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# Ion chromatographic separation of carboxylic acids Prediction of retention data

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**Abstract**

The retention behaviour of biologically relevant monovalent (formic, acetic, propionic, lactic and pyruvic) and divalent (oxalic, malonic, succinic, fumaric, maleic and tartaric acids) carboxylic acids together with inorganic analytes (chloride and sulphate) has been studied. The separation was performed on a latex-based strong anion-exchange resin using carbonate buffer systems in suppressed IC. The retention behaviour of analytes was investigated at different pH values and $[HCO_3^-]+[CO_3^{2-}]$ concentrations. A theoretical model, involving ion-exchange equilibria of sample and eluent anions, was derived and applied to the chromatographic data obtained. Chromatographic ion exchange selectivity values were determined and retention data were calculated for the anions using different carbonate eluent conditions. The average of errors between the predicted and the measured retention volumes of the analytes studied does not exceed 4.0%. The study effectively characterises the behaviour of different analytes under elution conditions of practical importance.

*Keywords:* Retention prediction; Carboxylic acids; Inorganic anions

## 1. Introduction

Inorganic and organic acids—carboxylic and other polar compounds—of fairly low molecular mass can be separated by several chromatographic techniques. A wide variety of the organic acids found in food products and in biological fluids have been resolved and quantitated by ion-pair [1–7], ion-exclusion [8–13] and ion chromatography [15–19].

Ion-pair chromatography has been used for the separation of ionised carboxylic acids both in isocratic or in gradient conditions [2,4] and with different detection techniques, such as UV–Vis [6], electrochemical and fluorimetry [4]. The pairing ions employed are commonly highly lipophilic cations such as hexadecyltrimethylammonium [1,2] and tetraethylammonium [3].

Ion-exclusion chromatography has been extensively used for the determination of carboxylic acids by potentiometric [8], UV [10] and both suppressed [9] and non-suppressed [10] conductivity detection. The separation mechanism is based on the partitioning of the acids in the molecular form between the interstitial and the stagnant mobile phase in the pores of a sulphonated resin [14]. Such a mechanism prevents the possibility of the simultaneous separation of weakly and strongly ionized species that elute unretained.

The other approach to organic acid separation is to convert them into anions, fractionate them on a pellicular anion-exchange column and elute them with alkaline solutions. Detection in the ion chromatographic system is generally performed by sup-

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pressed [16], non-suppressed conductivity [17] or by indirect photometry. The advantages of ion chromatography lie in the sensitive detection, the speed of separation and the ability to simultaneously determine organic and simple inorganic analytes, such as $Cl^-$ and $SO_4^{2-}$ ions that can be separated and detected together with the acids.

An additional feature is the prediction of the chromatographic data with the use of retention models that are able to describe the mechanism involved in the separation of carboxylate, focusing on the main factors that govern their retention.

The aim of this work is to study the chromatographic behaviour of monovalent and divalent carboxylic acids of practical importance by suppressed ion chromatography. This study allowed their elution in the presence of inorganic ions, chloride and sulphate, using carbonate eluents and latex-based strong anion-exchange resin. A retention model has been derived [20,21] in order to describe the chromatographic behaviour of the anions studied in the presence of multiple species eluent such as carbonate buffer. The model developed, involving ion-exchange equilibria of sample and eluent anions, was applied to the chromatographic data obtained. A non-linear regression method allowed the determination of chromatographic ion-exchange selectivity values. The selectivity constants were used to predict retention data of the anions considered under different carbonate eluent conditions.

## 2. Experimental section

The chromatographic equipment was a Dionex Series 2010i ion chromatograph (Dionex, Sunnyvale, CA, USA), a Dionex IonPac AS4A-SC anion separator column (250×4 mm), a Dionex AMMS-1 suppressor membrane, a Dionex CDM conductivity detector and a Carlo Erba SP 4270 data module integrator. The injection loop was 50 μl. The analytical column was a 13-μm polystyrene–divinylbenzene copolymer agglomerated with alkanol ammonium anion-exchange latex. The capacity was 20 μequivalents per column. The void volume, evaluated as the water deep at every eluent compositions studied, was 1.6 ml.

Eluents were daily prepared dissolving analytical grade $NaHCO_3$ and $Na_2CO_3$ (Merck) in triply distilled water obtained by a Milli-Q system (Millipore) supported with a 0.45-μm Millistack filter at the outlet. After optimisation, the eluent flow-rate was set at 1.7 ml $min^{-1}$.

Carboxylic standard solutions, formic, acetic, propionic, lactic, pyruvic oxalic, malonic, succinic, fumaric, maleic and tartaric acids, were from Sigma (St. Louis, MO, USA). Chloride and sulphate were from Fluka (Buchs, Switzerland).

## 3. Theory

The retention behaviour of ionizable analytes in buffered systems is dependent on several mobile phase parameters. Therefore, theoretical modeling must consider the effect of different ionic species in the eluent and different forms of analytes in the whole retention process.

The interaction between the analyte $A^{y-}$ and the eluent ion $E^{x-}$ bound in the resin R is expressed by the following ion-exchange equilibrium and its constant [22]:

$$yR_x\text{-}E + xA^{y-} \overset{K_{A/E}}{\rightleftharpoons} xR_y\text{-}A + yE^{x-} \tag{1}$$

$$K_{A/E} = \frac{(A^{y-})^x[E^{x-}]^y}{[A^{y-}]^x(E^{x-})^y} \tag{2}$$

where round and square brackets refer to the species concentration in the stationary and in the mobile phase, respectively.

From Eq. (2) it is possible to obtain the distribution coefficient $D$ of the analyte:

$$D_{A^{y-}} = \frac{(A^{y-})}{[A^{y-}]} = K_{A/E}^{1/x}\left(\frac{(E^{x-})}{[E^{x-}]}\right)^{y/x} \tag{3}$$

For analytes that undergo partial protonation, the molar fractions of the different solute species must also be considered [20]. In our study, experiments were performed at pH conditions of complete carboxylic acid dissociation ($pH > pK_a$) and so analyte protonation did not have to be considered.

In presence of multiple species eluent, such as the carbonate buffer system studied, $E^{x-}$ is the result of the following inter-eluent ion exchange equilibria:

$$CO_3^{2-} + 2R\text{-}HCO_3 \overset{K_{CO_3/HCO_3}}{\rightleftharpoons} R_2\text{-}CO_3 + 2HCO_3^- \quad (4)$$

$$K_{CO_3/HCO_3} = \frac{(CO_3^{2-})[HCO_3^-]^2}{[CO_3^{2-}](HCO_3^-)^2} \quad (5)$$

$$OH^- + R\text{-}HCO_3 \overset{K_{OH/HCO_3}}{\rightleftharpoons} R\text{-}OH + HCO_3^- \quad (6)$$

$$K_{OH/HCO_3} = \frac{(OH^-)[HCO_3^-]}{[OH^-](HCO_3^-)} \quad (7)$$

The capacity of the separation column is given by:

$$Q = 2(CO_3^{2-}) + (HCO_3^-) + (OH^-) \quad (8)$$

Obtaining $(CO_3^{2-})$ and $(OH^-)$ from Eqs. (5,7) and substituting them in Eq. (8), it is possible to rewrite it as:

$$Q = 2K_{CO_3/HCO_3}\frac{[CO_3^{2-}](HCO_3^-)^2}{[HCO_3^-]^2} + (HCO_3^-) + K_{OH/HCO_3}\frac{[OH^-](HCO_3^-)}{[HCO_3^-]} \quad (9)$$

from which the concentration of the bicarbonate eluent ion in the stationary phase is easily obtained:

$$(HCO_3^-) = \frac{-b + \sqrt{b^2 + 4aQ}}{2a} \quad (10)$$

where $a$ and $b$ are defined as:

$$a = 2K_{CO_3/HCO_3}\frac{[CO_3^{2-}]}{[HCO_3^-]^2} \quad (11)$$

$$b = 1 + K_{OH/HCO_3}\frac{[OH^-]}{[HCO_3^-]} \quad (12)$$

The stationary phase eluent concentration (Eq. (10)) must be substituted in the expression of the distribution coefficient of a monovalent (Eq. (13)) or a divalent (Eq. (14)) fully ionized analyte:

$$D_{A^-} = \frac{(A^-)}{[A^-]} = K_{A/E}\frac{(HCO_3^-)}{[HCO_3^-]} \quad (13)$$

$$D_{A^{2-}} = \frac{(A^{2-})}{[A^{2-}]} = K_{A/E}\left(\frac{(HCO_3^-)}{[HCO_3^-]}\right)^2 \quad (14)$$

With few mathematical rearrangements the final form of the model for retention behaviour of monovalent (Eq. (15)) and divalent (Eq. (16)) anions is obtained.

$$D_{A^-} = K_{A^-/HCO_3}\frac{-p + \sqrt{p^2 + q}}{4K_{CO_3/HCO_3}[CO_3^{2-}]} \quad (15)$$

$$D_{A^{2-}} = K_{A^{2-}/HCO_3}\left(\frac{-p + \sqrt{p^2 + q}}{4K_{CO_3/HCO_3}[CO_3^{2-}]}\right)^2 \quad (16)$$

where $p$ and $q$ are defined as:

$$p = [HCO_3^-] + K_{OH/HCO_3}[OH^-] \quad (17)$$

$$q = 8 \cdot K_{CO_3/HCO_3}[CO_3^{2-}]Q \quad (18)$$

For the description of the retention behaviour, the capacity factor, $k'$, is more suitable than the distribution coefficient:

$$k' = D\frac{V_{stat}}{V_0} \quad (19)$$

where $V_{stat}$ is the volume of the stationary phase (1.54 ml for the column used) and $V_0$ is the void volume.

## 4. Chromatographic separation

Retention times of saturated and unsaturated acids are influenced by many parameters such as molecular dimension, acidity of analytes and specific adsorption of organic molecules on the organic matrix of the ion exchangers. Moreover, thermodynamic properties of eluent ion and analytes (e.g. hydration enthalpy and entropy) play an important role in the control of selectivity. Examination of the chromatograms available, unfortunately, does not point out a clear correlation between these molecular parameters and retention times [19]. However, it is clear from the above derivation, that it contains a useful method by a phenomenological description of retention behaviour.

The analytes studied in this work have been chosen in order to get information on the relation between retention behaviour of the solutes, their chemical structure and their interaction with the stationary phase. The retention behaviour has been studied evaluating the effect of pH and carbonate

concentration on $k'$ over a relatively wide range of concentrations. The data collected were further used to verify the validity of the model derived for ion-exchange.

### 4.1. Eluent compositions studied

The chromatographic behaviour of the analytes has been investigated at three eluent pH values in the range 9.8–10.6 and at four different total carbonate concentrations, that is $[HCO_3^-]+[CO_3^{2-}]=2.5$ m*M*, 5.0 m*M*, 6.0 m*M* and 7.5 m*M*. The experimental pH values were chosen close to $pK_2$ value of $H_2CO_3$ ($pK_2=10.25$), in order to be able to easily vary the elution strength of the mobile phase by changing the ratio of $HCO_3^-$ and $CO_3^{2-}$ species. The analytical range of total carbonate concentration was considered suitable in order to avoid both too high and too short retention times that could lead to peak overlaps.

## 5. Results and discussion

All the carboxylates and the inorganic analytes have been considered within the 12 mobile phase compositions. Fig. 1 shows the dependence of $k'$ values of the dicarboxylates succinate, tartrate and maleate on pH, at a total carbonate concentration of 5.0 m*M*. The general trend of the curves is a decrease of $k'$ of all the analytes studied with increasing pH values. In fact, keeping the total carbonate and bicarbonate concentration constant, higher pH values increase the molar fraction of $CO_3^{2-}$, as plotted in the same graph, increasing the elution strength of the eluent. It can be easily calculated that an increase of pH from 9.8 to 10.6 corresponds to an increase of $[CO_3^{2-}]$ from 1.3 to 3.5 m*M*.

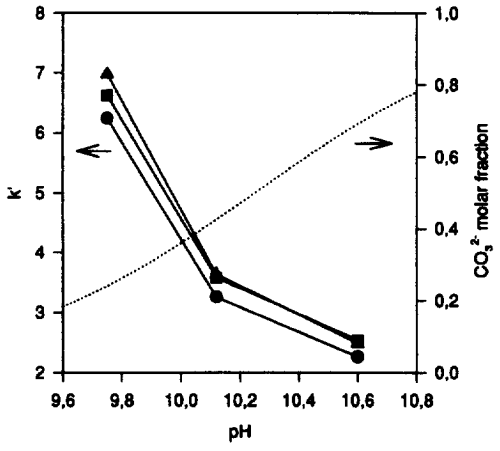


Fig. 1. Dependence of capacity factors ($k'$) on eluent pH. Stationary phase: IonPac AS4A-SC. Mobile phase: $[HCO_3^-]+[CO_3^{2-}]=5.0$ m*M*, pH as shown. ● = succinate; ■ = tartrate; ▲ = maleate.

At every eluent composition studied, the retention order of divalent analytes was succinate ≤ malonate < sulphate < tartrate < maleate < oxalate < fumarate. Regarding to the behaviour of maleate and succinate, it was observed that $k'$ values of maleate are higher than $k'$ values of succinate. These analytes have the same number of carbon atoms (C4) and of carboxylic groups, whilst the difference lies in the unsaturation of the maleate anion. The presence of the double bond leads to higher capacity factors probably due to stronger hydrophobic interactions of this bond with the polymeric matrix of the resin. The shorter length of the chain induced by the unsaturation could also contribute to stronger electrostatic interactions between ionic groups.

Succinate and tartrate have almost the same chromatographic behaviour, with tartrate slightly more retained than succinate. These divalent anions have four carbon atoms and differ in their chemical structure by the presence of two hydroxyl substituents in C2 and C3 in tartrate. The increased polarity of tartrate caused by the –OH groups could result in stronger interactions both with the aqueous mobile phase (leading to lower $k'$ for tartrate) and the alkanol substituent of the quaternary ammonium functional group of the resin (leading to higher $k'$). The retention behaviour seems to be the result of these complex and opposite effects.

Fig. 2 shows the chromatographic behaviour of oxalic and succinic acids, as a function of total carbonate concentration, obtained at pH 10.6. The total eluent concentration has been increased keeping constant the ratio $[HCO_3^-]:[CO_3^{2-}]=1:3$. The decreasing $k'$ at higher carbonate concentration is due, as usual in anion-exchange chromatography, to the competition effect of eluent ions on the analytes for the column cationic sites.

The elution order for succinate, malonate and

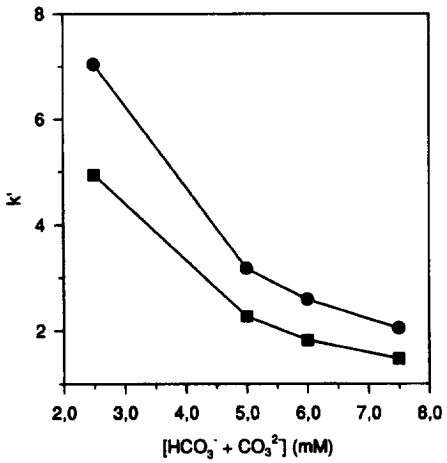


Fig. 2. Dependence of capacity factors ($k'$) on eluent concentration. Stationary phase: IonPac AS4A-SC. Mobile phase: $[HCO_3^-]:[CO_3^{2-}] = 1:3$, pH 10.6, total carbonate concentration as shown. ● = oxalate; ■ = succinate.

oxalate cannot be explained by the hydrophobic interaction between the resin and the hydrophobic chains of the analytes alone—that would suggest a reversed elution order. In this case, the interactions between the column and the shorter and more polar analytes are preferred.

The monovalent ions were less retained than all the divalent species studied and the retention order observed was lactate < acetate < propionate < formate < pyruvate < chloride. For formate, acetate and propionate there is no correlation between the elution order and their structure, making it difficult to relate retention to the dimension and the hydrophobicity of the acids. A combination of both these parameters contribute to the whole retention behaviour, as for formate electrostatic interactions are dominant while for propionate hydrophobic interactions are important. The retention times of pyruvate higher than those of propionate, which has the same number of carbon atoms, can be explained considering the polar interaction between the carbonylic group of the pyruvate and the OH substituent of the resin.

As an example of the effect of pH and total carbonate concentration on solutes separation previously considered, Figs. 3 and 4 show typical chromatograms obtained.

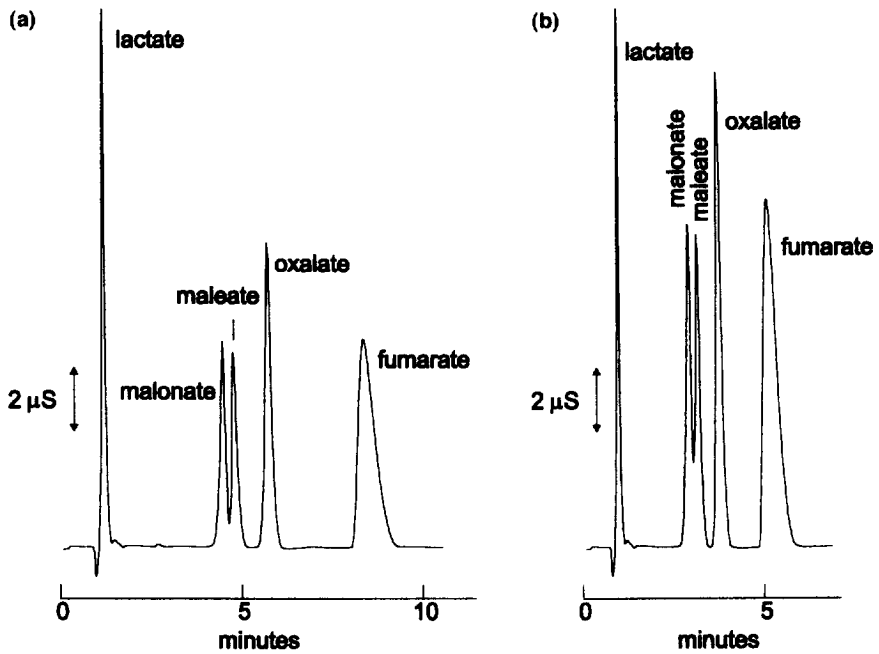


Fig. 3. Effect of pH on analyte separation. Stationary phase: IonPac AS4A-SC. Mobile phase: $[HCO_3^-] + [CO_3^{2-}] = 7.5$ m*M*. Sample loop 50 µl, conductivity detector, 0.5 m*M* lactate; 0.25 m*M* malonate; 0.25 m*M* maleate; 0.25 m*M* oxalate; 0.5 m*M* fumarate. (a) pH 9.8 (b) pH 10.1.

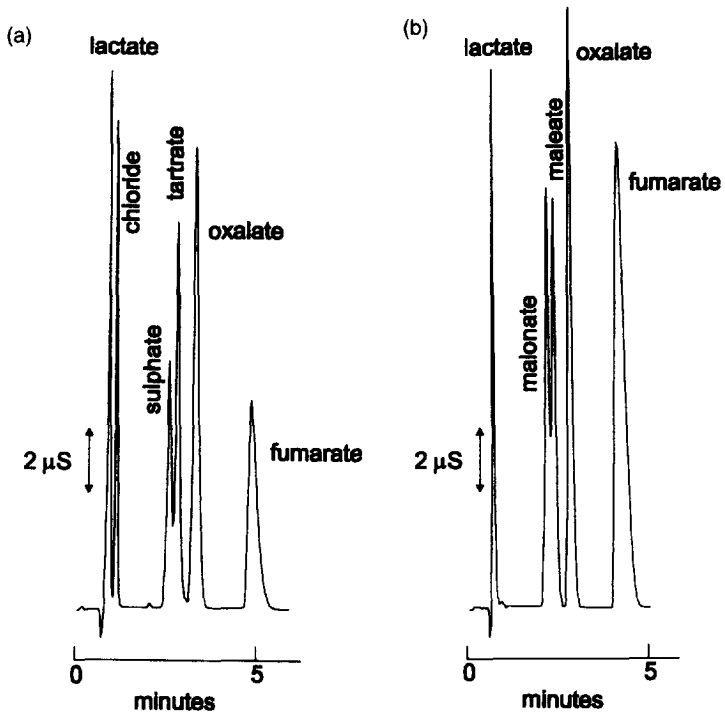


Fig. 4. Effect of total carbonate concentration on analyte separation. Stationary phase: IonPac AS4A-SC. Eluent pH 10.6. Sample loop 50 µl, conductivity detector, 0.5 m*M* lactate; 0.25 m*M* malonate; 0.25 m*M* maleate; 0.25 m*M* oxalate; 0.5 m*M* fumarate; 0.25 m*M* tartrate; $5.2 \cdot 10^{-2}$ m*M* sulphate; 0.15 m*M* chloride. (a) $[HCO_3^-]+[CO_3^{2-}]=6.0$ m*M*; (b) $[HCO_3^-]+[CO_3^{2-}]=7.5$ m*M*.

### *5.1. Retention model validation*

The validity of Eqs. (15,16) for data prediction of monovalent and divalent anions, respectively, has been checked by the chromatographic observations obtained for carboxylic acids at different mobile phase composition. A non-linear regression method, based on the Marquardt Levenberg algorithm, allowed the determination of chromatographic ion exchange selectivity values, $K_{A/HCO_3}$ $K_{OH/HCO_3}$ $K_{CO_3/HCO_3}$, for each analyte studied.

The mathematical procedure was first applied to the retention data of each divalent ion. The intereluent constants, $K_{OH/HCO_3}$ $K_{CO_3/HCO_3}$, calculated from the $k'$ values of the dicarboxylates, were averaged and the mean of the values ($K_{OH/HCO_3}=8.7\pm1.4$, $K_{CO_3/HCO_3}=14.0\pm3.8$) obtained from the 7 analytes was imposed in the iterative calculations of monovalent anions, obtaining the values of $K_{A/HCO_3}$ of singly charged analytes. Table 1 shows the chromatographic ion exchange selectivity constants determined for each solute ion. The values of $K_{CO_3/HCO_3}$ and $K_{OH/HCO_3}$ calculated for all the different analytes are quite in good agreement each other, meaning that intereluent constants are little influenced from the analyte considered and that they

Table 1
Chromatographic ion specific and intereluent ion-exchange constants for carboxylic and inorganic analytes

| Analyte | $K_{A/HCO_3}$ | $K_{CO_3/HCO_3}$ | $K_{OH/HCO_3}$ |
|---|---|---|---|
| Pyruvate | 0.93 | 14.0 | 8.7 |
| Formate | 0.79 | 14.0 | 8.7 |
| Lactate | 0.54 | 14.0 | 8.7 |
| Chloride | 1.88 | 14.0 | 8.7 |
| Acetate | 0.60 | 14.0 | 8.7 |
| Propionate | 0.68 | 14.0 | 8.7 |
| Fumarate | 107.3 | 15.3 | 8.1 |
| Maleate | 67.7 | 19.5 | 6.3 |
| Succinate | 43.8 | 12.9 | 10.7 |
| Malonate | 40.3 | 11.9 | 9.1 |
| Oxalate | 58.8 | 12.6 | 8.6 |
| Tartrate | 31.59 | 8.04 | 9.76 |
| Sulphate | 58.55 | 17.70 | 8.14 |

are representative of the chromatographic system chosen (column, eluent). The high value for the intereluent constant $K_{CO_3/HCO_3}$ means a high affinity of $CO_3^{2-}$ for the resin in respect to $HCO_3^-$ ion (Eq. (4)). This in turn means a stronger elution power of $CO_3^{2-}$ mobile phases, as experimentally verified (Fig. 1).

The low value for $K_{OH/HCO_3}$ is due to the similar affinity for the resin which endows similar elution power to the two ions. As the value obtained is >1, some considerations can be made on the selectivity of the resin for the $HCO_3^-$ and $OH^-$ ions. It is established [23] that the selectivity of a particular packing is the end result of several factors such as the chemical nature of the monomers in both the substrate resin and the latex, the ratios of the functionalised monomers to crosslinking monomers and the chemical nature of the ion-exchange functionalities. The value of $K_{OH/HCO_3}$ suggests that a preferential interaction between the $OH^-$ ion and the resin takes place in the column. The presence of hydroxyl groups at the beta position relative to the quaternary nitrogen definitely does increase the affinity of the stationary phase for $OH^-$ relative to $HCO_3^-$. This can be attributed either to hydrogen bonding or to the fact that the resin–OH group is relatively acidic and may convert the quaternary site to a zwitterion in the presence of $OH^-$ ion.

The values of $K_{A/HCO_3}$ are characteristic of the analyte ions and they represent the affinity of the solutes for the stationary phase (Eq. (1)). It must be pointed out that the values are related to the experimental elution order of the analytes (e.g. fumarate, the most retained analyte, has the highest $K_{A/HCO_3}$ value), within the standard deviation of the iterative calculation process.

The importance of the calculation of ion-specific and inter-eluent selectivity constants consists in the possibility of predicting the retention volumes of the analytes at different pH values and carbonate concentration elution conditions. In fact, substituting the values of the constants of Table 1 in Eqs. (15,16), the predicted retention volumes ($V_{R,calc}$) for monovalent and divalent analytes, respectively, can be calculated. This process has been applied to all the analytes studied and the values for experimental ($V_{R,meas}$) and calculated retention volumes obtained are shown in Tables 2–5. At the bottom of each table the average errors (%), calculated as $100\cdot(V_{R,meas} - V_{R,calc}/V_{R,meas})$, has been indicated. The errors for each analyte do not exceed 4.0% and they are homogenously distributed at lower and higher eluent concentrations, confirming the validity of the equation proposed and therefore the values obtained for the constants.

The simultaneous effect of the parameters of mobile phase, influencing the retention behaviour of the analytes, can be easily visualized by the calcu-

Table 2
Comparison of observed and predicted retention volumes for pyruvate, formate and lactate

| Eluent concn. ($M$) | pH | Pyruvate | | | Formate | | | Lactate | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_{R,calc}$ | $V_{R,meas}$ | Error[a] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[b] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[c] (%) |
| 0.0025 | 9.8 | 2.38 | 2.52 | 5.6 | 2.26 | 2.38 | 5.0 | 2.05 | 2.18 | 6.0 |
| 0.0025 | 10.2 | 2.19 | 2.19 | 0.0 | 2.10 | 2.11 | 0.5 | 1.94 | 1.94 | 0.0 |
| 0.0025 | 10.5 | 2.08 | 2.04 | 2.0 | 2.01 | 1.99 | 1.0 | 1.88 | 1.89 | 0.5 |
| 0.0050 | 9.8 | 2.14 | 2.14 | 0.0 | 2.06 | 2.04 | 1.0 | 1.91 | 1.90 | 0.5 |
| 0.0050 | 10.1 | 2.02 | 1.94 | 4.1 | 1.96 | 1.89 | 3.7 | 1.84 | 1.80 | 2.2 |
| 0.0050 | 10.6 | 1.93 | 1.87 | 3.2 | 1.88 | 1.82 | 3.3 | 1.79 | 1.75 | 2.3 |
| 0.0060 | 9.9 | 2.05 | 2.02 | 1.5 | 1.98 | 1.97 | 0.5 | 1.86 | 1.84 | 1.1 |
| 0.0060 | 10.4 | 1.94 | 1.92 | 1.0 | 1.88 | 1.84 | 2.2 | 1.79 | 1.77 | 1.1 |
| 0.0060 | 10.7 | 1.90 | 1.84 | 3.3 | 1.85 | 1.80 | 2.8 | 1.77 | 1.72 | 2.9 |
| 0.0075 | 9.8 | 2.02 | 1.99 | 1.5 | 1.95 | 1.90 | 2.6 | 1.84 | 1.85 | 0.5 |
| 0.0075 | 10.2 | 1.92 | 2.02 | 5.0 | 1.87 | 2.01 | 7.0 | 1.79 | 1.75 | 2.3 |
| 0.0075 | 10.6 | 1.87 | 1.80 | 3.9 | 1.83 | 1.77 | 3.4 | 1.76 | 1.68 | 4.8 |

[a] Mean = 2.6.
[b] Mean = 2.8.
[c] Mean = 2.0.

Table 3
Comparison of observed and predicted retention volumes for chloride, acetate and propionate

| Eluent concn. ($M$) | pH | Chloride | | | Acetate | | | Propionate | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_{R,calc}$ | $V_{R,meas}$ | Error[a] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[b] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[c] (%) |
| 0.0025 | 9.8 | 3.18 | 3.30 | 3.6 | 2.10 | 2.19 | 4.1 | 2.17 | 2.28 | 4.8 |
| 0.0025 | 10.2 | 2.80 | 2.87 | 2.4 | 1.98 | 1.99 | 0.5 | 2.04 | 2.07 | 1.4 |
| 0.0025 | 10.5 | 2.58 | 2.60 | 0.8 | 1.91 | 1.90 | 0.5 | 1.96 | 1.96 | 0.0 |
| 0.0050 | 9.8 | 2.69 | 2.60 | 3.5 | 1.95 | 1.94 | 0.5 | 2.00 | 1.99 | 0.5 |
| 0.0050 | 10.1 | 2.45 | 2.35 | 4.3 | 1.87 | 1.82 | 2.7 | 1.91 | 1.84 | 3.8 |
| 0.0050 | 10.6 | 2.27 | 2.21 | 2.7 | 1.81 | 1.79 | 1.1 | 1.84 | 1.79 | 2.8 |
| 0.0060 | 9.9 | 2.52 | 2.52 | 0.0 | 1.89 | 1.89 | 0.0 | 1.93 | 1.92 | 0.5 |
| 0.0060 | 10.4 | 2.28 | 2.28 | 0.0 | 1.82 | 1.79 | 1.7 | 1.85 | 1.84 | 0.5 |
| 0.0060 | 10.7 | 2.20 | 2.16 | 1.9 | 1.79 | 1.75 | 2.3 | 1.82 | 1.75 | 4.0 |
| 0.0075 | 9.8 | 2.44 | 2.41 | 1.2 | 1.87 | 1.85 | 1.1 | 1.91 | 1.89 | 1.1 |
| 0.0075 | 10.2 | 2.25 | 2.26 | 0.4 | 1.81 | 1.82 | 0.5 | 1.84 | 1.87 | 1.6 |
| 0.0075 | 10.6 | 2.15 | 2.09 | 2.9 | 1.77 | 1.72 | 3.3 | 1.80 | 1.73 | 4.0 |

[a] Mean = 2.0.
[b] Mean = 1.5.
[c] Mean = 2.1.

lated retention surfaces (Figs. 5 and 6). The figures clearly show the difference of the magnitude of retention of divalent analytes when compared with monovalent species of similar chemical structure (e.g.: propionate and malonate in Fig. 5, acetate and oxalate in Fig. 6). This is mainly due to stronger electrostatic interactions (Eq. (1)) which lead to higher $D$ values (compare Eqs. (15,16)) for doubly negatively charged analytes. The resulting effect is that both pH and carbonate concentration influence the retention of $A^{2-}$ to a larger extent than the retention of $A^-$ species. Moreover, it has been discussed that $CO_3^{2-}$ concentration is the most effective parameter for the reduction of the retention (Fig. 1 and $K_{CO_3/HCO_3}$ value obtained by the iterative computation). This is also evident in Eqs. (15,16) in which the term $[CO_3^{2-}]$ has its higher weight in the denominator. Retention surfaces of Fig. 5 show the

Table 4
Comparison of observed and predicted retention volumes for fumarate, maleate and succinate

| Eluent concn. ($M$) | pH | Fumarate | | | Maleate | | | Succinate | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_{R,calc}$ | $V_{R,meas}$ | Error[a] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[b] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[c] (%) |
| 0.0025 | 10.2 | 27.80 | 28.46 | 2.3 | 15.01 | 15.52 | 3.3 | 13.77 | 14.21 | 3.1 |
| 0.0025 | 10.5 | 19.13 | 19.04 | 0.5 | 10.72 | 10.76 | 0.4 | 9.53 | 9.50 | 0.3 |
| 0.0050 | 9.8 | 23.34 | 23.12 | 1.0 | 12.75 | 12.75 | 0 | 11.78 | 11.58 | 1.7 |
| 0.0050 | 10.1 | 14.94 | 12.85 | 16.3 | 8.41 | 7.41 | 13.5 | 7.84 | 6.80 | 15.3 |
| 0.0050 | 10.6 | 9.90 | 9.61 | 3.0 | 5.89 | 5.58 | 5.6 | 5.39 | 5.22 | 3.3 |
| 0.0060 | 9.9 | 17.06 | 17.85 | 4.4 | 9.52 | 9.88 | 3.6 | 8.84 | 9.49 | 6.8 |
| 0.0060 | 10.4 | 10.09 | 10.86 | 7.1 | 5.94 | 6.21 | 4.3 | 5.55 | 5.87 | 5.5 |
| 0.0060 | 10.7 | 8.28 | 8.25 | 0.4 | 5.06 | 4.85 | 4.3 | 4.65 | 4.51 | 3.1 |
| 0.0075 | 9.8 | 14.67 | 14.62 | 0.3 | 8.32 | 8.23 | 1.1 | 7.71 | 7.63 | 1.0 |
| 0.0075 | 10.2 | 9.42 | 9.84 | 4.3 | 5.59 | 5.37 | 4.1 | 5.26 | 5.12 | 2.7 |
| 0.0075 | 10.6 | 7.14 | 6.82 | 4.7 | 4.45 | 4.15 | 7.2 | 4.15 | 3.94 | 5.3 |

[a] Mean = 4.0.
[b] Mean = 4.3.
[c] Mean = 4.4.

Table 5
Comparison of observed and predicted retention volumes for malonate, oxalate, tartrate and sulphate

| Eluent concn. ($M$) | pH | Malonate | | | Oxalate | | | Tartrate | | | Sulphate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $V_{R,calc}$ | $V_{R,meas}$ | Error[a] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[b] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[c] (%) | $V_{R,calc}$ | $V_{R,meas}$ | Error[d] (%) |
| 0.0025 | 10.2 | 13.80 | 14.28 | 3.4 | 18.63 | 19.40 | 4.0 | 15.04 | 15.42 | 2.5 | 14.10 | 14.60 | 3.4 |
| 0.0025 | 10.5 | 9.66 | 9.62 | 0.4 | 12.91 | 12.85 | 0.5 | 10.34 | 10.27 | 0.7 | 9.97 | 9.98 | 0.1 |
| 0.0050 | 9.8 | 11.65 | 11.44 | 1.8 | 15.63 | 15.44 | 1.2 | 12.48 | 12.19 | 2.4 | 12.05 | 11.90 | 1.3 |
| 0.0050 | 10.1 | 7.82 | 6.80 | 15.0 | 10.27 | 8.87 | 15.8 | 8.44 | 7.33 | 15.1 | 7.98 | 7.00 | 14.0 |
| 0.0050 | 10.6 | 5.43 | 5.27 | 3.0 | 6.97 | 6.66 | 4.7 | 5.79 | 5.66 | 2.3 | 5.56 | 5.30 | 4.9 |
| 0.0060 | 9.9 | 8.76 | 9.33 | 6.1 | 11.59 | 12.10 | 4.2 | 9.36 | 9.93 | 5.7 | 9.02 | 9.62 | 6.2 |
| 0.0060 | 10.4 | 5.55 | 5.80 | 4.3 | 7.12 | 7.46 | 4.6 | 5.96 | 6.27 | 4.9 | 5.65 | 5.88 | 3.9 |
| 0.0060 | 10.7 | 4.68 | 4.56 | 2.6 | 5.92 | 5.73 | 3.3 | 4.97 | 4.98 | 0.2 | 4.79 | 4.64 | 3.2 |
| 0.0075 | 9.8 | 7.62 | 7.62 | 0.0 | 10.01 | 10.13 | 1.2 | 8.06 | 8.14 | 1.0 | 7.89 | 7.80 | 1.2 |
| 0.0075 | 10.2 | 5.24 | 5.08 | 3.1 | 6.68 | 6.29 | 6.2 | 5.61 | 5.88 | 4.6 | 5.34 | 5.02 | 6.4 |
| 0.0075 | 10.6 | 4.17 | 3.91 | 6.6 | 5.19 | 4.85 | 7.0 | 4.42 | 4.23 | 4.5 | 4.24 | 3.96 | 7.1 |

[a] Mean = 4.2.
[b] Mean = 4.8.
[c] Mean = 4.0.
[d] Mean = 4.7.

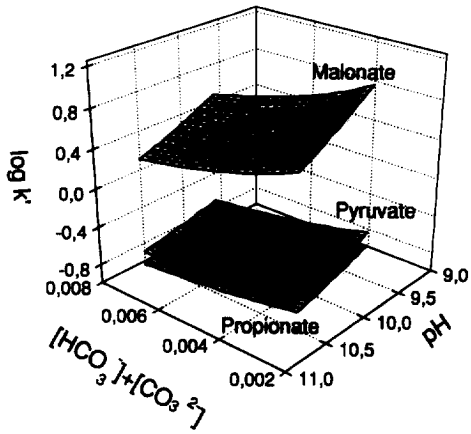


Fig. 5. Retention surfaces of monovalent (propionate, pyruvate) and divalent (malonate) carboxylates at different total carbonate molar concentrations and pH, calculated according to Eqs. (15,16), respectively.

effect of different chemical structure of analytes on the extent of retention, as already discussed in the text.

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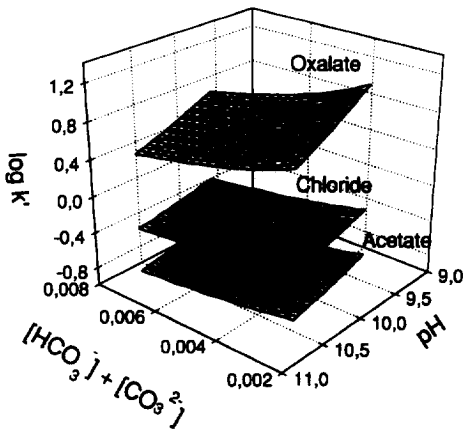


Fig. 6. Retention surfaces of monovalent (acetate, chloride) and divalent (oxalate) analytes at different total carbonate molar concentrations and pH, calculated according to Eqs. (15,16), respectively.

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