_b is the buffer concentration and K_b is its dissociation constant.

Taking the most simple case where pure water is used as the mobile phase, the occluded stationary and void volumes of the column are equal, and the functional groups on the resin dissociate completely, Eq. (1) simplifies to Eq. (2), where the solute

distribution coefficient is related to its concentration at the peak maximum, c_{max}, and dissociation constant, K_a [5]:

$$K_d = \frac{4c_{max} + K_a - \sqrt{K_a^2 + 8K_a c_{max}}}{4c_{max} - K_a + \sqrt{K_a^2 + 8K_a c_{max}}} = \frac{2c_{max} + K_a - \sqrt{K_a^2 + 8K_a c_{max}}}{2c_{max} - 2K_a}. \quad (2)$$

From Eq. (2), it can be shown that the solute distribution coefficient is a function of only one experimental quantity — the ratio of its concentration to its dissociation constant. An increase in the sample concentration and a decrease of the sample dissociation constant will increase the distribution coefficient.

In our previous paper, we derived an equation which describes the dependence of retention factor, k', for a solute on the concentration of hydrogen ions, $[H^+]_M$, and organic modifier, φ, in the mobile phase [9]:

$$\ln k' = \ln \frac{V_S + \left(K_{ads[HR]} + \frac{K_a}{[H^+]_M} K_{ads[R^-]}\right) \cdot V_R}{\left(1 + \frac{K_a}{[H^+]_M}\right) \cdot V_M} - s\varphi \quad (3)$$

where V_M, V_S and V_R are the volumes of the mobile, stationary and resin phases, respectively. The two adsorption coefficients quantify the magnitude of the contribution of adsorption to the retention mechanism of the carboxylic acid for both the undissociated, $K_{ads[HR]}$, and the dissociated, $K_{ads[R^-]}$, forms of the acid. In addition, it was also assumed that the mobile and stationary phases are characterised by equal concentrations of the neutral form of the solute, that is, these two phases are identical in composition and the partition coefficient is therefore equal to unity. Eq. (3) is valid only when the acid–base dissociation of the solute is independent of the concentration of the solute: that is, when the eluent is well-buffered or the eluent concentration is high with respect to that of the solute. In our previous study [9], Eq. (3) was found to give reasonable predictions of k', but the values of $K_{ads[HR]}$ and $K_{ads[R^-]}$ obtained by non-linear regression were inconsistent with expected trends.

In ion-exclusion chromatography it is common to use relatively dilute solutions of mineral acids (e.g., sulfuric acid) as eluents and since these eluents are not buffered, Eq. (3) applies only for relatively concentrated eluents. It is therefore of interest to extend this model to also describe the retention mechanism in a system using an unbuffered mobile phase. The extended model will include the influence of adsorption effects and it will be assumed that the adsorption coefficients for the neutral and ionised forms of the solute are a function of the concentration of organic modifier in the mobile phase [10].

2. Theory

2.1. Starting equations and assumptions

When a weak acid, HR, is injected onto the column, dissociation proceeds according to:

$$HR \rightleftharpoons H^+ + R^- \quad K_a = \frac{[H^+][R^-]}{[HR]} \quad (4)$$

where K_a is the acid dissociation constant of the acid.

In a chromatographic system, the distribution coefficient, K_d, is given by Eq. (5), which is the ratio of the solute concentration in the stationary phase, denoted by subscript S, to that in the mobile phase, denoted by subscript M:

$$K_d = \frac{[HR]_S + [R^-]_S}{[HR]_M + [R^-]_M} \quad (5)$$

The adsorption coefficients of the dissociated and undissociated forms of acid can be expressed in Eqs. (6) and (7), respectively:

$$K_{ads[HR]} = \frac{[HR]_S}{[HR]_M} \quad (6)$$

$$K_{ads[R^-]} = \frac{[R^-]_S}{[R^-]_M} \quad (7)$$

Since the mobile phase has an overall neutral charge, the concentration of protons is a sum of the protons contributed by the dissociation of acid analyte, as well as the acid used as a buffer:

$$[H^+]_M = c_b + [R^-]_M \quad (8)$$

where c_b denotes the concentration of a strong acid (completely dissociated) used as a buffer.

The conservation of solute mass means that its injected mass, defined as the solute concentration multiplied by its injection volume, is equal to that eluted from the column, defined by its actual concentration multiplied by the hypothetical peak volume. Therefore, the mass of solute, $c_i v_i$, can be written in terms of the total mass of all forms of the acid in both the stationary and mobile phases as a fraction of the peak volume:

$$c_i v_i = ([R^-]_M + [HR]_M)V_P \frac{V_M}{V_M + V_S} + ([R^-]_S + [HR]_S)V_P \frac{V_S}{V_M + V_S} \quad (9)$$

where the peak volume is described by Eq. (10) for a Gaussian peak:

$$V_P = V_R \sqrt{\frac{2\pi}{N}} \quad (10)$$

Finally, the retention volume, V_R, of the solute can be expressed as:

$$V_R = V_M + K_d V_S \quad (11)$$

2.2. Solution of equation

Taking Eqs. (5) and (6), and substituting Eq. (4) for $[R^-]_M$ and $[R^-]_S$, Eq. (12) is obtained as an expression for the distribution coefficient, K_d:

$$K_d = \frac{1 + K_a^S/[H^+]_S}{1 + K_a^M/[H^+]_M} \times K_{ads[HR]} \quad (12)$$

where K_a^M and K_a^S are the acid dissociation constants for the solute in the mobile and stationary phases, respectively. Eq. (13) can be obtained from Eqs. (5) and (7) by substituting Eqs. (4) and (6) for $[HR]_M$:

$$K_d = \frac{K_{ads[HR]}[H^+]_M + K_a^M K_{ads[R^-]}}{[H^+]_M + K_a^M} \quad (13)$$

2.3. Buffered mobile phase

Both Eqs. (12) and (13) express the distribution coefficient, K_d, using different sets of parameters even though they are theoretically equivalent. In order to solve Eq. (12), it is necessary to determine the concentration of hydrogen ions in the stationary phase and the value of solute dissociation constant in the same phase. It is usually assumed that the solute dissociation constant in the stationary phase is equivalent to that in the mobile phase. The concentration of hydrogen ions can therefore be calculated from the ion-exchange capacity of the resin:

$$K_a^M = K_a^S = K_a \quad (14)$$

$$[H^+]_S = c_f \quad (15)$$

$$[H^+]_M = c_b \quad (16)$$

Substituting Eqs. (14)–(16) into (12) and (13) yields:

$$K_d = \frac{1 + \frac{K_a}{c_f}}{1 + \frac{K_a}{c_f}} \cdot K_{ads[HR]} \quad (17)$$

$$K_d = \frac{K_{ads[HR]}c_b + K_a K_{ads[R^-]}}{c_b + K_a} \quad (18)$$

However, the dielectric constant of the stationary phase can be influenced by the high concentration of the functional groups on the resin surface as well as by the resin network itself. Therefore, activities should be used instead of concentrations when there are high concentrations of ions in the stationary phase. Furthermore, changes in the dielectric constant will also influence the acid dissociation constant of the solute.

2.4. Unbuffered mobile phase

In the case of an unbuffered mobile phase, Eqs. (4)–(11) can be solved by substituting Eqs. (6) and (7) for $[HR]_S$ and $[R^-]_S$ into Eq. (9). In this equation there are only two unknowns, $[HR]_M$ and $[R^-]_M$. Multiplying both sides of Eq. (9) by $(V_M + V_S)/V_P$, gives Eq. (19):

$$[HR]_M = \alpha - \beta [R^-]_M \quad (19)$$

where:

$$\alpha = \frac{c_i v_i (V_M + V_S)}{(V_M + K_d V_S)(V_M + K_{ads[HR]} V_S)} \cdot \sqrt{\frac{N}{2\pi}} \quad (20)$$

$$\beta = \frac{V_M + K_{ads[R^-]} V_S}{V_M + K_{ads[HR]} V_S} \quad (21)$$

Now, $[H^+]$ can be obtained from Eq. (4) and substituted into Eq. (8) to yield Eq. (22):

$$K_a[HR]_M = c_b[R^-]_M + [R^-]_M^2 \quad (22)$$

Combining Eqs. (19) and (22), Eq. (23) is obtained:

$$[R^-]_M^2 + (c_b + K_a\beta)[R^-]_M - K_a\alpha = 0 \quad (23)$$

Finally, by substituting for $[R^-]_M$ and $[HR]_M$ from Eq. (19) and solving the quadratic Eq. (23), the solution for $[H^+]_M$ using Eq. (8) is obtained as Eq. (24):

$$[H^+]_M = c_b + \frac{\sqrt{(c_b + K_a\beta)^2 + 4K_a\alpha} - (c_b + K_a\beta)}{2} \quad (24)$$

where α and β are defined by Eqs. (20) and (21), respectively.

3. Experimental

The ion chromatograph employed comprised a Waters Model 6000A pump, Model U6K injector, Model 717 plus autosampler, Model 484 tunable absorbance detector operated at 220 nm and Model TCM temperature control module (Milford, MA, USA). Chromatograms were recorded using a Waters Maxima 820 Chromatographic Workstation. Manual injections were performed using a 100-μl syringe (Scientific Glass Engineering, Ringwood, Australia).

The ion-exclusion columns used were a 300×7.8-mm I.D. Tosoh TSKGel SCX (Tokyo, Japan) (a 5-μm polystyrene–divinylbenzene (PS–DVB) co-polymer, functionalised with sulfonate groups, capacity 4.2 mequiv/g), a 300×7.8-mm I.D. Tosoh TSKGel SP-5PW (a 5-μm polymethacrylate co-polymer, functionalised with sulfonate groups, capacity 0.3 mequiv/ml), and a 300×7.8-mm I.D. sulfonated silica column, packed with 5 μm Develosil silica (Nomura, Japan) that was sulfonated in our laboratories to a capacity of 0.275 mequiv./g.

The chromatographic data used in this work consisted of measuring the retention volumes of 13 carboxylic acids (formic, acetic, propionic, butyric, valeric, malonic, succinic, glutaric, adipic, citric, benzoic, salicylic and phthalic acids) in the above columns at 14 eluent conditions achieved by varying pH (3–5) and %MeOH (0–20%). The injection volume for each analyte was 100 μl and the analyte concentration varied between 0.1 and 1 m*M* [9].

Non-linear regression analysis of the retention data and calculation of adsorption coefficients were performed using SigmaPlot for Windows, version 6.00 (SPSS, IL, USA).

4. Results and discussion

4.1. Buffered mobile phase

In ion-exclusion chromatography, the use of pure water or a very dilute solution of an acid as eluent gives asymmetrical peak shapes for most solutes due to variations in the degree of ionisation of the solute throughout the chromatographic peak, as shown in Fig. 1a. In addition, the retention volume of the solute is dependent on its concentration, which is inconvenient from an analytical point of view. To obtain a constant degree of solute dissociation throughout the chromatographic peak, a buffer or a suitably high concentration of a strong acid is added to the mobile phase. Fig. 1b shows the separation of five carboxylic acids on a PS–DVB column using 0.5 m*M* sulfuric acid solution as the eluent. The peak shapes are symmetrical and retention volumes are independent of solute concentration.

Assuming that the dissociation constant of the acid solute is the same in both the mobile and stationary phases, and that the concentration of hydrogen ions in the stationary phase is equal to the concentration of functional groups present in the resin, it is possible to use Eq. (17) to calculate the adsorption coefficient of the undissociated form of the acid analyte, $K_{ads[HR]}$. Using retention data acquired for mobile phases containing different amounts of methanol, the influence of methanol on the adsorption coefficient can be determined and data for five

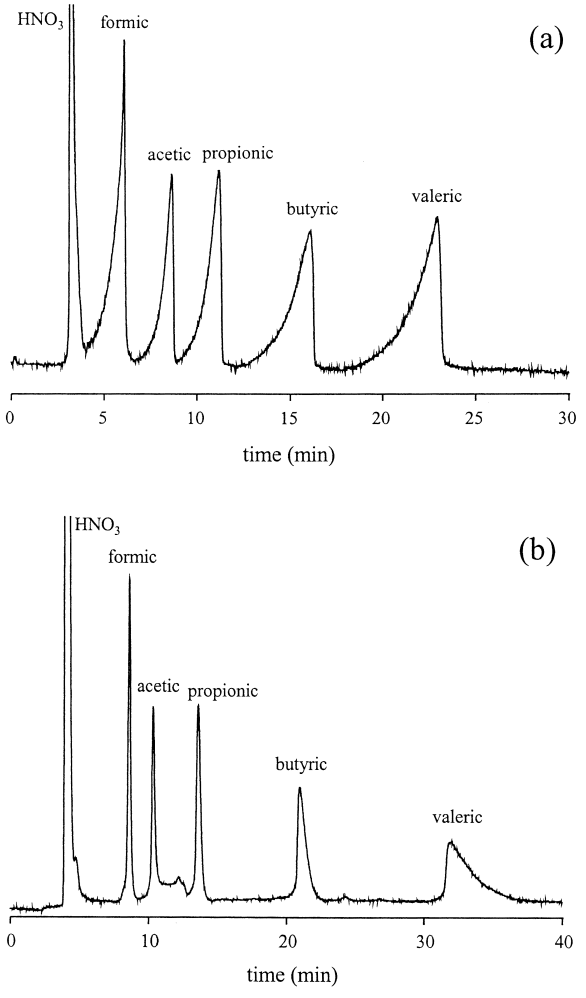

Fig. 1. Chromatograms showing the separation of monocarboxylic acids on the PS–DVB column using (a) 5×10^{-6} *M* sulfuric acid and (b) 0.5 m*M* sulfuric acid as the eluent.

carboxylic acids are shown in Fig. 2. In the case of acetic, propionic, butyric and valeric acids, a linear relationship was observed between the logarithm of the adsorption coefficient of the undissociated form of the acid, $\ln(K_{ads[HR]})$, and the concentration of methanol in the mobile phase. This suggested that hydrophobic adsorption was a major contributing factor to the retention mechanism for these acids. Fig. 2 also shows that with increasing chain length of the carboxylic acid, the slope of the regression line increased from 0.703 for acetic acid, to 0.930 for valeric acid. This demonstrated that the retention of

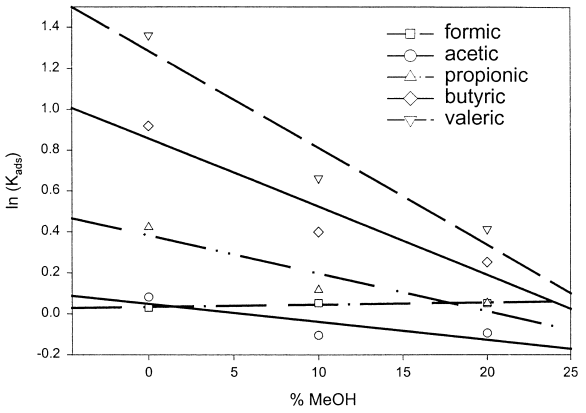

Fig. 2. Relationship between the logarithm of the adsorption coefficient of the undissociated form of the acid ($K_{ads[HR]}$) and the concentration of methanol in the eluent for five carboxylic acids on a PS–DVB column using 0.5 m*M* sulfuric acid (pH 3) as the eluent.

short-chain acids was based on a mixed ion-exclusion and hydrophobic adsorption mechanism whilst in the case of valeric acid, the retention mechanism was dominated by hydrophobic adsorption alone.

However, Fig. 2 shows that an increase in the adsorption coefficient of formic acid was observed with increasing methanol in the mobile phase, in contrast to the trends described above. Formic acid is the smallest and strongest of the five investigated acids ($pK_a = 3.75$) and shows the least hydrophobic adsorption. We suggest that the observed effect was due to a decrease in the dissociation of formic acid in the presence of methanol rather than as a result of any influence of methanol on its hydrophobic adsorption. It has been shown that increasing the methanol content in the mobile phase decreases the dielectric constant of the medium and this has the effect of increasing the pK_a value of formic acid [11].

The adsorption coefficients of the carboxylic acids also increased with increasing number of carbon atoms in the molecule. It is possible to estimate the molecular surface area of a molecule by assuming the cavity shape is spherical and summing the partial molar volumes of its fragments using the following equation:

$$A = N^{-2/3} \cdot 4.836\left(\sum_i V_i\right)^{2/3} \quad (25)$$

where A is the cavity surface area in liquid, N is Avogadro's number, and V_i are the increments of partial molar of volumes of the fragments [12].

Fig. 3 shows the linear dependency between the logarithm of the adsorption coefficient of the undissociated form of the acid analyte, $\ln(K_{ads[HR]})$, and the molecular surface area, A. The slopes of the lines suggest that hydrophobic adsorption plays an important role in the retention mechanism of those acids.

4.2. Unbuffered mobile phase

The trends that have been observed above are applicable only to the adsorption coefficients calculated for a buffered eluent. However, the retention data set we have selected included conditions using relatively dilute solutions of sulfuric acid as the eluent, and in previous cases, the calculated values of the adsorption coefficients did not follow the expected trends. For example, the values of the adsorption coefficient of the dissociated form of the acid, $K_{ads[R^-]}$, were generally higher than those for the undissociated form of the acid, $K_{ads[HR]}$ [9]. This discrepancy in the calculations was attributed to the use of an unbuffered eluent.

Eq. (13) can be used to calculate the distribution coefficient of the solute, K_d, in an unbuffered mobile phase. In this case, the concentration of hydrogen ions in the mobile phase can be calculated from Eqs. (20), (21) and (24). We have assumed that the adsorption coefficients of the undissociated, $K_{ads[HR]}$, and dissociated, $K_{ads[R^-]}$, forms of the acid are a linear function of the methanol concentration in the mobile phase, φ:

$$\ln K_{ads[HR]} = \ln (K^{aq}_{ads[HR]}) - s_{[HR]}\varphi \quad (26)$$

$$\ln K_{ads[R^-]} = \ln (K^{aq}_{ads[R^-]}) - s_{[R^-]}\varphi \quad (27)$$

where $s_{[HR]}$ and $s_{[R^-]}$ are constants which represent the slopes of the solvophobic plots.

The calculated values of the adsorption coefficients and slopes for each analyte on each of the three columns are presented in Table 1, with only limited data being shown for the silica column because of the reduced retention data being obtained with this column. The values of the adsorption coefficients for both undissociated and dissociated forms of the acid are improved considerably over those obtained using the previous model [9]. Table 1 shows that the adsorption coefficients of the undissociated form of the acid, $K^{aq}_{ads[HR]}$, are consistently larger than those for the dissociated form, $K^{aq}_{ads[R^-]}$, as expected from considerations of the effects of the ionisation of the functional group on hydrophobicity. The adsorption coefficients also increased as the chain length of the carboxylic acids increased, and provide a quantitative description of the influence of hydrophobic adsorption on the retention mechanism. Finally, Table 1 shows that adsorption effects were generally higher on the PS–DVB stationary phase than the polymethacrylate material, with silica showing the smallest adsorption effects.

It can be noted that some of values of the adsorption coefficients are negative, for which there are two possible explanations. Firstly, it was observed in some cases that the retention volumes measured were nearly independent of the methanol concentration and this gave rise to large errors in the calculations. The second source of error has already been detailed for the case of formic acid. The addition to the mobile phase of organic modifiers, characterised by smaller dielectric constants than water, increases the pK_a of the acid solutes and thus increases their retention. In cases where this effect

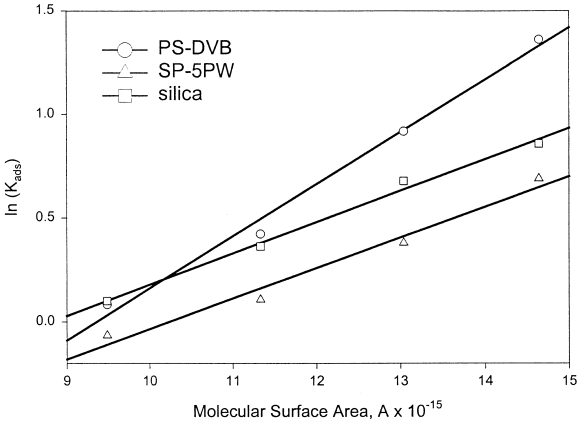

Fig. 3. Relationship between the logarithm of the adsorption coefficient of the undissociated form of the acid ($K_{ads[HR]}$) and the molecular surface area of the acid for acetic, propionic, butyric and valeric acids on three different columns. Other conditions were as in Fig. 2.

Table 1
Values of the adsorption coefficients, $K^{aq}_{ads[HR]}$ and $K^{aq}_{ads[R^-]}$, for a range of acid analytes separated on three different sulfonated stationary phases using dilute sulfuric acid as the eluent[a]

Acid	TSKGel SCX		TSKGel SP-5PW		Sulfonated silica	
	$K^{aq}_{ads[HR]}$	$K^{aq}_{ads[R^-]}$	$K^{aq}_{ads[HR]}$	$K^{aq}_{ads[R^-]}$	$K^{aq}_{ads[HR]}$	$K^{aq}_{ads[R^-]}$
Formic	0.96	0.10	0.98	0.12	0.55	0.47
Acetic	1.05	0.25	0.91	0.36		
Propionic	1.47	0.31	1.08	0.37		
Butyric	2.41	0.39	1.42	0.45		
Valeric	3.72	0.64	1.93	0.41		
Malonic	0.86	0.01	1.40	−0.12	0.78	−0.02
Succinic	0.82	0.03	0.96	0.02		
Glutaric	1.06	0.05	1.07	0.01		
Adipic	1.48	0.01	1.22	0.08		
Citric	0.72	−0.17	0.72	0.38	0.63	−0.05
Benzoic	34.81	1.31	10.80	−2.02		
Salicylic	41.39	0.31	25.50	−1.61	1.44	−0.09
Phthalic	10.75	−2.65	1.06	0.96	1.83	−0.56

[a] Retention data used for non-linear regression solution of the retention model were taken from Ref. [9].

overshadows hydrophobic adsorption of the acid, an overall increase in retention volume is observed with increasing concentration of methanol in the mobile phase. Furthermore, the addition of organic modifiers to the mobile phase also influences other parameters which were not considered in this model, for example, the column performance and mobile and stationary phase volumes. These issues will be resolved in subsequent models.

Fig. 4 shows the relationship between the adsorption coefficients of the undissociated and dissociated forms of the acid analyte and the number of carbon atoms present in the acid. These plots show that both the undissociated and dissociated forms of the acid adsorb onto the resin, although the adsorption of the dissociated form is significantly less than for the undissociated form. When the ratio of the two adsorption coefficients is plotted against the number of carbon atoms (also in Fig. 4), only moderate changes were observed for analytes with more than two carbon atoms. This shows that for the tested analytes an increase in the hydrophobicity of the undissociated analyte leads to a similar increase in the hydrophobicity of the dissociated analyte.

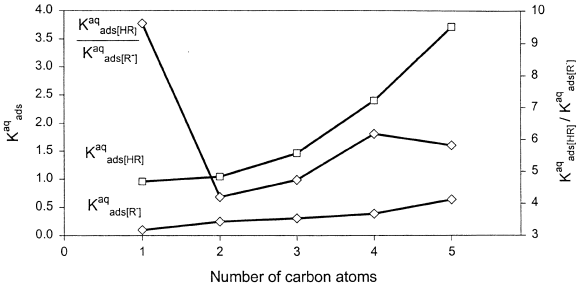

Fig. 4. Relationship between adsorption coefficients of the undissociated ($K^{aq}_{ads[HR]}$) and dissociated ($K^{aq}_{ads[R^-]}$) forms of the acid and the number of carbon atoms present for formic, acetic, propionic, butyric and valeric acid. The ratio of the two adsorption coefficients is also plotted. Conditions as in Fig. 2.

5. Conclusions

The retention model derived in this paper describes the retention mechanism of carboxylic acids in ion-exclusion chromatography more precisely than any previously published models in that it considers adsorption effects of the neutral and ionised solute, the effect of methanol added to the mobile phase, and finally the model can be applied when the mobile phase is buffered or unbuffered. The derived equations were used to estimate the values of the adsorption coefficients of the neutral and ionised solute, and the slopes of their dependencies on the concentration of methanol in the mobile phase. The values of the adsorption coefficients calculated indicate that both the undissociated and dissociated forms of the acid solutes adsorb onto the resin,

although adsorption of the dissociated form is significantly smaller. The retention model presented in this paper provides an improved description of the retention mechanism of carboxylic acids in ion-exclusion chromatography, and offers the potential for improved predictability of retention data. Further work is being undertaken to evaluate the suitability of this model for computer optimisation of separations of carboxylic acids by ion-exclusion chromatography.

References

[1] P.R. Haddad, P.E. Jackson, in: Ion Chromatography–Principles and Applications, Elsevier, Amsterdam, 1991.
[2] B.K. Glód, Neurochem. Res. 22 (1997) 1237.
[3] K. Tanaka, T. Ishizuka, H. Sunahara, J. Chromatogr. 174 (1979) 153.
[4] B.K. Glód, Acta Chromatogr. 7 (1997) 72.
[5] B.K. Glód, W. Kemula, J. Chromatogr. 366 (1986) 39.
[6] K. Kihara, S. Rokushika, H. Hatano, J. Chromatogr. 410 (1987) 103.
[7] B.K. Glód, J. Stafiej, J. Chromatogr. A 654 (1993) 197.
[8] B.K. Glód, Acta Chromatogr. 6 (1996) 101.
[9] K.L. Ng, B. Paull, P.R. Haddad, K. Tanaka, J. Chromatogr. A 850 (1999) 17.
[10] Cs. Horvath, W. Melander, I. Molnar, J. Chromatogr. 125 (1976) 129.
[11] E. Bosch, P. Bo, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[12] S.V. Galushko, J. Chromatogr. 552 (1991) 91.