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Effect of stationary phase hydrophobicity and mobile phase composition on the separation of carboxylic acids in ion chromatography

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Abstract

In a previous work, we studied the retention behavior of monovalent and divalent carboxylic acids on a highly cross-linked polystryene–divinylbenzene anion-exchange column (IonPac AS4A-SC) using a carbonate-based buffer, and a retention model was applied to the chromatographic data obtained. In this work we characterized the retention of carboxylates (formic, acetic, propionic, lactic, pyruvic, oxalic, malonic, succinic, fumaric, maleic, tartaric, glutaric, adipic, malic, mucic, *trans*-β-hydromuconic, *trans*,*trans*-muconic acids) on a column with higher hydrophilicity (IonPac AS11) according to analyte and stationary phase properties, using previously investigated eluent compositions and comparing the retention data obtained. Moreover, the effect of organic modifiers (CH_3OH and CH_3CN) in the eluent on the retention factors was also evaluated. The chromatographic data obtained on the IonPac AS11 column were fitted by the retention model and allowed one to obtain and to compare ion-specific selectivity constants (parameters of the model) with the ones obtained with the previous column.

Keywords: Stationary phases, LC; Mobile phase composition; Carboxylic acids

1. Introduction

The interest in the analysis of substances containing carboxylic groups is continuously increasing. Carboxylic acids, hydrophilic weak electrolytes, belong to a class of compounds of biological and applicative importance. In the pharmaceutical industry, they are used as antioxidants, acidifiers and drug adsorption modifiers. Carboxylic acids are also extensively used for manufacturing of technical products. Carboxylates can also be found as natural compounds or additives as preservatives in food and beverages.

The most widely used methods for the determination of carboxylic acids include liquid chromatographic techniques based on anion-exchange, ion-exclusion and reversed-phase (RP) mechanisms. Among separative analysis, capillary electrophoresis has become a powerful technique owing to its high separation ability. Anion-exchange chromatography

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offers high sensitivity and reproducibility together with the possibility to simultaneously determine strong electrolytes. Whichever the separation technique, it is desirable to have the option of manipulating a variety of parameters affecting retention in order to control the quality of a separation. A review dealing with the main results reached in the prediction of retention data of carboxylic acids, focusing on the main separation methods, has recently been presented [1].

In a previous work [2] we studied the retention behavior of formic, acetic, propionic, lactic, pyruvic, oxalic, malonic, succinic, fumaric, maleic and tartaric acids on a highly cross-linked polystyrene–divinylbenzene anion-exchange column (IonPac AS4A-SC) using a carbonate-based buffer, and a retention model [3] was applied to the chromatographic data obtained.

The aim of this work was to characterize the retention of analytes according to analyte and stationary phase properties. For this reason other analytes such as glutaric, adipic, malic, mucic, *trans*-β-hydromuconic, *trans*,*trans*-muconic acids have also been added and studied with a column of different (higher) hydrophilicity, namely IonPac AS11. For a significant comparison, the eluent had the same composition as in Ref. [2]. The retention model [3] was applied to the chromatographic data for the IonPac AS11 column and the ion-specific selectivity constants (parameters of the equation of the model) obtained by non-linear regression were compared with the ones previously obtained. The results allowed several considerations on other effects affecting retention besides pure ion-exchange.

Since the improvement and the development of highly cross-linked substrate beads which minimize swelling (solving the problems related to high back-pressure on the column and to the headspace created when the column had water pumped through it after use with solvents) organic solvents are known to play an important role in ion chromatography (IC) with selectivity mediation for the ion-exchange process being their most important usage. Unfortunately, little work has been done on the use of organic solvents in IC. Rabin and Stillian [4] after the description of the main effects of organic solvent on the parameters involved in retention, considered the practical aspects on the use of organic solvents in IC. Examples of the use of methanol or acetonitrile in the eluent are provided for the following columns: OmniPac PAX-100, PAX-500, IonPac AS11 and CS14. In particular, the effect of the organic solvent on the selectivity of inorganic anions on the OmniPac PAX-100 column in the presence of hydroxide eluents has been previously evaluated by Stillian and Pohl [5]. Organic modifiers have been used in anion chromatography with crown ether-based stationary phase [6]. In that case, column capacity of macrocycle columns was increased by incorporation of the organic solvent in the eluent system. Examples on the use of modifiers in anion-exchange separations are available for poly(vinyl alcohol) gel-based columns [7], where the retention of nitrite ion has been evaluated. In that work it was pointed out that a nitrite ester is formed by the reaction with alcohol in the mobile phase and in the solvated layer on the stationary phase. Dumont et al. have performed the cation-exchange of *n*-alkylamine [8] in non-aqueous solvents, showing that in the presence of organic solvents, a plot of $\log t'_R$ vs. $\log H^+$ is linear with a slope close to -1, affirming that under these conditions the separation mechanism is pure ion-exchange and not partition based on hydrophobic attraction. Dumont and Fritz studied the non-aqueous cation-exchange of alkali metals in macroporous resin [9], observing that retention increases with the amount of organic modifiers in the eluent, due to ion-pair associations in a system of lower dielectric constant, and finally decreases if 100% organic solvent content is reached. This decrease has been explained considering that the larger radii of the analytes when they are solvated by the organic instead of water solvent molecules inhibits the approach of the cations to the resin and therefore cause a decrease of the retention times. The effect of methanol has also been evaluated in the ion-exclusion of hydrophobic aliphatic carboxylic acids [10], pointing out that the reduction of retention of carboxylic acids with organic solvent is due to the lipophilic property of the alkyl group in methanol rather than that of the hydrophilic property of the alcoholic hydroxyl group in methanol.

As a characterization of the retention behavior of carboxylic acids when an organic modifier is present

in the eluent, in this work, the effect of methanol and acetonitrile concentrations on the retention of the analytes has also been evaluated and compared.

2. Experimental

The apparatus consisted of a Model QIC ion chromatograph (Dionex, CA, USA) equipped with a single-piston DQP pump. The volume of the sample loop was 50 μl. Eluent conductivity was suppressed by an Anion MicroMembrane Suppressor (AMMS-II, Dionex). The regenerant solution was 12.5 m*M* sulfuric acid (Merck, Darmstadt, Germany). Chromatograms were recorded by a data system (AI-450, Dionex), also used for data collection and processing. The retention times represent the average values of at least three injections. Dead volume, 1.55 ml, was evaluated by the water dip. Chromatograms were recorded at ambient temperature. The analytical columns included a guard column Dionex AG11 (50×4 mm I.D.) and a separator column Dionex AS11 (250×4 mm I.D.). The resin of the columns is composed of 13 μm ethylvinylbenzene cross-linked with 55% divinylbenzene substrate, aminated with a latex containing hydroxyl groups. Ion exchange capacity is approximately 45 μequiv./column.

Eluents were prepared dissolving analytical grade $NaHCO_3$ and Na_2CO_3 (Merck) in distilled water obtained by a Milli-Q system (Millipore). Methanol and acetonitrile for HPLC were purchased from BDH (Dorset, UK). Formic and acetic acids were from Carlo Erba (Milan, Italy); propionic acid sodium salt, lactic acid disodium salt, pyruvic acid sodium salt, 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 and *trans,trans*-muconic acids were from Aldrich (Milwaukee, WI, USA).

The performance of the separator column used throughout this work will often be compared with that of the IonPac AS4A-SC column.

3. Results and discussion

Details of the two column packings are giving in Table 1. Particle diameter, substrate cross-linking and the type of functional group (alkanolammonium) in the ion-exchange latex are the same for both materials. Nevertheless, the molecule in the ion-exchange functionality and the latex parameters are different for the two columns. If only the cross-linking percentage is considered, the IonPac AS11 column had a higher % of cross-linking, consequently a much lower water uptake, when compared with the AS4A-SC column. The expected selectivity of AS11 differs from the AS4A-SC. As an example, two comparative chromatograms for some carboxylates obtained under the same mobile phase conditions with the IonPac AS4A-SC and IonPac AS11 columns are shown in Fig. 1.

For a significant comparison between the two columns, the same mobile phases have been prepared and studied: $[NaHCO_3]+[Na_2CO_3]=2.5$, 5.0, 6.0 and 7.5 m*M* (Fig. 2) For each concentration, pH values of 9.6, 9.9 and 10.3 have been studied (Fig. 3). At these pH values, dissociation of carboxylic groups for the acids is complete and therefore they can be separated as anionic species.

Table 1
Specifications of the columns IonPac AS4A-SC and IonPac AS11

	IonPac AS4A-SC	IonPac AS11
Particle diameter (nm)	13	13
Substrate X-linking (%)	55	55
Latex diameter (nm)	160	85
Latex X-linking (%)	0.5	6
Capacity per column	20	45
Functional group	Alkanol quaternary ammonium	Alkanol quaternary ammonium
Hydrophobicity	Medium–low	Very low

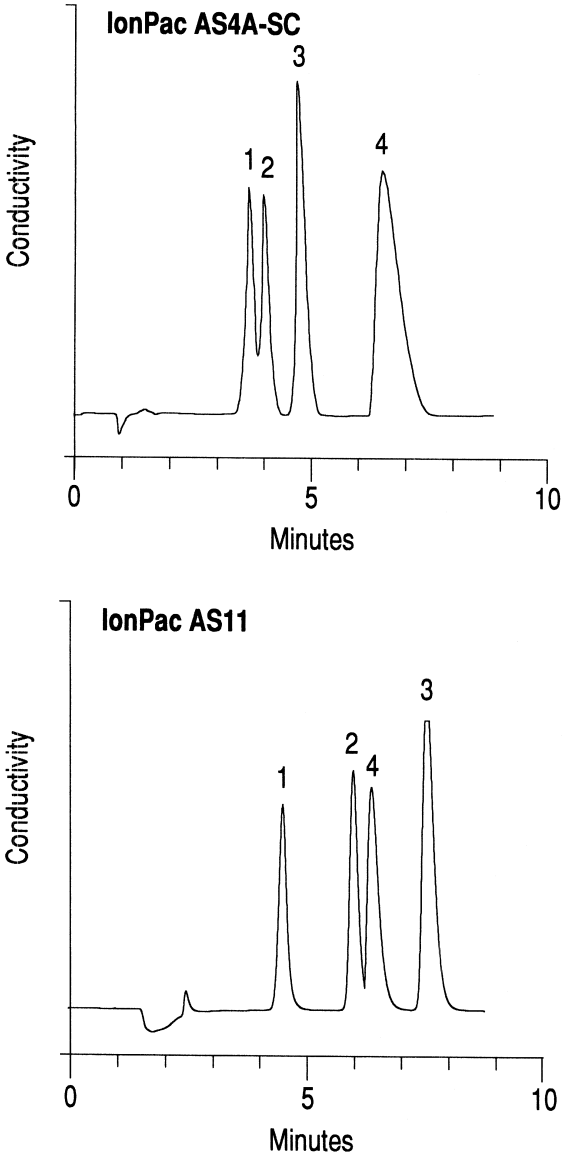

Fig. 1. Chromatograms of carboxylates obtained under identical mobile phase conditions ($[HCO_3^- + CO_3^{2-}] = 7.5$ m*M*, pH 10.1, flow-rate 1 ml/min) with the IonPac AS4A-SC and IonPac AS11 columns. Peaks: 1 = Malonic, 2 = maleic, 3 = oxalic, 4 = fumaric.

For each of the 12 mobile phases studied, the concentration of each eluent species was calculated and reported in Table 2. The results obtained for the retention behavior of carboxylic acids will be first shown for the AS11 column and then compared with those previously obtained with the AS4A-SC column. Standard deviations for the AS11 column are included in a ±4% range.

3.1. IonPac AS11 column

The elution order for dicarboxylic acids was: mucic<glutaric<succinic≤malic<adipic<tartaric< malonic<*trans*-β-hydromuconic<maleic<fumaric< oxalic<*trans*,*trans*-muconic acids.

The introduction of hydroxyl groups on the same molecular structure (succinic, malic and tartaric acids) leads to a higher retention factor (*k*). Interactions of analytes both with the mobile and stationary phase via hydrogen bonding, molecular size and hydration enthalpy, affecting charge density, have to be considered. Mucic acid, the analyte having more hydroxyl groups, is the less retained analyte. An increase of the number of carbon atoms on the same molecular structure (fumaric/*trans*-β-hydromuconic and oxalic/malonic) reduces *k*. The same happens with the homologous series from oxalic to glutaric, with adipic acid being the only exception.

The introduction of a double bond in the same structure (*trans*-β-hydromuconic→*trans*,*trans*-muconic) increases hydrophobic interaction with the polymeric matrix of the stationary phase.

The elution order for monovalent acids was: lactic<acetic<propionic≤formic<pyruvic acids. For formic, acetic and propionic acids there is no evidence of correlation between the retention order and structure. Comparing the retention of pyruvic and propionic acids (having the same number of carbon atoms) it can be noted that pyruvic is more retained due to the polar interaction between the carbonyl group of pyruvic acid and the hydroxyl groups of the resin.

3.2. Comparison between IonPac AS4A-SC and IonPac AS11 columns

The discussion reported hereafter will refer to the results obtained and shown in Table 3.

Generally, the *k* values for analytes are greater when the IonPac AS11 rather than the IonPac AS4A-SC column is used. This trend is not observed for succinic and fumaric acids, at low eluting power eluents. The higher retention on AS11 is due both to its higher capacity (see Table 1) and to its enhanced

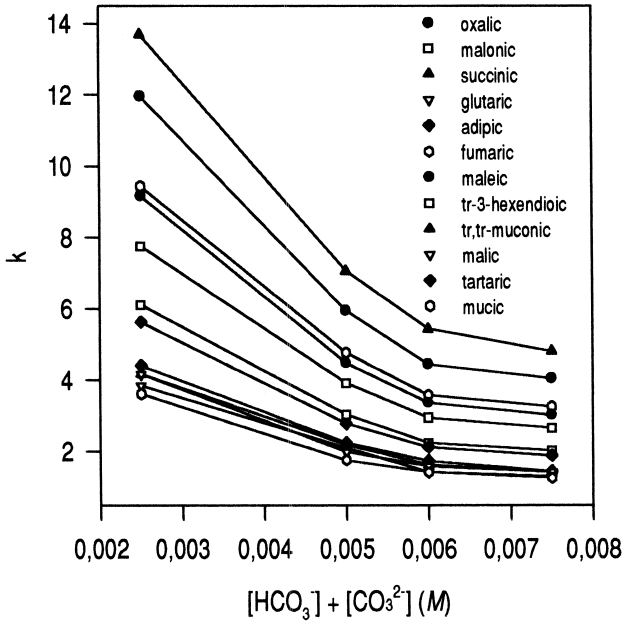

Fig. 2. Effect of total carbonate concentration on k for dicarboxylic acids. Stationary phase: IonPac AS11 (250×4 mm I.D.). Eluent: $NaHCO_3$–Na_2CO_3 (1:3), pH 10.35. $NaHCO_3 + Na_2CO_3$ as shown.

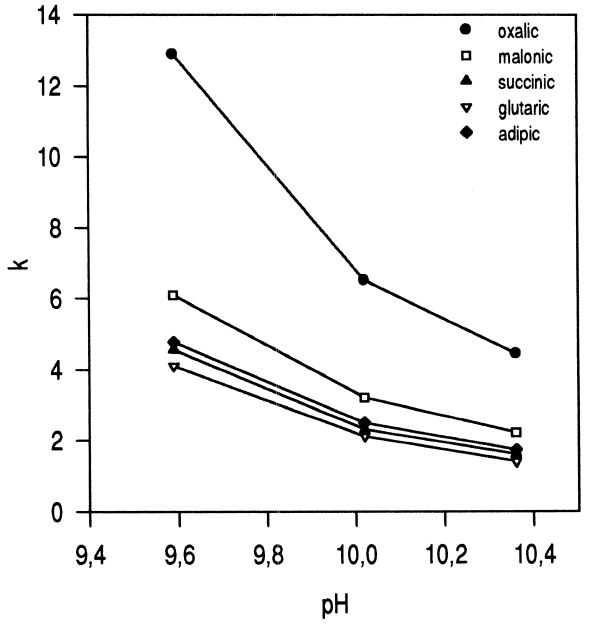

Fig. 3. Effect of eluent pH on retention of some dicarboxylic acids. Stationary phase: IonPac AS11 (250×4 mm I.D.). Eluent: $NaHCO_3 + Na_2CO_3 = 6$ mM, pH as shown.

hydrophilicity. In fact, since ion-exchange sites of AS11 column contain more hydroxyl groups than AS4A-SC, they are more effective in the interactions with carboxyl groups of the analytes mainly via the hydrogen bond. For monovalent ions the elution order is the same in both columns. For divalent anions, instead, the retention order is: AS11:succinic<tartaric<malonic<maleic<fumaric <oxalic acids and AS4A-SC:succinic<malonic <tartaric<maleic<oxalic<fumaric acids.

Significant inversion of elution order takes place between oxalic/fumaric acids and tartaric/malonic acids. While the retention of fumaric and tartaric acids does not change significantly on the two columns, the retention of malonic and, even more, oxalic acids is enhanced when the AS11 column is used. As a consequence of the reverse of the elution order, selectivity between malonic and succinic is enhanced on the AS11 column. Moreover, the differences among the homologous series oxalic, malonic, succinic, glutaric are magnified. Among them there is not a linear dependence log k vs. $n(CH_2)$.

The different selectivity exhibited by the two columns for maleic and oxalic suggests that different

Table 2
Concentration of eluent species (OH^-, HCO_3^- and CO_3^{2-}) at each mobile phase investigated, calculated according to $pK_w = 14$ and $pK_{2aH_2CO_3} = 10.25$

$[HCO_3^-]+[CO_3^{2-}]$ (*M*)	$[HCO_3^-]:[CO_3^{2-}]$	pH	(*M*)			
			$[H^+]$	$[OH^-]$	$[HCO_3^-]$	$CO_3^{2-}]$
0.0025	3:1	9.51	$3.09\cdot10^{-10}$	$3.24\cdot10^{-5}$	$2.11\cdot10^{-3}$	$3.85\cdot10^{-4}$
0.0025	1:1	9.88	$1.32\cdot10^{-10}$	$7.59\cdot10^{-5}$	$1.75\cdot10^{-3}$	$7.48\cdot10^{-4}$
0.0025	1:3	10.30	$5.01\cdot10^{-11}$	$2.00\cdot10^{-4}$	$1.18\cdot10^{-3}$	$1.32\cdot10^{-3}$
0.0050	3:1	9.50	$3.16\cdot10^{-10}$	$3.16\cdot10^{-5}$	$4.24\cdot10^{-3}$	$7.55\cdot10^{-4}$
0.0050	1:1	10.00	$1.00\cdot10^{-10}$	$1.00\cdot10^{-4}$	$3.20\cdot10^{-3}$	$1.80\cdot10^{-3}$
0.0050	1:3	10.30	$5.01\cdot10^{-11}$	$2.00\cdot10^{-4}$	$2.36\cdot10^{-3}$	$2.64\cdot10^{-3}$
0.0060	3:1	9.59	$2.57\cdot10^{-10}$	$3.89\cdot10^{-5}$	$4.92\cdot10^{-3}$	$1.08\cdot10^{-3}$
0.0060	1:1	10.02	$9.55\cdot10^{-11}$	$1.05\cdot10^{-4}$	$3.78\cdot10^{-3}$	$2.22\cdot10^{-3}$
0.0060	1:3	10.36	$4.36\cdot10^{-11}$	$2.29\cdot10^{-4}$	$2.62\cdot10^{-3}$	$3.38\cdot10^{-3}$
0.0075	3:1	9.63	$2.34\cdot10^{-10}$	$4.27\cdot10^{-5}$	$6.05\cdot10^{-3}$	$1.45\cdot10^{-3}$
0.0075	1:1	9.75	$1.78\cdot10^{-10}$	$5.62\cdot10^{-5}$	$5.70\cdot10^{-3}$	$1.80\cdot10^{-3}$
0.0075	1:3	10.45	$3.55\cdot10^{-11}$	$2.82\cdot10^{-4}$	$2.90\cdot10^{-3}$	$4.60\cdot10^{-3}$

retention mechanisms (e.g., additional interactions) occur on the two columns.

In contrast to what has been observed with the AS4A-SC column, the AS11 column does not show any difference in the selectivity between maleic (*cis* isomers) and fumaric (*trans* isomer) acids. Nevertheless, selectivity between succinic and maleic acid (same chemical structure, but double bond for maleic acid) is enhanced with the AS11 column.

The presence of hydroxyl groups on the same chemical structure (succinic–tartaric) increases *k* on the AS4A-SC column by about 6% and on the AS11 column by about 25%, according to the hydrophobic characteristics of the two columns.

Comparing lactic and pyruvic acids it can be seen that from a hydroxyl group to a ketonic group, retention increases on both columns, indicating that the main parameters involved in the retention are those related to the ion-exchange mechanism (e.g., ionic radius).

3.3. Effect of organic solvent on k

In this work the effect of the presence of organic solvents in the eluent has also been studied. Since the substrate is ethylvinylbenzene cross-linked with 55% divinylbenzene, packing of the IonPac AS11 is HPLC solvent compatible, 0–100%.

The two organic modifiers used are methanol and acetonitrile and they have been added to eluents containing $[NaHCO_3]+[Na_2CO_3]=5.0$ m*M*, $[NaHCO_3]:[Na_2CO_3]=1.1$. This concentration does not provide either short or long retention times, allowing one to modulate properly the elution power of the mobile phase when an organic solvent is added.

Methanol. The effect of CH_3OH % in the eluent on *k* has been studied at 7.5, 15, 25, 30% (v/v) (Fig. 4A). For all the analytes (except those containing unsaturations) *k* decreases up to 15% CH_3OH and then increases. The retention order of malic, tartaric and mucic acids with no methanol added to the mobile phase (mucic<malic<tartaric) changes when 15% CH_3OH is used (malic<mucic<tartaric). The *k* values of analytes containing unsaturations decrease with the increase in methanol content. Organic solvent decreases polarity of the eluent and hence the hydrophobic interactions between analytes and stationary phase. Conversely, among unsaturated acids, maleic acid increases its retention for methanol concentrations higher than 15%.

Acetonitrile. Due to the different polarity exhibited by acetonitrile, in order to compare the *k* values obtained with each solvent for each analyte, we studied percentages of 6, 12, 20, 24%, that represent isoeluotropic eluents [11] with those methanol-based, previously used.

The study on the effect of CH_3CN % in the mobile phase on *k* shows that, in contrast to methanol, retention values for every analyte decrease with

Table 3
Retention factors for the analytes studied. obtained with IonPac AS11 (this work) and IonPac AS4A-SC [2] columns

Analyte	Eluent (*M*)	$[HCO_3^-]:[CO_3^{2-}]$ (3:1)		$[HCO_3^-]:[CO_3^{2-}]$ (1:1)		$[HCO_3^-]:[CO_3^{2-}]$ (1:3)	
		k_{AS11}	$k_{AS4A-SC}$	k_{AS11}	$k_{AS4A-SC}$	k_{AS11}	$k_{AS4A-SC}$
Fumaric	0.0025	31.69	–	14.85	16.79	9.44	10.9
	0.0050	12.85	13.45	7.49	7.03	4.77	5.01
	0.0060	10.38	10.17	5.2	5.79	3.58	4.16
	0.0075	9.21	8.14	4.86	5.15	3.25	3.26
Maleic	0.0025	32.11	–	13.54	8.7	9.17	5.72
	0.0050	12.46	6.97	7.4	3.63	4.48	2.49
	0.0060	9.98	5.17	4.98	2.88	3.35	2.03
	0.0075	8.74	4.14	4.51	2.36	3.01	1.59
trans-β-Hydromuconic	0.0025	26.38	–	11.5	–	7.76	–
	0.0050	10.47	–	6.62	–	3.92	–
	0.0060	8.07	–	4.36	–	2.95	–
	0.0075	7.38	–	4.00	–	2.65	–
trans,*trans*-Muconic	0.0025	53.09	–	21.31	–	13.69	–
	0.0050	18.93	–	11.49	–	7.06	–
	0.0060	15.19	–	7.83	–	5.43	–
	0.0075	13.96	–	7.22	–	4.81	–
Oxalic	0.0025	41.26	–	19.07	11.12	11.96	7.03
	0.0050	16.54	8.65	9.6	4.54	5.95	3.16
	0.0060	12.91	6.56	6.52	3.66	4.45	2.58
	0.0075	11.88	5.33	6.05	2.93	4.05	2.03
Malonic	0.0025	22.89	–	8.99	7.92	6.11	5.01
	0.0050	8.21	6.15	4.75	3.25	3.03	2.29
	0.0060	6.10	4.83	3.22	2.62	2.23	1.85
	0.0075	5.89	3.76	2.99	2.17	2.02	1.44
Succinic	0.0025	14.25	–	6.09	7.88	4.17	4.94
	0.0050	5.79	6.24	3.37	3.25	2.18	2.26
	0.0060	4.56	4.93	2.31	2.67	1.62	1.82
	0.0075	4.02	3.77	2.13	2.2	1.42	1.46
Glutaric	0.0025	14.69	–	5.68	–	3.85	–
	0.0050	5.29	–	3.22	–	2.09	–
	0.0060	4.11	–	2.12	–	1.41	–
	0.0075	3.84	–	1.97	–	1.30	–
Adipic	0.0025	15.01	–	6.4	–	4.41	–
	0.0050	6.08	–	3.22	–	2.24	–
	0.0060	4.77	–	2.5	–	1.73	–
	0.0075	4.18	–	2.29	–	1.44	–
Malic	0.0025	15.48	–	6.27	–	4.17	–
	0.0050	5.58	–	3.5	–	1.98	–
	0.0060	4.33	–	2.29	–	1.59	–
	0.0075	4.04	–	1.9	–	1.41	–

(*Continued on next page*)

Table 3. (*Continued*)

Analyte	Eluent (*M*)	$[HCO_3^-]:[CO_3^{2-}]$ (3:1)		$[HCO_3^-]:[CO_3^{2-}]$ (1:1)		$[HCO_3^-]:[CO_3^{2-}]$ (1:3)	
		k_{AS11}	$k_{AS4A-SC}$	k_{AS11}	$k_{AS4A-SC}$	k_{AS11}	$k_{AS4A-SC}$
Tartaric	0.0025	19.00	–	8.18	8.64	5.62	5.42
	0.0050	7.25	6.62	4.35	3.58	2.77	2.54
	0.0060	5.96	5.21	3.07	2.92	2.11	2.11
	0.0075	5.17	4.09	2.69	2.67	1.88	1.64
Mucic	0.0025	11.98	–	5.21	–	3.61	–
	0.0050	4.56	–	2.67	–	1.75	–
	0.0060	4.02	–	2.03	–	1.41	–
	0.0075	3.27	–	1.70	–	1.25	–
Formic	0.0025	0.52	0.49	0.33	0.32	0.28	0.24
	0.0050	0.45	0.27	0.42	0.18	0.33	0.14
	0.0060	0.27	0.23	0.21	0.13	0.18	0.12
	0.0075	0.40	0.19	0.33	0.26	0.31	0.11
Acetic	0.0025	0.32	0.37	0.25	0.24	0.20	0.19
	0.0050	0.37	0.21	0.35	0.14	0.29	0.12
	0.0060	0.21	0.18	0.15	0.12	0.15	0.09
	0.0075	0.35	0.16	0.28	0.14	0.28	0.07
Propionic	0.0025	0.44	0.42	0.30	0.29	0.25	0.22
	0.0050	0.43	0.24	0.36	0.15	0.34	0.12
	0.0060	0.27	0.20	0.21	0.15	0.18	0.09
	0.0075	0.41	0.18	0.33	0.17	0.31	0.08
Lactic	0.0025	0.27	0.36	0.19	0.21	0.15	0.18
	0.0050	0.32	0.19	0.27	0.12	0.25	0.09
	0.0060	0.16	0.15	0.12	0.11	0.11	0.07
	0.0075	0.31	0.16	0.253	0.09	0.25	0.05
Pyruvic	0.0025	0.65	0.57	0.43	0.37	0.28	0.27
	0.0050	0.55	0.34	0.49	0.21	0.39	0.17
	0.0060	0.37	0.26	0.28	0.20	0.23	0.15
	0.0075	0.53	0.24	0.40	0.26	0.37	0.12

the increase of acetonitrile content. For analytes containing unsaturations, a change of selectivity for CH_3CN % higher than 20% takes place. In fact, elution order without organic solvent is: *trans*-β-hydromuconic<maleic<fumaric<*trans*,*trans*-muconic acids, while at CH_3CN % higher than 20% the elution order is: *trans*-β-hydromuconic<fumaric<*trans*,*trans*-muconic<maleic acids. As an example of the different behavior of acetonitrile in respect to methanol, the effect of CH_3CN on k for malic, tartaric and mucic acids is shown in Fig. 4B.

The effect of the organic solvent in the eluent is difficult to interpret and predict. Since organic solvent type and concentration determine the solvation of analyte, eluent ions and functional groups of the stationary phase, different degrees of solvation occur if methanol or acetonitrile is used. The solvated radii of each ion are involved in the ion-exchange mechanism and selectivity. Besides polarity, other characteristics such as hydrogen bonding properties and dispersive (London) forces also contribute to solvating strength. All of these factors are taken into account in the solubility parameter [12].

The organic modifiers in the eluent also affect the degree of dissociation of H_2CO_3 in the eluent, due to the changes of pK_{acid} values ($pK_2=10.25$ in water)

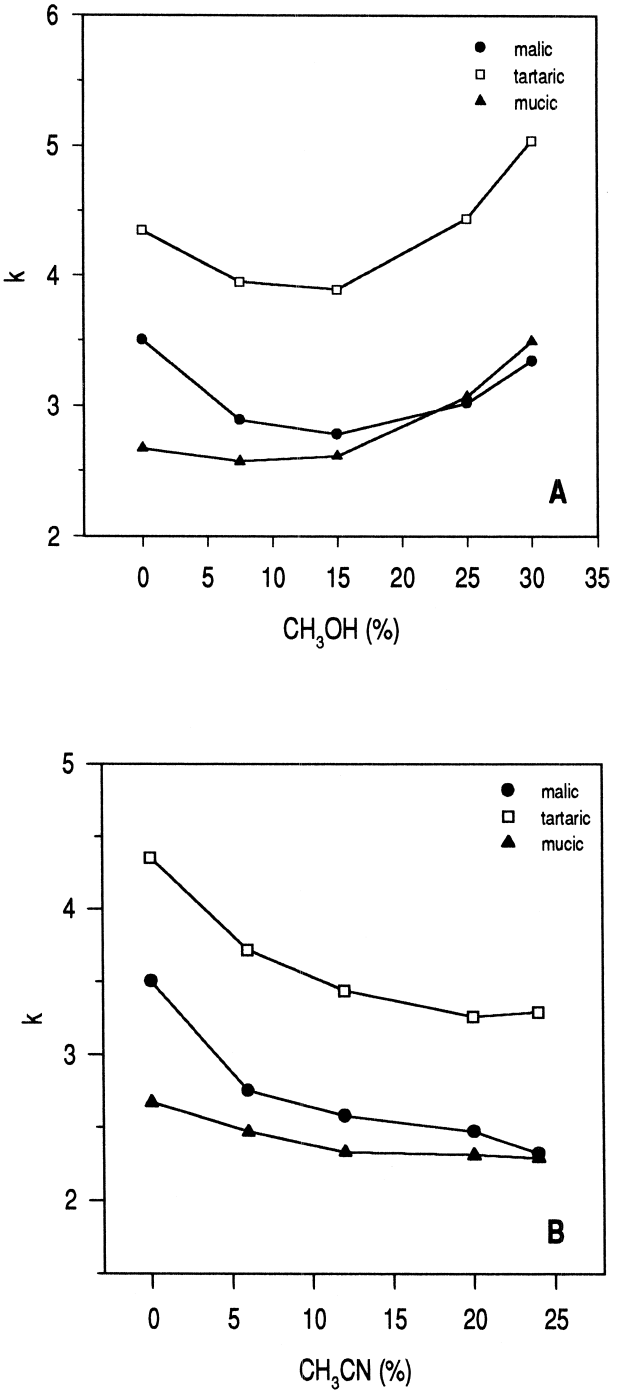

Fig. 4. Effect of organic solvents on retention factors for malic, tartaric and mucic acids. Stationary phase: IonPac AS11 (250×4 mm I.D.). Eluent: $NaHCO_3 + Na_2CO_3 = 5$ m*M*, aqueous pH 10.0. (A) Methanol, (B) acetonitrile.

with solvent composition [13]. Considering that the pH of the eluents studied ranged around 10.0, a different dissociation degree can occur when organic modifier type and concentration are changed, determining a different elution power. Nevertheless, this effect is not significant for the analytes studied, because their pK_a values are much lower than the eluent pH. In fact it has been reported [13] that
$pK_{2\text{succinic acid, } H_2O}=5.60$ and
$pK_{2\text{succinic acid, 50\% } CH_3OH}=6.71$,
$pK_{\text{formic acid, } H_2O}=3.73$ and
$pK_{\text{formic acid, 50\% } CH_3OH}=4.35$,
$pK_{\text{acetic acid, } H_2O}=4.77$ and
$pK_{\text{acetic acid, 50\% } CH_3OH}=5.54$.

In addition to the effects which are mobile phase-related, those stationary phase-related effects should also be considered. In the 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.

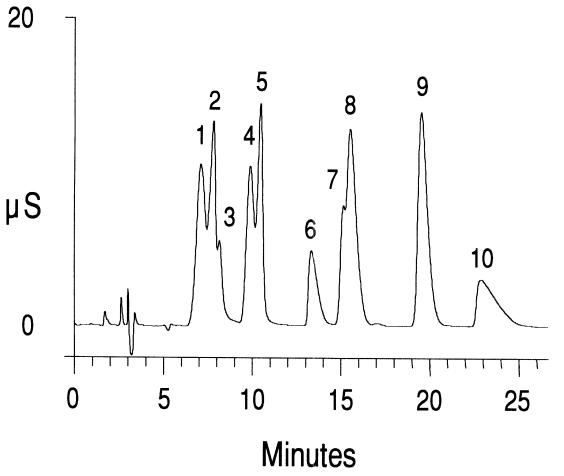

Fig. 5. Separation of the divalent carboxylic acids. Column: IonPac AS11 (250×4 mm I.D.). Eluent: $[NaHCO_3]+[Na_2CO_3]=$ 6.0 m*M*, $[NaHCO_3]:[Na_2CO_3]=1:1$, pH 10.0. Detection: suppressed conductivity. Peaks: 1=Mucic, glutaric; 2=malic, succinic; 3=adipic; 4=tartaric; 5=malonic; 6=*trans*-β-hydromuconic; 7=maleic; 8=fumaric; 9=oxalic; 10=*trans,trans*-muconic.

3.4. Optimization of separation of mixture of analytes

The optimization of the chromatographic method allowed one to identify the best chromatographic conditions for the separations of the analytes considered.

According to the results obtained during the experimental work, a mobile phase containing $[NaHCO_3]+[Na_2CO_3]=6.0$ m*M*, $[NaHCO_3]:[Na_2CO_3]=1:3$, pH 10.35 was first used to elute the mixture of the 12 dicarboxylic acids. Under these conditions, the separations of mucic, glutaric, malic, succinic and adipic acids which eluted as a single peak at about 4 min proved particularly difficult. Further coelution between tartaric and malonic acids occurred. An improvement of this separation has been obtained using a mobile phase containing $[NaHCO_3]+[Na_2CO_3]=6.0$ m*M*, $[NaHCO_3]:[Na_2CO_3]=1:1$, pH 10.0 (Fig. 5). Selectivity between tartaric and malonic acids has been enhanced, even if they are not baseline resolved. A decrease of resolution has been observed instead for maleic and fumaric acids, if compared with the previous eluent used. The separation of mucic–glutaric, malic–succinic can not be improved with weaker aqueous or hydroalcoholic eluents.

3.5. Retention model for calculation of chromatographic parameters

The retention model [3] has been applied to the chromatographic data for the IonPac AS11 column and the ion-specific selectivity constants (parameters of the equation of the model) obtained by non-linear regression were compared with the ones previously obtained with the IonPac AS4A-SC column [2].

The complexity of a retention model is a function of the complexity of the elution system involved. In order to facilitate the comprehension of the discussion, we report the equilibria on which the model is based:

$$y\text{R}_x\text{-E} + x\text{A}^{y-} \overset{K_{\text{A/E}}}{\rightleftharpoons} x\text{R}_y\text{-A} + y\text{E}^{x-}$$

ion-exchange equilibrium

$$\text{CO}_3^{2-} + 2\text{R-HCO}_3 \overset{K_{\text{CO}_3/\text{HCO}_3}}{\rightleftharpoons} \text{R}_2\text{-CO}_3 + 2\text{HCO}_3^-$$

inter-eluent ion-exchange equilibria

$$OH^- + R\text{-}HCO_3 \overset{K_{OH/HCO_3}}{\rightleftharpoons} R\text{-}OH + HCO_3^-$$

inter-eluention-exchange equilibria

and the final form of the model for divalent anionic species:

$$k_{A^{2-}} = \frac{V_{stat}}{V_0} \cdot K_{A^{2-}/HCO_3} \left(\frac{-p + \sqrt{p^2 + q}}{4K_{CO_3/HCO_3}[CO_3^{2-}]} \right)^2$$

where p and q represent:

$$p = |HCO_3^-| + K_{OH/HCO_3}[OH^-]$$

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

where Q = capacity of the column.

To calculate the values of selectivity constants, a non-linear regression method based on the Marquardt Levenberg algorithm was used. The values obtained for the IonPac AS11 column are reported in Table 4 where they are compared with those previously obtained for the IonPac AS4A-SC column [2].

The results allowed several considerations on other effects affecting retention besides pure ion-exchange.

The K_{A/HCO_3} values for the AS4A-SC column follow the retention order observed. The same does not happen in some cases with the IonPac AS11 column. The values of K_{A/HCO_3} in fact are related to the ion-exchange equilibrium of the analyte where purely electrostatic interactions are involved. When additional interactions are not present, or at least when they are negligible with respect to the electrostatic ones, the K_{A/HCO_3} values reflect the elution order of each analyte (AS4A-SC). Due to the characteristics of the stationary phase (different latex properties and different hydrophilicity) and of the analytes considered (unsaturations), additional interactions (not accounted by the purely electrostatic model) of hydrophobic and hydrogen-bond type can not be neglected in the AS11 column.

The K_{OH/HCO_3} values for the AS11 column are higher than the AS4A-SC and even greater than the values of K_{CO_3/HCO_3}. This is in agreement with the characteristics of the column used. In fact the IonPac AS11 column is more hydroxide-selective than the AS4A-SC column. It means that minor concentrations of OH^- in the eluent are required for the AS11 column to obtain comparable retention volumes in the two stationary phases. The variability of the K_{CO_3/HCO_3} values for AS11 has to be related to the additional interactions, besides ion-exchange, unaccounted for by the model.

The constants obtained can also be used to predict retention behavior and to choose eluent condition.

4. Conclusion

It has been shown that the hydrophobicity and ion-exchange capacity of the stationary phases have a great influence on the separation of carboxylic acids, especially for divalent and unsaturated anions.

Table 4
Ion specific and inter-eluent selectivity constants for divalent carboxylic acids in IonPac AS11 and IonPac AS4A-SC columns.

Analyte	K_{A/HCO_3}		K_{CO_3/HCO_3}		K_{OH/HCO_3}	
	AS11	AS4A-SC	AS11	AS4A-SC	AS11	AS4A-SC
Fumaric	18.2	107	9.33	15.3	17.9	8.10
Oxalic	17.2	58.8	6.30	12.6	22.4	8.60
Adipic	14.3	–	16.7	–	22.0	–
Maleic	13.7	67.7	6.48	19.5	27.0	6.30
Succinic	12.3	43.8	14.8	12.9	22.1	10.7
trans-β-Hydromuconic	11.6	–	6.81	–	21.7	–
trans,*trans*-Muconic	9.49	–	2.00	–	33.7	–
Tartaric	7.86	31.6	6.41	8.04	21.3	9.76
Mucic	6.68	–	9.20	–	17.9	–
Malonic	4.30	40.3	2.22	11.9	32.2	9.10
Glutaric	2.99	–	2.44	–	33.9	–
Malic	2.86	–	2.17	–	30.7	–

Some correlations have been observed between the retention order of analytes and their structure. The type of water miscible organic solvent (CH_3CN, CH_3OH) used as modifiers in the isoeluotropic eluent has an effect on retention as well as selectivity. Practical application of multiple eluent/analyte retention model to the determination of selectivity constants of the ionic species demonstrates some physicochemical aspects and also reflects interactions in the system.

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