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# Mixed-mode liquid chromatography of carboxylic acids and inorganic anions on a latex-based pellicular stationary phase<sup>1</sup>

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## Abstract

The retention behaviour of mono-, dicarboxylate and inorganic anions on latex-based pellicular anion-exchanger was investigated. This paper is focused on the possible retention mechanism providing a retention database on a variety of mobile phase compositions. It has been demonstrated that anions can interact with the cation-exchange layer on the highly crosslinked latex-based pellicular phase as well as with the anion-exchange sites. A hypothesis is proposed to explain a mixed-mode retention of the series of analytes. A combination of two mechanisms has been found responsible for the enhanced selectivity: ion-exchange and ion-exclusion. Applications include the separation of formate, acetate, propionate, lactate, pyruvate, oxalate, malonate, succinate, tartrate, fumarate, maleate, chloride, nitrate and sulphate with NaOH eluent using suppressed conductivity detection. The study effectively characterizes the behaviour of different analytes under elution conditions of practical importance.

**Keywords:** Inorganic anions; Carboxylic acids

## 1. Introduction

Both carboxylic acids and their carboxylate salts are widely used in chemistry and in commercial products [1–3]. In the pharmaceutical industry, organic acids are used as antioxidants, preservatives, acidifiers and drug adsorption modifiers. Various diseases have been correlated with increased concentrations of organic acids in biological fluids. Organic acids are also fundamental components of fruits. Therefore a rapid and simple method for determining these compounds is required.

Carboxylic acids are usually separated by three

different liquid chromatographic methods: anion-exchange [4,5], ion-exclusion and reversed-phase chromatography [6–8]. Traditional high-performance liquid chromatography methods use refractive index (RI), ultraviolet (UV) absorption and derivatization fluorescence techniques for the detection of the acids. The most common application is the ion-exclusion technique. It is especially attractive as an adjunct to ion-exchange chromatography since selectivities obtained by these methods are quite different. Strong inorganic acid anions are excluded from the resin phase in a single peak according to the Donnan principle and elute at the void volume. Weaker and protonated species existing largely in the molecular form are retained on the stationary phase by a combination of ion-exclusion and hydrophobic interactions. For low-molecular-mass components, ion-

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exclusion is the preferred retention mechanism and all fully ionized solutes will elute at the same retention, without resolution. Simultaneous determination of short-chain carboxylic acids in mixtures with the mono- and divalent inorganic anions still represents a rather difficult separation problem.

A popular but complicated method for simultaneous separation of inorganic and organic anions is to couple ion-exclusion and ion-exchange separation modes in a multidimensional method [9]. The weakly ionized carboxylic acids pass rapidly through an anion-exchange column and suppressor membrane, where they are not retained. This early part of the chromatogram separated on an ion-exclusion column. At the same time, the fully ionized inorganic anions are separated on the anion-exchange column.

Recently, ion-chromatography has developed rapidly as a sensitive and simple separation method for inorganic and weak aliphatic organic acids. Gradient elution anion-exchange IC is a powerful technique and also yields separation of inorganic anions with many mono-, diprotic carboxylic acids [5]. In general, the anion-exchange and ion-exclusion modes of ion-chromatography by isocratic elution cannot provide a complete chromatogram of the homologous series of weak acids and inorganic anions. The combination of anion-exchange separation and eluent-suppressed electrical conductivity detection using latex-based pellicular stationary phase offers several advantages over existing techniques. Latex-based pellicular covering is a term used to describe the attachment of colloidal ion-exchange resin of one charge to a much larger substrate particle of the opposite charge [10]. These packings offer high speed, high efficiency and moderate loading capacity while maintaining very short diffusion paths. These features combine to give better resolution and shorter separation time. The chemically and mechanically stable substrate core is ethylvinylbenzene cross-linked with 55% divinylbenzene, which permits the use of these packings with HPLC solvents [11].

In this paper we describe a simple, rapid HPIC method for the simultaneous separation of organic and inorganic anions using ion-exchange chromatography with suppressed conductivity detection. The retention behaviour of aliphatic mono-, dicarboxylate anions and inorganic anions on a latex-based strong

anion-exchange resin column has been studied. There is no known literature discussing retention behaviour of carboxylic anions using highly cross-linked latex-based anion-exchange stationary phase. To the best of our knowledge, there has only been one systematic study on retention of inorganic analytes by Slingsby and Pohl [10], where they studied selectivity variations with differences in the nature of quaternary ammonium ion-exchange sites of the latexes. A hypothesis will be proposed to explain a mixed mode retention of analyte anions. We consider the influence of ion-exchange equilibria and electrostatic repulsion on the retention behaviour of the series of mono- and dicarboxylate anions. We wanted to provide a better understanding of the retention mechanism occurring in this system.

## 2. Experimental

### 2.1. Reagents and solutions

Organic chemicals were from Sigma (St. Louis, MO, USA) and inorganic chemicals were from Fluka (Buchs, Switzerland). Eluents were prepared by using analytical grade NaOH. Triply distilled water was purified by a Milli-Q system (Millipore, Bedford, MA, USA) containing a 0.45- $\mu\text{m}$  Millistack filter at the outlet. The sodium hydroxide eluent was prepared by diluting 10 M NaOH with deionized water that had been boiled and degassed. It should be noted that dilute solutions of NaOH will absorb carbon dioxide from the atmosphere to make carbonate, which is a stronger eluent than NaOH. However, the solubility of  $\text{Na}_2\text{CO}_3$  is limited in 10 M NaOH, so carbonate-free eluent can be prepared by dilution from a concentrated NaOH solution. Concentration range studied was 1–75 mM NaOH (pH 11.0–12.9). Ten different NaOH eluent concentrations were used: 1, 2.5, 5, 7.5, 10, 15, 25, 35, 50 and 75 mM. Sample solutions were prepared by dissolution of analytical grade sodium salts. The concentrations of the anions in the samples varied in the range  $5 \cdot 10^{-5}$ – $10^{-4}$  M.

### 2.2. Instrumentation

Chromatography was performed on a Dionex 2010i ion chromatograph (Dionex, Sunnyvale, CA,

USA) equipped with conductivity detector and Dionex-4270 integrator.

Dionex AS4A-SC polymer-based anion-exchange separator phase with alkanol amine functional groups was used. The separator column (250×4 mm) was based on a 13  $\mu\text{m}$  polystyrene–divinylbenzene copolymer agglomerated with completely aminated anion-exchange latex. The ion-exchange capacity of the column was 20  $\mu\text{equiv./column}$ . Chemically suppressed conductivity detection was accomplished using a Dionex AMMS-1 cation micromembrane suppressor which was continuously regenerated with 0.025 *M* sulphuric acid with a flow-rate of 3.5 ml/min. The injection loop was 50  $\mu\text{l}$ . All samples were analyzed in triplicate with a flow-rate of 1.7 ml/min.

### 3. Results and discussion

#### 3.1. Separation

Classical anion-exchange chromatography is often the method of choice for the hydrophilic carboxylic acids. However, resolution of the weakest acids is limited. The more hydrophilic carboxylic acids are those with  $\text{p}K_{\text{a}}$  values below 4 with aliphatic character and the hydroxy substituted acids (e.g., malic, lactic, tartaric etc.). Different groups of series e.g., saturated, unsaturated, hydroxy-, keto-, mono- and diprotic acids and inorganic components were chosen for separation (Table 1)

Because ionization of organic acids depends on the pH, their elution behaviour would be affected by changing the pH of the mobile phase in the pH range determined by their  $\text{p}K_{\text{a}}$  values. Using an eluent pH higher than 8 all species – basic eluents and sample analytes – are in anionic form. Conductivity detec-

Table 1

Dissociation constants of organic acids in aqueous solutions ( $T=25^{\circ}\text{C}$ ) [12]

Carboxylic acid	$\text{p}K_{\text{a}1}$	$\text{p}K_{\text{a}2}$	$\text{p}K_{\text{a}3}$
Formic	3.75		
Acetic	4.75		
Propionic	4.87		
Lactic	3.86		
Pyruvic	2.49		
Oxalic	1.23	4.19	
Malonic	2.83	5.69	
Succinic	4.16	5.61	
Tartaric	2.98	4.34	
Fumaric	3.02	4.39	
Maleic	1.83	6.07	
Citric	3.13	4.76	6.40

tion using NaOH eluent is insensitive in a single-column system because of the high background conductivity. Suppressed system however yields to a sensitive method as the background conductivity is reduced, NaOH is transformed into water and sample anions remain mostly in anionic form. The pH of the eluent after passing the suppressor device is between 7 and 8. The separation system used can be seen in Fig. 1. Typical chromatograms are shown in Fig. 2(a), Fig. 2(b) and Fig. 2(c).

Fig. 2(a) shows a separation of eight mono-, di- and trivalent carboxylate anions and sulphate ions using 50 mM NaOH eluent. Mostly baseline resolution was achieved between every peak. Under these conditions, citrate is strongly retained. Separation ability for carboxylic acids can be based on their ionic character as the main interaction mechanism. Fig. 2(b) can be used to show the enhanced selectivity of the alkanolammonium latex-bonded phase. The retention with 25 mM sodium hydroxide as eluent increases in the order monoprotic carboxylate<inorganic anions<diprotic carboxyl-

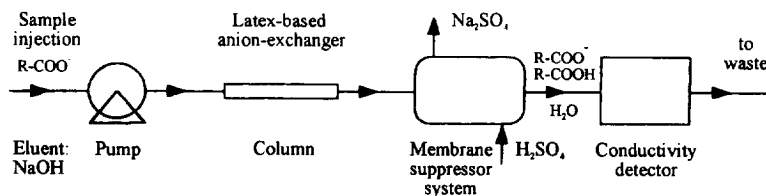


Fig. 1. Separation system.

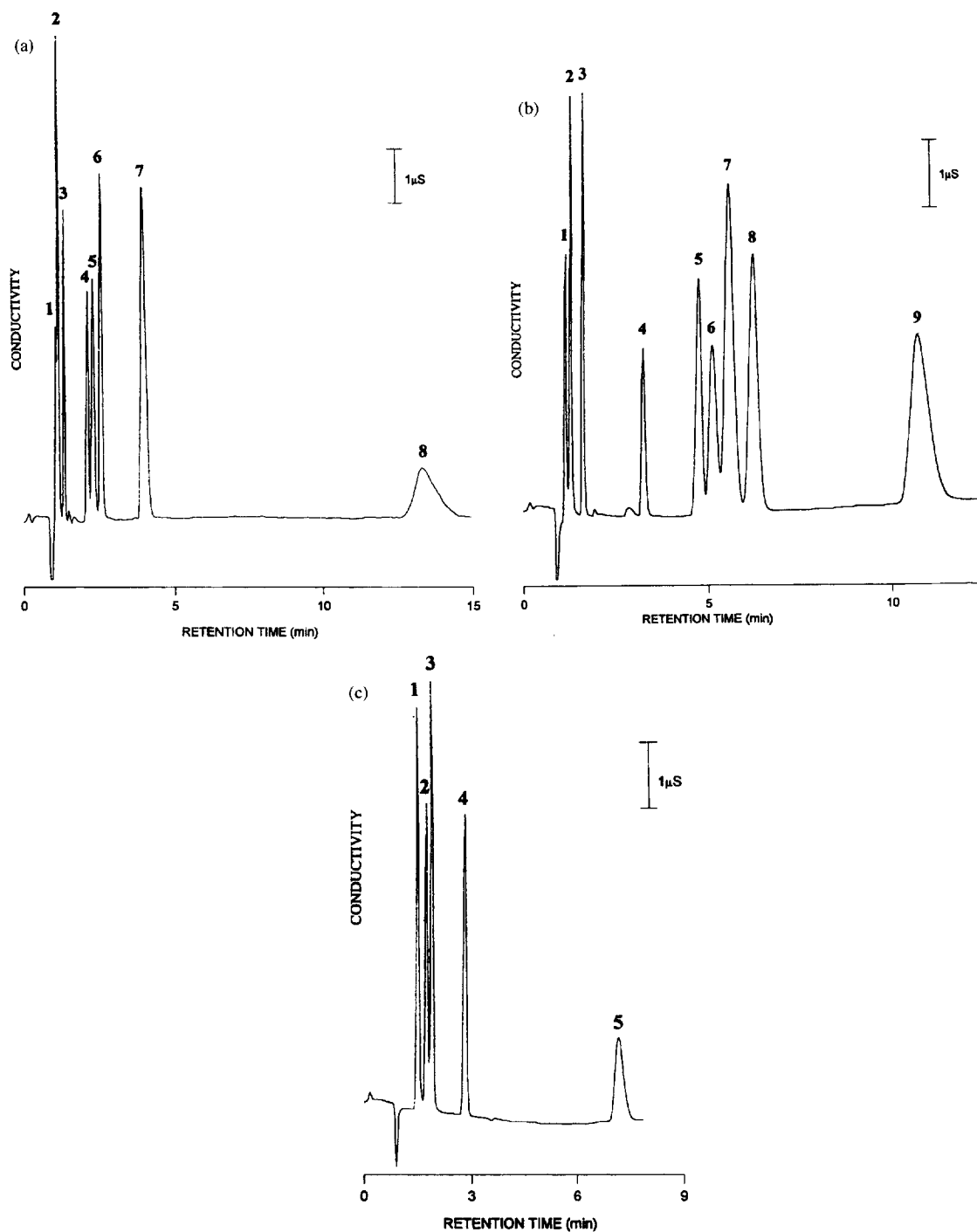


Fig. 2. Chromatograms of carboxylate- and inorganic anions. The concentrations of the anions in the samples varied in the range 0.05–0.1 mM. Detection was by suppressed conductivity. (a)  $c_{\text{eluent}} = 50 \text{ mM NaOH}$ ; peaks: 1 = acetate, 2 = pyruvate, 3 = chloride, 4 = sulphate, 5 = succinate, 6 = oxalate, 7 = fumarate, 8 = citrate. (b)  $c_{\text{eluent}} = 25 \text{ mM NaOH}$ ; peaks: 1 = lactate, 2 = pyruvate, 3 = chloride, 4 = nitrate, 5 = sulphate, 6 = succinate, 7 = tartrate, 8 = oxalate, 9 = fumarate. (c)  $c_{\text{eluent}} = 5 \text{ mM NaOH}$ ; peaks: 1 = lactate, 2 = formate, 3 = pyruvate, 4 = chloride, 5 = nitrate.

ate < unsaturated carboxylate. By decreasing the eluent strength to 5 mM (Fig. 2(c)), monoprotic acids such as lactate, formate, pyruvate can also be separated. The low eluent concentration is required to resolve the early eluting anions.

### 3.2. Retention behaviour of analytes

The observed capacity factors of the analytes are summarized in Fig. 3. Close examination of these figures highlights several interesting aspects of the retention characteristics of the analytes studied. Plotting the  $\log k'$  values, where  $k' = (V_R - V_0)/V_0$ , as the function of  $\log c_{\text{eluent}}$  the following conclusions are established:

- The changes of the eluent concentration in turn had a significant effect on the analyte retention characteristics. Increasing eluent concentration leads to decreased capacity factors. Inorganic

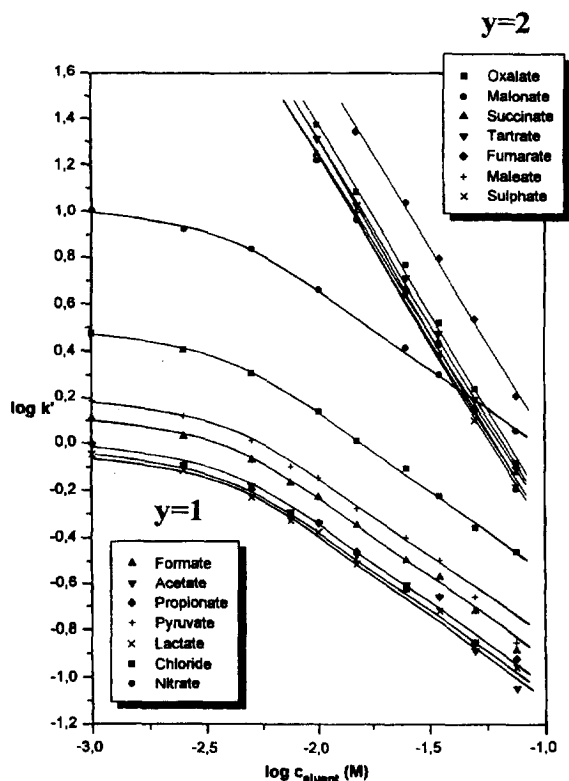


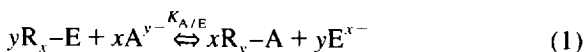
Fig. 3. Effect of eluent concentration on the retention behaviour of monovalent ( $y=1$ ) and divalent ( $y=2$ ) anions.

anions such as chloride, nitrate and sulphate have  $k'$  different to those of mono- and dicarboxylate anions and they can be separated simultaneously in one run.

- The fact that several of the plots cross one another indicates that the elution order of the anions can be reversed by increasing the eluent concentration. At NaOH concentrations below 35 mM nitrate elutes before diprotic carboxylate. When the eluent concentration is increased to 75 mM NaOH, elution order is reversed and nitrate is retained past all anions, except for fumarate.
- Monocarboxylate anions eluted before dicarboxylate anions. Increased solute charge leads to increased capacity factor.
- Retention is longer when there is an other functional group in the molecule. In general, keto- or OH groups on the hydrocarbon chain give higher retention volumes (pyruvate vs. propionate and tartrate vs. succinate).
- The retention behaviour of dicarboxylic acids depends on the relative position of the carboxyl groups on the hydrocarbon chain. Geometric isomers (maleate and fumarate) can be separated.
- Retention is higher when there are  $\pi$ -bonds in the hydrocarbon chain (fumarate, maleate vs. succinate).

### 3.3. Theoretical and practical treatment of mixed retention mechanisms

The ion-exchange equilibria for the eluent ( $E^{x-}$ ) and analyte ( $A^{y-}$ ) species:



where  $R$  refers to the stationary phase,  $x$  and  $y$  are the charges of a given anion.

The selectivity coefficient,  $K_{A/E}$ , is given by:

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

where  $()$  is the concentration in the stationary phase and  $[\ ]$  is the concentration in the mobile phase.

The volumetric distribution coefficient for solute  $A$  is designated as  $D_A$  and is given by:

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

Analytical trace conditions are achieved when the number of moles of  $A^{y-}$  ions is much less than the number of moles of  $E^{x-}$  ions. Supposing that  $c_A \leq c_E$  in the mobile and in the stationary phase, respectively;  $K_{A/E} = \text{const.}$  and  $E^{x-}$  occupies  $x$  ion-exchange sites on the stationary phase, the ion-exchange capacity of the column,  $Q$ , is given by:  $(E^{x-}) = Q/x$ ; then Eq. (3) becomes:

$$D_A = K_{A/E}^{1/x} \left(\frac{Q}{x}\right)^{y/x} [E^{x-}]^{-y/x} \quad (4)$$

The logarithm of Eq. (4) provides the relationship:

$$\log D_A = \text{const} - \frac{y}{x} \log[E^{x-}] \quad (5)$$

where  $D_A = k' \frac{V_0}{V_{st}}$  and

$$\log k' = \text{const}' - \frac{y}{x} \log[E^{x-}] \quad (6)$$

The slopes of these linear plots should be equal to  $-1$  times the charge of the sample anions divided by the charge of the eluent as with  $-y/x$  in Eq. (6). Since the charge of the  $OH^-$  eluent is  $-1$ , the slopes should be equal to the charge of the analytes. Studies of retention in ion-chromatography showed results which were in general agreement with the theoretical ion-exchange model [13,14], but in this case some significant deviations from the predicted dependences indicated that the actual mechanism was more complex. All slopes in Fig. 3 are definitely less than those predicted on the basis of the stoichiometric pure ion-exchange model.

A logical hypothesis for this unusual retention behaviour of anions may be made based on chemical structure of highly crosslinked latex-based pellicular phase. This polymer configuration concentrates a vast number of anion-exchange sites into a very narrow layer on the surface sulfonated highly crosslinked (55%) core. It can be seen in Fig. 4 that there are two sites of interaction on the stationary phase, namely the anion-exchange in latex-bonded layer and electrostatic repulsion or ion-exclusion in an oppositely charged under-layer which excludes anions through Donnan potential. In general, highly crosslinked polymers show stronger Donnan exclusion

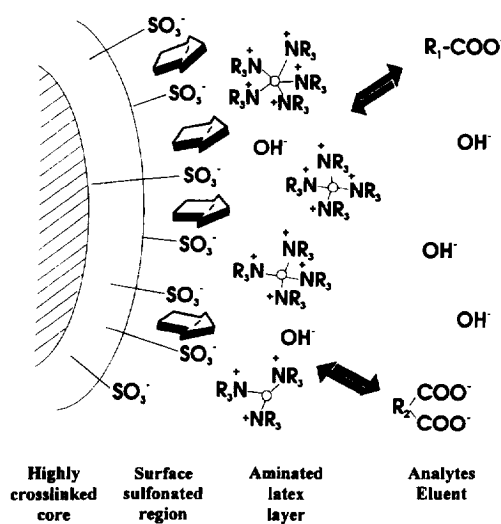


Fig. 4. Schematic view of the mixed mode retention effects on latex based pellicular phase.

effects than resins of lower crosslinking [15].

The observed  $k'$  of each of the 14 mono- and divalent analytes at ten different eluent concentrations was fitted to a second order polynomial function. The following form was investigated:

$$\log k' = a_0 + a_1 \log c + a_2 (\log c)^2 \quad (7)$$

where  $a_0$  is the intercept and  $a_1, a_2$  are coefficients. Results are presented in Table 2. Fig. 3 shows that data are well fitted by Eq. (7) which lends support to the proposed dual- or mixed-retention mechanism of anions on a latex-bonded phase.

To evaluate the influence of the electrostatic repulsion on the ion-exchange mechanism, the observed capacity factor of the analytes should be subtracted from the calculated values of pure ion-exchange model according to Eq. (6). Consequently, a negative sorption "correction" must be made to the ion-exchange distribution term in the form of an anion-exclusion capacity factor ( $k'_{excl}$ )

$$k'_{excl} = k'_{iex} - k'_{obs} \quad \text{or} \quad k'_{obs} = k'_{iex} - k'_{excl} \quad (8)$$

This phenomenon corresponds to the process of "electrolyte exclusion" or "Donnan exclusion" in gel-type ion-exchangers. (See Fig. 4)

The results are illustrated for two different cases in Fig. 5. Curves  $k'_{iex}$  are calculated contribution from

Table 2  
Relationships of capacity factors and NaOH concentration for anions

Analytes	$\log k' = a_0 + a_1 \log c + a_2 (\log c)^2$	$R^2$
<i>Monovalent</i>		
Formate	$\log k' = -2.20 - 1.41 \log c - 0.21 (\log c)^2$	0.999
Acetate	$\log k' = -2.47 - 1.56 \log c - 0.25 (\log c)^2$	0.998
Propionate	$\log k' = -2.10 - 1.21 \log c - 0.17 (\log c)^2$	0.998
Pyruvate	$\log k' = -2.26 - 1.54 \log c - 0.24 (\log c)^2$	0.999
Lactate	$\log k' = -2.13 - 1.22 \log c - 0.17 (\log c)^2$	0.999
Chloride	$\log k' = -1.72 - 1.31 \log c - 0.19 (\log c)^2$	0.999
Nitrate	$\log k' = -1.23 - 1.35 \log c - 0.20 (\log c)^2$	0.999
<i>Divalent</i>		
Oxalate	$\log k' = -2.64 - 2.62 \log c - 0.31 (\log c)^2$	0.999
Malonate	$\log k' = -3.12 - 3.14 \log c - 0.49 (\log c)^2$	0.999
Succinate	$\log k' = -2.47 - 2.37 \log c - 0.26 (\log c)^2$	0.999
Tartrate	$\log k' = -2.56 - 2.48 \log c - 0.28 (\log c)^2$	0.999
Fumarate	$\log k' = -2.66 - 3.11 \log c - 0.50 (\log c)^2$	0.999
Maleate	$\log k' = -3.38 - 3.54 \log c - 0.62 (\log c)^2$	0.999
Sulphate	$\log k' = -2.58 - 2.41 \log c - 0.25 (\log c)^2$	0.999

A Dionex AS4A-SC column (250 × 4 mm) was used with NaOH as mobile phase.

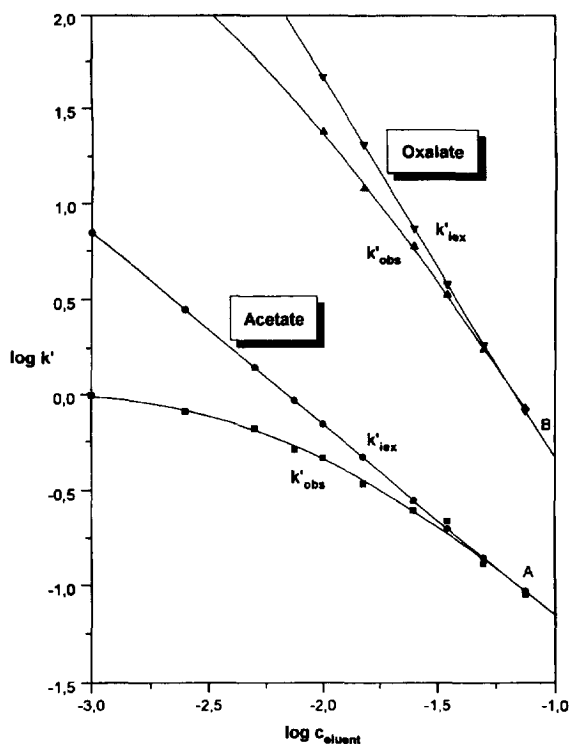


Fig. 5. Dependence of experimentally observed  $k'_{obs}$  and calculated (Eq. (6))  $k'_{ieq}$  on eluent concentration using NaOH as mobile phase.

ion-exchange. Curves  $k'_{obs}$  are for overall capacity factor, which show the contributions of ion-exchange and ion-exclusion to the retention.

The slopes of the tangents at points A and B of  $\log k'_{obs}$  vs.  $\log c$  curves indicate a pure ion-exchange process for acetate and oxalate anions, respectively. The two values,  $k'_{ieq}$  and  $k'_{obs}$  are equal only at these points and thus  $k'_{excl} = 0$ . The calculated values of eluent concentrations at these points were 98 mM and 74 mM for oxalate and acetate, respectively. With decreasing eluent concentration the contribution of  $k'_{excl}$  to the total capacity factor increases progressively. This means that the ion-exclusion is the dominant factor at low concentration interval of the eluent. This interaction – between the sulfonate groups and the anionic solutes – tends to decrease their overall capacity factors. Retention therefore appears to be a combination of ion-exchange and ion-exclusion effects. The above mentioned parameters which have been shown to contribute to the retention of aliphatic carboxylate- and inorganic anions can be applied to further separation on latex-based stationary phase.

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