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Effect of ion-exchange site and eluent modifiers on the anion-exchange of carboxylic acids

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Abstract

The chromatographic behavior of carboxylic acids has been investigated, on three different latex-based anion-exchange columns, in order to define the effect of the ion-exchange site structure on selectivity. The analytical columns produced are characterized by alkyl amines containing zero, one or two hydroxyl groups on the anion-exchange functional site. Divalent carboxylic acids, namely fumaric, maleic, *trans*-β-hydromuconic, *trans*,*trans*-muconic, oxalic, malonic, succinic, glutaric, adipic, malic, tartaric and mucic acids, have been chosen as test solutes. The performance of the three stationary phases has been studied employing NaOH eluents and has been discussed with respect to the different hydrophilicity of the ion-exchange sites and analytes. Considering on previous results obtained using organic solvents (methanol and acetonitrile) with carbonate eluents on a highly hydrophilic column, the performance of the three exchangers has also been studied using acetonitrile, methanol and *n*-propanol. The chromatographic behavior was similar for the three columns studied, but the different organic solvents gave variations in selectivity. In order to characterize these differences, particle size measurements of the latices were performed both in pure water and in the presence of each organic solvent studied.

Keywords: Ion-exchange site structures; Eluent modifiers; Carboxylic acids

1. Introduction

Selectivity in ion chromatography (IC) separations is still an important matter of study, due to its impact in governing retention and for the development of ion-exchange columns for selected applications. Reichenberg studied selectivity in anion exchange [1] and considered several factors involved in the selectivity, but concluded that selectivity order for anions can be explained by a theory which considers both the electrostatic interactions between functional group of the resin and counter-ion, as well as hydration energies of competing counter-ions. Diamond and Whitney [2], although evidencing the importance of hydration, focused on the differences between the resin and the external phase structures, and the availability of water molecules in the two phases. Water in the resin phase is used for hydration of the ionic groups and therefore solutes that require high hydration have higher affinity for the external phase rather than for the resin. The external phase is considered responsible for selectivity; ions of lower

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hydration energy interact with the resin phase. For *simple* ions, hydration is related to the dimension of the ions. For ions having greater dimensions and hydrophobic moieties, the resin has a more direct role and hydrophobic interactions between functional groups and counter-ions become important. With decreasing crosslinkage of the resin, strongly and weakly hydrated ions are less discriminated, probably because by increasing the water content in the resin, differences between resin and external phases decrease. Generally, ions possessing low hydration enthalpies (nitrate, bromide, chlorate) are more retained by hydrophobic resins.

In previous work [3], we studied the retention behavior of formic, acetic, propionic, lactic, pyruvic, oxalic, malonic, succinic, fumaric, maleic, and tartaric acids on a column composed of highly crosslinked polystyrene–divinylbenzene substrate with a low crosslinked latex-based anion-exchange phase (IonPac AS4A-SC, Dionex, Sunnyvale, CA, USA) using carbonate based buffers, and a stoichiometric retention model [4] has been applied to the chromatographic data obtained. Subsequently [5] retention of the same analytes has been characterized according to analyte and stationary phase properties. In order to define the effect of some characteristics of analytes such as alkyl chain, unsaturations, –OH substituents in governing retention, glutaric, adipic, malic, mucic, *trans*-β-hydromuconic, *trans*,*trans*-muconic acids have also been studied as additional analytes with a column of different (higher) hydrophilicity, namely the IonPac AS11 (Dionex). For the purpose of comparison, the eluent had the same composition as that in Ref. [3]. The retention model [4] has also been applied to the chromatographic data of the IonPac AS11 column and the ion-specific selectivity constants obtained by non-linear regression were compared with the ones previously obtained. The results allowed several considerations of other effects, besides pure ion-exchange, that affect retention.

Ion-exchange chromatography can also be performed in the presence of organic solvents which act on selectivity, giving another parameter to be manipulated to adjust retention.

While the effect of organic solvents in reversed-phase chromatography is well established, the same cannot be said for IC. Rabin and Stillian [6] provide examples for the use of methanol or acetonitrile in the eluent for different anion and cation-exchange columns. When macrocycle columns are used, it has been shown that their capacity is increased by incorporating the organic solvent of the eluent system [7].

Particular attention has been devoted to the separation mechanism involved in the cation-exchange of *n*-alkylamine [8] and alkali metals [9] in non-aqueous solvents.

A comparison of retention behavior of alkylsulfonic acids in anion-exchange chromatography when acetonitrile and methanol are present in sodium hydroxide eluents has been provided by Aceto et al. [10]. Comparing the results obtained with the two organic solvents, they evidenced two different mechanisms involving ionic and hydrophobic interactions during the anion-exchange separation.

The aim of this work is to define the effect of the ion-exchange site structure on the selectivity of dicarboxylic acids (fumaric, maleic, *trans*-β-hydromuconic, *trans*,*trans*-muconic, oxalic, malonic, succinic, glutaric, adipic, malic, tartaric and mucic acids) when NaOH eluents are used. For this purpose, three different anion-exchange latices were prepared for three different columns. The latices are characterized by alkyl amines containing zero, one and two hydroxyl groups on the anion-exchange functional site. Discussion is focused on selectivity based on differences in the hydrophilicity of the ion-exchange sites and of the analytes.

The effect of methanol, *n*-propanol and acetonitrile concentrations on the retention of the analytes has also been evaluated and compared for characterization of the retention behavior for carboxylic acids when an organic modifier is present. Similar chromatographic behavior was obtained for the three columns studied, but differences were obtained as a function of the organic solvent used. In order to explain these difference characterization of latex size has been performed by particle size measurements in pure water and in the presence of each organic solvent studied.

A complete description of selectivity variations of inorganic anions with non hydroxy selective columns as a function of the analytes is available [11].

2. Experimental

The chromatographic system used was a 4000i gradient pump (Dionex) equipped with a 25-μl sample loop and a CDM-3 conductimetric detector (Dionex). For aqueous eluents, suppressed conductometric detection has been performed in the autosuppression recycle mode with an Anion Self-Regenerating Suppressor (ASRS-II, 4-mm, Dionex). When eluent modifiers were used, detection has been performed in the autosuppression external water mode. For each experiment, current setting was matched to the eluent concentration and flow-rate.

Three analytical separation columns (250×4 mm I.D.) were using the divinylbenzene–ethylvinylbenzene substrate. Each column was agglomerated with one of the three latices. The only difference among the stationary phases was the alkyl amine that contained zero, one and two hydroxyl groups on the anion-exchange functional site. After preparations, the retention and asymmetry for common inorganic anions F^-, Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-} with a 21 m*M* NaOH eluent was compared for each column produced. Anion-exchange capacity of each of the three columns were determined by loading the column with chloride, eluting the chloride with NaOH, and subsequent determining the concentration of chloride using an IonPac AS11 (250×4 mm I.D.) column (Dionex).

The void volumes for each column have been calculated by the water dip, while the stationary phase volumes have been calculated by subtraction of the void volume from the geometrical volume of the columns.

Particle size measurements have been performed with a BI-90 particle sizer (Brookhaven Instruments, NY, USA).

Malonic acid sodium salt, fumaric acid disodium salt, maleic acid disodium salt, tartaric acid disodium salt dihydrate were from Fluka (Buchs, Switzerland); oxalic acid dihydrate was from Merck, while succinic, glutaric, adipic, malic, mucic, *trans*-β-hydromuconic, *trans,trans*-muconic acids were from Aldrich (Milwaukee, WI, USA). Sodium hydroxide, methanol and acetonitrile were from Merck, while *n*-propanol was from BDH (Germany).

After preparation, eluents have been degassed and sonicated in an ultrasonic bath for 15 min. Eluent flow-rate was 2.0 ml/min and all chromatograms were obtained at room temperature. Depending on hydroxide selectivity of the columns prepared, solutions of 10–100 m*M* NaOH were used. Methanol, acetonitrile and *n*-propanol were used as eluent modifiers.

3. Results and discussion

Selectivity in an ion-exchange system (Eq. (1)) can be quantified through the selectivity coefficient, $K_{A/E}$:

$$xR_y - E + yA^{x-} \overset{K_{A/E}}{\rightleftharpoons} yR_x - A + xE^{y-} \quad (1)$$

The characteristics of the columns produced are summarized in Table 1. Column capacity has been calculated through the uptake of chloride ions by each column according to the following procedure. A 60-ml volume of 0.1 *M* NaCl was eluted through each column to convert all ion-exchange sites of the column to the chloride form, 30 ml water to remove the excess of chloride in the eluent, 50 ml 0.1 *M* NaOH to elute Cl^- ions by the sites. The NaOH

Table 1
Characteristics of the columns developed

Column	–OH groups in the amine	Capacity (μequiv./column)		V_{stat} (ml)	V_0 (ml)	Latex size (nm)
		Q	r^2			
1	2	43.5	0.9997	2.00	1.14	85
2	1	40.5	0.9998	2.03	1.11	86
3	0	38.1	0.9822	2.03	1.11	89

eluate was collected, 1:10 diluted, injected into an IonPac AS11 and eluted by a 10 m*M* NaOH solution. This eluent concentration has been chosen to match the hydroxide concentration injected. The concentration of the chloride ion has been calculated by the standard addition method. In Table 1, for each column, the capacity (Q) obtained and the regression coefficient (r^2) of the standard addition plot have been reported.

3.1. Common inorganic anions

After preparation, the columns were tested by injecting common inorganic anions. Elution was performed varying NaOH concentration as shown in Table 2. Peak asymmetry has been checked to verify the performance of the columns.

With increasing hydroxyl content, the sites show higher affinity for OH^- ions in the eluent, as a result of increased hydrogen bonding. Nitrate, as a hydrophobic ion, sulfate and phosphate, as multivalent ions, most clearly show the effect of OH^- selectivity on anion-exchange sites.

3.2. Carboxylic acids

The chromatographic behavior of carboxylic acids of different hydrophobicity has been further evaluated in the three columns. According to the hydroxide selectivity of the phases prepared, a 10–20 m*M* NaOH range has been studied for column 1, 20–50 m*M* NaOH for column 2, and 50–100 m*M* NaOH for column 3. These ranges of concentration were chosen in order to have a comparison for column performance with, at least, one concentration value (20 m*M* NaOH columns 1 and 2, 50 m*M* columns 2 and 3).

From the retention order obtained, stationary phases with more hydrophilic ion-exchange sites exhibit lower retention for analytes. This is due to the fact that a highly hydrated eluting ion (OH^-) is a less effective eluent on less hydrophilic ion exchangers, such as column 3.

The characteristics of the latex act on selectivity causing inversions in the retention order of some anions. In fact, with decreasing hydrophilicity of the stationary phase (from column 1 to 3), fumaric acid gradually reverses its elution order with *trans*,*trans*-muconic acid. The characteristics of the latex of column 3 are such that interactions of hydrophobic type with the alkyl chains of the amine and interactions of H-bonding type are reduced to a minimum. With this column, fumaric acid is more retained than *trans*,*trans*-muconic acid, as expected by the differences in charge density of the two analytes.

When column 1 is used, succinic acid is more retained than mucic acid, while the opposite occurs using column 3. This can be explained by considering the difference in the structure of column 1, the lower molecular size of succinic acid and the repulsive effect between the dissociated –OH groups of mucic and of the alkanolamine of the site. Moreover, dissociation of OH groups in the ionic site of column 1 can induce electrostatic repulsion with the alcoholic groups of mucic acids, making it less retained. In the same way the inversion of elution order between malonic and tartaric acids can be explained. These inversions of elution order are also dependent on hydration enthalpy of the analytes and of the anion-exchange site. To explain the phenomenon, we can consider the retention of mucic and malic acids in the three columns. In columns 1 and 2 malic acid elutes before mucic acid. When a less hydrophilic column is used (column 3), the elution order is reversed. Based on heat of hydration data for crystalline salts, the tetrabutyl ammonium ion has a relatively high heat of hydration, which is hence indicative for a high enthalpy of hydration. Moreover, the osmotic pressure can be related to the hydration enthalpy. Based on our experience, osmotic pressure of an anion-exchange latex based on a quaternary group containing no hydroxyl substituents is higher than that of a latex based on a quaternary

Table 2
Retention data with 21 m*M* NaOH as eluent

Column	k'				
	F^-	Cl^-	SO_4^{2-}	NO_3^-	PO_4^{3-}
1	0.04	0.34	0.88	1.34	3.08
2	0.20	1.91	16.6	8.27	–[b]
3	0.69	7.84	–[a]	38.7	–[b]

[a] Not eluted within 30 min.
[b] Not eluted within 40 min.

group containing hydroxyl substituents. In our study, this means that hydration enthalpy is higher in the less hydrophilic column (column 3).

Presumably, in the case of quaternary groups without any hydroxyl substituent the high enthalpy of hydration results in incorporation of the hydroxyl substituents from organic hydroxy acids into the solvation sphere, making use of all available solvation options. On the other hand, the lower osmotic pressure observed with quaternary groups containing a hydroxyl substituent suggests a lower hydration enthalpy, presumably, because these hydroxyl substituents partially serve this need. As a result, there is a lower tendency for ion-exchange sites of this type to incorporate into the solvation sphere the hydroxyl substituent present in organic hydroxy acids. As a consequence, less retention of hydroxy carboxylic acids is observed as the number of hydroxyl groups attached to the quaternary center increases. In agreement with these observations, tartaric acid retention increases when passing from column 1 to column 3.

According to the previous statements, selectivity between tartaric–malic acids and mucic–adipic acids increases from columns 1 to 3, that is with the decrease of hydrophilicity of the functionality of the ion-exchange site (e.g.,: $\alpha_{\text{mucic/adipic,column 1}} = 1.10$, $\alpha_{\text{mucic/adipic,column 2}} = 1.34$, $\alpha_{\text{mucic/adipic,column 3}} = 1.84$).

The retention of the homologous series from oxalic to adipic acids suggests that the elution agrees with an anion-exchange mechanism. Selectivity between oxalic and malonic is higher than that of the other acids of the series and decreases with the length of the alkyl chain of the acid for each column and for each NaOH concentration evaluated (e.g.,: for column 3 at 50 m*M* NaOH $\alpha_{\text{oxalic/malonic}} = 2.19$, $\alpha_{\text{malonic/succinic}} = 1.31$, $\alpha_{\text{succinic/glutaric}} = 1.29$, $\alpha_{\text{glutaric/adipic}} = 1.11$).

The capacity factors obtained for carboxylic acids at the different NaOH concentrations in the three columns, have been used to calculate the selectivity coefficients of each analyte for each stationary phase. The selectivity coefficients ($K_{A/E}$) have been calculated according to Eq. (2) commonly used in IC [12,13] by linear regression on the data previously obtained:

$$\log k'_A = 1/y \log K_{A/E} + x/y \log Q + \log V_{stat}/V_0 - x/y \log[E^{y-}] \quad (2)$$

The results obtained are collected in Table 3. In the same table, the regression coefficients (r^2) for each analyte are also shown. The selectivity coefficients are characteristic of each analyte and express the net effect of all parameters operating in both stationary and mobile phases that are responsible for determining selectivity. Parameters affecting selectivity affect the activity coefficients of the ions in one phase relative to the other.

The data clearly show that selectivity coefficients

Table 3
Selectivity coefficients $K_{A/E}$ (calculated according to Eq. (2)) and r^2 for analytes in the three ion exchangers

Analyte	Column 1[a]		Column 2[b]		Column 3[c]	
	$K_{A/E}$	r^2	$K_{A/E}$	r^2	$K_{A/E}$	r^2
Oxalic acid	0.24	0.9996	5.74	0.9997	72.56	0.9942
Malonic acid	0.12	0.9996	3.38	0.9999	38.08	0.9944
Succinic acid	0.12	0.9999	3.23	0.9998	32.41	0.9939
Glutaric acid	0.09	0.9999	2.68	0.9996	26.96	0.9938
Adipic acid	0.13	0.9999	2.51	0.9991	27.59	0.9932
Fumaric acid	0.47	0.9999	9.10	0.9997	112.4	0.9940
Maleic acid	0.17	0.9999	3.23	0.9989	38.97	0.9932
trans-β-Hydromuconic acid	0.24	0.9999	4.35	0.9998	53.79	0.9938
trans,*trans*-Muconic acid	0.55	0.9999	10.44	0.9999	115.0	0.9936
Malic acid	0.18	0.9999	3.15	0.9997	32.41	0.9940
Tartaric acid	0.10	0.9992	3.79	0.9999	39.88	0.9939
Mucic acid	0.12	0.9998	3.15	0.9999	34.73	0.9942

Experiments performed in [a]10–20 m*M* NaOH, [b]20–50 m*M* NaOH, [c]50–100 m*M* NaOH.

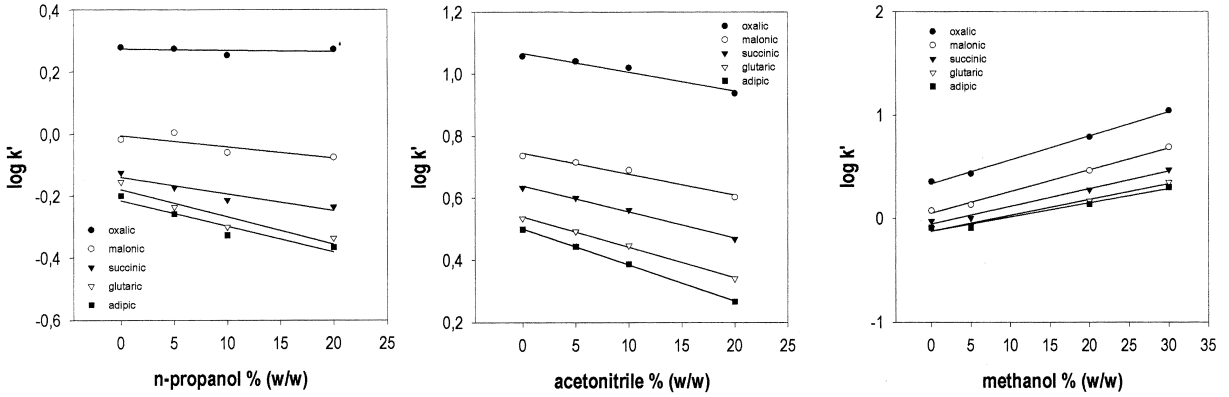

Fig. 1. Effect of *n*-propanol, acetonitrile and methanol % on k' of carboxylates in column 1. Eluent: 15 m*M* NaOH.

increases with a decrease in column hydrophilicity. The hydroxyl groups on the ion-exchange functionality make the resin more hydroxide selective.

In the presence of purely electrostatic interactions, that is in the presence of a pure ion-exchange mechanism from which Eq. (2) is derived, the $K_{A/E}$ values should be related to the retention order observed. The data obtained show that this is mainly true for column 3, where, as previously mentioned, additional interactions are reduced to the minimum.

3.3. Effect of organic solvents

The behavior of carboxylic acids in the three exchangers has also been studied in the presence of three different organic solvents: *n*-propanol, methanol and acetonitrile. In order to better evaluate the effect of organic solvents and according to the results obtained without organic modifiers, the following NaOH eluent concentrations have been chosen for the three columns: 15 m*M* NaOH for column 1, 50 m*M* NaOH for column 2, and 100 m*M* NaOH for column 3. The concentration ranges investigated were *n*-propanol (0–20%, w/w), acetonitrile (0–20%, w/w) and methanol (0–30%, w/w).

The behavior of k' and the selectivity among the analytes depend on the type of solvent studied.

While k' decreases at increasing concentrations of acetonitrile (see Figs. 1–3) the opposite occurs for methanol, for each column studied. When *n*-propanol is present, for each column only the retention of unsaturated analytes (fumaric, maleic, *trans*-β-hydro-

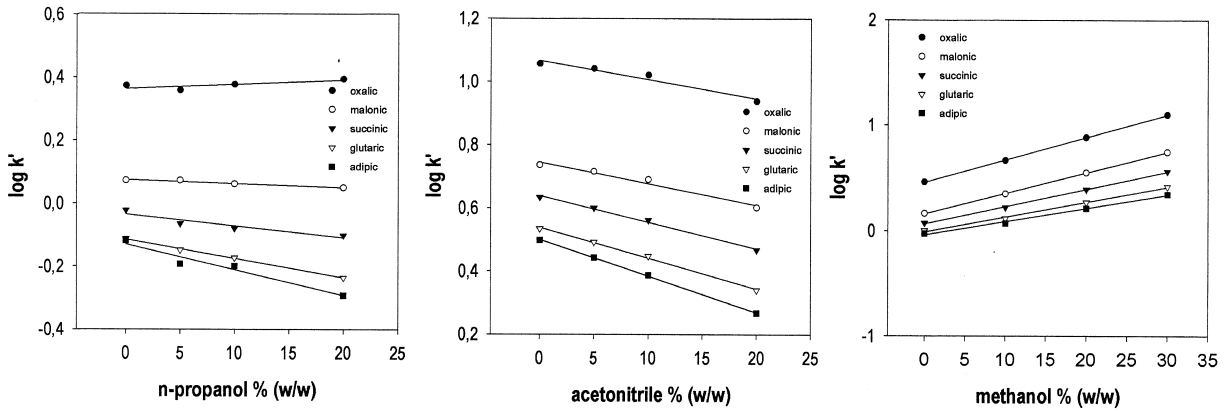

Fig. 2. Effect of *n*-propanol, acetonitrile and methanol % on k' of carboxylates in column 2. Eluent: 50 m*M* NaOH.

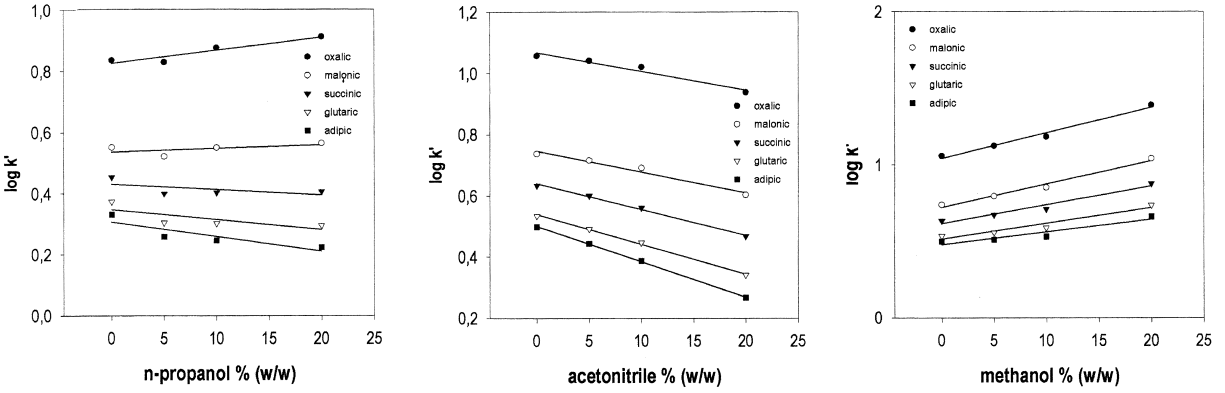

Fig. 3. Effect of *n*-propanol, acetonitrile and methanol % on k' of carboxylates in column 3. Eluent: 100 m*M* NaOH.

muconic and *trans,trans*-muconic acids) decreases for an increase of eluent modifier concentrations. Retention of the other analytes is generally unaffected or slightly decreasing for column 1 and slightly increasing for column 3.

Comparing these results with those previously obtained [5], it can be readily inferred that, the increase of k' with the percentage of methanol in mobile phase is more enhanced in the presence of NaOH eluents, than with carbonate buffer. In fact, while k' for oxalic, malonic, succinic, glutaric, adipic and maleic acids were almost unaffected or slightly increasing for methanol content higher than 15% (v/v) in carbonate buffers, with sodium hydroxide eluents, the retention increases even at lower methanol percentages. The behavior of fumaric, *trans*, *trans*-muconic and *trans*-β-hydromuconic acids appear completely different for the two eluent systems. In fact, with carbonate based buffers, their retention decreases with the increase of methanol content, leading to preferential interactions with the mobile phase rather than the stationary phase. The same analytes with NaOH eluents, increase their retention with the increase of organic solvents content. Considering that the properties of the stationary phase used in the previous work [5] were more similar to those of column 1 in this work, and that no differences were noted for the three columns when the effect of methanol in the eluent was studied, the differences in retention behavior can be ascribed only to the characteristics of the eluents. It can be proposed that the pK_2 of carbonic acid increases in a methanol–water system, changing (decreasing) the ratio of carbonate/hydrogencarbonate eluent ions, giving a mobile phase with a lower elution power. This effect is particularly important if experiments are performed in a pH value close to that of pK_2, as it was the case of our previous work [5].

The effects of organic solvents in the hydroxide mobile phase are related to its solvation. Hydroxide ions are less hydrated in an organic medium with its hydration enthalpy depending on several parameters such as the dielectric constant of the medium. Considering the tabulated values of dielectric constants for the organic solvents studied [14], ϵ_{water} (25°C)=78.5, $\epsilon_{\text{acetonitrile}}$ (20°C)=38.8, $\epsilon_{\text{methanol}}$ (25°C)=32.6, $\epsilon_{n\text{-propanol}}$ (25°C)=20.1, we would expect a minor hydration degree in the presence of *n*-propanol. This means that OH^- would have lower eluting strength in the presence of *n*-propanol thus increasing retention times more than the other organic solvents. A high dielectric constant favors interactions between polar groups of the solute and the mobile phase, but also the ion association between the ionic groups of the analytes and the functional site of the stationary phase. Solvation can be defined as the ability of a solvent to disperse electrostatic charges by ion–dipole interactions. Water-lean mobile phases tend to exhibit more pronounced solvation effects because solvation is related to the dielectric constant. Hydrogen-bonding solvents, such as water or alkyl alcohols, tend to preferentially solvate ions with a high charge-to-radius ratio. Acetonitrile is a dipolar solvent and exhibits poor solvating ability for small inorganic ions but solvates well the hydrophobic ions which

can participate in electron bonding (e.g., via π-bonds). The efficacy of acetonitrile in decreasing retention times with respect to *n*-propanol can be evaluated comparing the behavior of oxalic, malonic, succinic, glutaric and adipic acids in the two solvents for each of the column studied (Figs. 1–3).

The lower polarity of the modifiers minimize any adsorptive interaction with the stationary phase, making ion-exchange the prevalent separation mechanism. The presence of hydrophobic interactions when no organic modifier is present is readily evidenced by the fact that oxalic acid becomes the most retained ion in the presence of any of the three solvents used. The decrease of retention for *trans*, *trans*-muconic and *trans*-β-hydromuconic acids, when passing from aqueous to organic-containing eluents, points out the presence of hydrophobic interactions.

Assuming the basic concept of the reversed-phase chromatography that the eluent strength of a given solvent increases with its retention factor, the following eluotropic series, normalized to methanol with water as eluent in a C_{18} stationary phase, can be considered: methanol 1.0, acetonitrile 3.1, *n*-propanol 10.1 [15]. On the basis of this scale, the reduction of hydrophobic interactions is more efficient for *n*-propanol, acetonitrile and methanol, respectively.

Generally, hydrophobic interactions are governed by bulk surface tension. Based on an approximation [16], it can be assumed that the greater the surface tension of the eluent, the greater the retention of the given eluent.

Due to the great number of mobile and stationary phase parameters involved in the retention mechanism, the effect of the presence of the organic solvent in the eluent is of difficult interpretation and prediction, as deeply discussed in this and our previous work [5]. To summarize, solvated radii of analyte ion, eluent ions and functional groups of the stationary phase, determined also by hydrogen bonding properties and dispersive (London) forces, greatly influence the retention mechanism in the presence of different organic solvents. Differences in selectivity can be found according to the column and to the type of modifier used. Whichever the column and the organic modifier used, oxalic and fumaric acids are the most retained analytes, differently from what happened without organic solvent, in agreement with a pure ion-exchange mechanism.

It is interesting to note that curves of fumaric and maleic, *trans*-β-hydromuconic and *trans,trans*-muconic acids increase with a parallel slope at increasing methanol % in the eluent for columns 2 and 3, indicating that no variation of selectivity is acting, different from what happens in the most hydrophilic column (column 1) where at high methanol content (30%) a decrease of selectivity between fumaric and maleic acids occurs.

It has been noticed that selectivity between malic and mucic acids is governed both by the structure of the functional site and by the eluent compositions. In fact, as previously noted, when aqueous eluents are used, malic is more retained in the more hydrophilic column (column 1) and then inversion of elution order takes place: $(\alpha_{\text{malic/mucic}})_{\text{column 1}} = 1.11$, $(\alpha_{\text{malic/mucic}})_{\text{column 2}} = 1.03$, $(\alpha_{\text{malic/mucic}})_{\text{column 3}} = 0.88$. When organic solvents are present, the same occurs. Selectivity is enhanced in the least hydrophilic column, in the presence of water, acetonitrile and *n*-propanol. Minor variation of selectivity among the three columns has been noted in the presence of methanol. As an example of performance and selectivity of the stationary phases prepared, an example of separation is shown in Fig. 4.

In order to characterize the differences of k'

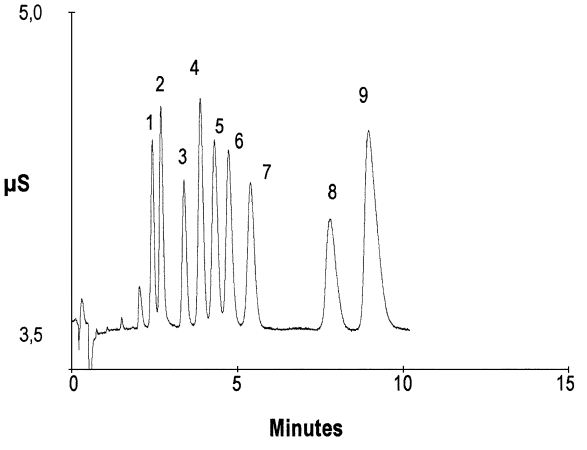

Fig. 4. Example of performance of column 3 in the separation of carboxylic acids. Eluent: 100 m*M* NaOH, 10% (w/w) methanol. Peaks: 1=adipic, 2=glutaric, 3=succinic, 4=malic, 5=*trans*-β-hydromuconic, 6=maleic, 7=tartaric, 8=*trans,trans*-muconic, 9=oxalic acids. Analyte concentrations: 10 mg/l each.

Table 4
Size lattices and polydispersity of column 2 without and in the presence of eluent modifiers

Column 2	Water	30% (v/v) CH_3OH	20% (v/v) CH_3CN	10% (v/v) $CH_3(CH_2)_2OH$
Diameter (nm)	86±0[a]	81±1[a]	89±0[a]	86±0[a]
Polydispersity	0.065±0.008	0.079±0.017	0.058±0.016	0.039±0.004

[a] $n=6$.

behavior according to the type of eluent modifier used, and, in particular, to explain the increase of retention with increasing methanol percentage, the capacity of the column in the presence of each organic modifier were measured following the procedure previously explained. Measurements have been performed in column 2, obtaining $Q_{30\%\ (w/w)CH_3OH}=42.5$ μequiv./column ($r^2=0.9963$), $Q_{20\%(w/w)\ CH_3CN}=45.8$ μequiv./column ($r^2=0.9832$) and $Q_{10\%\ (w/w)\ n\text{-propanol}}=29.65$ μequiv./column ($r^2=0.9999$). These results evidence that capacity values do not change significantly to explain the increasing k' values of analytes when methanol is added to the eluent.

As we discussed, the effects of the organic solvents on the mobile phase composition, have a great impact in the selectivity among analytes, but we should also take into account the stationary phase-related effects. In fact, in presence of organic solvents, the latex of the anion-exchange column, which contains the ion-exchange sites, can swell (or shrink) reducing (or increasing) the number of the sites per unit area of ion-exchange polymer. In these conditions, the charge density, that is a function of number of sites and latex size, is influenced.

On the basis of these considerations, and in order to correlate the variations of latex size as a function of the solvent type, and hence to the different chromatographic behaviors of analytes, further experiments have been aimed to latex size measurements in methanol–water, acetonitrile–water and *n*-propanol–water mixtures (Table 4). In the same table, polydispersity, that is related to the homogeneity of the system, has also been indicated.

The results obtained show that in the presence of methanol, latex size is smaller than in a pure water system, indicating a shrinking of the latex. Methanol reduces the number of molecules of water required to solvate the latex. The ion-exchange sites are hence distributed in a smaller surface, leading to a higher charge density than in pure water systems. This in turn induces higher retention times, if only electrostatic interactions are considered. Conversely, in presence of acetonitrile, latex dimensions are higher, confirming a swelling of the latex particles. Density charge is now lower than in pure water and retention times shorter, in agreement with the trends obtained.

Latex size in the presence of *n*-propanol remains constant, indicating that no changes in the electrostatic properties of the resin are acting. As a consequence, the effects of *n*-propanol on retention of analytes should be mainly related to the hydrophobic interactions. This statement is in agreement with the behavior of k' that decrease only for double bond-containing analytes (fumaric, maleic, *trans*-β-hydromuconic and *trans,trans*-muconic acids).

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