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Anion chromatography on hydroxyethyl methacrylate-based sorbents

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ABSTRACT

The selectivity of sorbents plays an important role in the design of ion chromatographic separations. The selectivity of the hydroxyethylmethacrylate-based sorbent Tessek Separon HEMA-S 1000 Q-L was compared with those of other commonly used sorbents and the role of the eluent charge was investigated. The selectivity of this sorbent was found to be satisfactory for most commonly used ion chromatographic detection modes, including indirect photometry and both suppressed and non-suppressed conductivity detection.

INTRODUCTION

Ion chromatography (IC) is a widely used technique and the methods used and applications are described in several books [1–3] and reviews [4–6]. An interesting aspect of anion chromatography is the selectivity of separation. An understanding of the rules that influence selectivity is essential for optimizing the conditions for difficult separations. The most important factors influencing selectivity are eluent anion, its charge, eluent concentration and pH, as well as sorbent matrix, functional group and its content.

The selectivity of separations in anion chromatography was shown to be determined essentially by the above factors. The influence of the character of the functional group [7–9], spacer length [10] and character of the support [11] on selectivity has been studied earlier. Retention models for ion chromatography were evaluated and only partial agreement was found between theoretical models and experimental data [12,13]. There is still much to be investigated, the problem of the selectivity of mono- and divalent anions having been neglected so far.
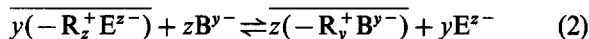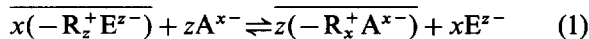
The potential of the hydroxyethyl methacrylate-based Tessek Separon HEMA-S 1000 Q-L sorbent (Tessek, Prague, Czech Republic) in Alltech universal anion columns for both suppressed and single-column ion chromatography has been extensively studied by Saari-Nordhaus and co-workers [14–16]. During the development and production of this sorbent, an extreme dependence of sorbent selectivity on minor variations in the matrix properties was observed. In this work, the selectivity of this sorbent was compared with those of other common sorbents based on published data [12]. The influence of the matrix surface character and eluent charge on selectivity was also studied. Utilization of selectivity changes is demonstrated in several applications.

THEORY

Equations describing retention and selectivity in IC can be derived from ion-exchange equilibrium and chromatographic theory [8,17,18] for separa-

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tion of anions A^{x-} and B^{y-} on a sorbent with functional groups R^+ with eluent anion E^{z-}:

$$x(\overline{-R_z^+ E^{z-}}) + zA^{x-} \rightleftharpoons z(\overline{-R_x^+ A^{x-}}) + xE^{z-} \quad (1)$$

$$y(\overline{-R_z^+ E^{z-}}) + zB^{y-} \rightleftharpoons z(\overline{-R_y^+ B^{y-}}) + yE^{z-} \quad (2)$$

where the overbars refer to species bound in the stationary phase. The capacity factor, k_A, with certain assumptions, can be expressed in the form

$$k_A = K_{xE}^{zA^{1/x}} \left(\frac{Q_g / z}{c_E} \right)^{x/z} \frac{m_s}{V_m} \quad (3)$$

After combining eqns. 1 and 2, an equation for the dependence of the separation factor on the capacity factor can be derived [8]:

$$\alpha_{A,B} = (K_{xB}^{yA})^{1/x} k_A^{(x-y)/x} \left(\frac{V_m}{m_s} \right)^{(x-y)/x} \quad (4)$$

where K_{xB}^{yA} and K_{xE}^{zA} are selectivity coefficients, $\alpha_{A,B}$ is the separation factor, Q_g is the specific ion-exchange capacity of the sorbent, c_E is the eluent concentration and m_s and V_m are the mass of sorbent and mobile phase volume in column, respectively.

In logarithmic coordinates, linear dependences of capacity factor on eluent concentration or sorbent capacity and of selectivity factor on capacity factor can be derived from eqn. 3. Based on eqn. 4, diagrams for the dependence of selectivity coefficient on chloride capacity factor are well suited for comparison of the selectivities of different sorbents. The slopes of these dependences should be related to the charges of the eluting and eluted anions. Nevertheless, these models do not consider the common situation where both eluent and analyte anions can be present in different ionic states. For the situation where multiple eluent anions are present, equations were derived by Hoover [19] and used by Jenke and Pagenkopf [13,20] and Maruo et al. [21]. However, simplifying assumptions had to be made and the fit of the models was usually unsatisfactory. Consideration of all the equilibria present (dissociation of eluent anion, dissociation of analyte anion, dissociation of sorbent) will give a very complicated system of equations. For this reason, only a qualitative approach was adopted in this study.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used for selectivity measurements consisted from Model 1350 soft start pump (Bio-Rad Labs., Richmond, CA, USA), a Model 7125 injection valve (Rheodyne, Cotati, CA, USA), and a Model 731.87 spectrophotometer (Knauer, Berlin, Germany). An APEX version 2.0 PC computer-based chromatographyic integrator (DataApex, Prague, Czech Republic) was used for data collection and evaluation. A modular IC system, consisting of Model 325 HPLC pump, Model 330 column heater and Model 320 conductivity detector (Alltech, Deerfield, IL, USA), a Model 9125RV injection valve (Rheodyne) and a Model 4400 ChromJet integrator (Spectra-Physics, Santa Clara, CA, USA), was employed.

Columns

Compact glass cartridge (CGC) columns (70 or 150 × 3.3 mm I.D.) were packed by an aqueous slurry technique in the laboratory and used with an appropriate column holder (Tessek). The sorbents used were Tessek Separon HEMA-S 1000 Q-L (10 μm) (Tessek) with a 0.15 mmol/g ion-exchange capacity and an experimental batch of Tessek Separon HEMA-BIO 1000 Q-L (Tessek) with a 0.1 mmol/g ion-exchange capacity. PEEK Alltech Universal Anion columns (150 × 4.6 mm I.D.) (Alltech) packed with the first sorbent were used in applications.

Eluents and analytes

Potassium hydrogenphthalate (analytical-reagent grade) (Merck, Darmstadt, Germany) and distilled, deionized water were used for the preparation of eluents. Phthalic acid was used for the preparation of eluents with pH 4.0. The pH was adjusted with sodium hydroxide (analytical-reagent grade) (Merck) with the use of an Acidimeter 333 (Druopta, Prague, Czech Republic) equipped with a Crytur 01-29 combined glass pH electrode (Monokrystaly, Turnov, Czech Republic) unless stated otherwise. The eluents were filtered before use through a Schleicher & Schüll (Dassel, Germany) ME 25 membrane filter with 0.45-μm pores. Each column was equilibrated with eluent for several hours. EZ-LUTE buffer concentrates (Alltech) were used for the preparation

of other eluents; the pH was adjusted with lithium hydroxide.

As analytes, typical inorganic anions were selected. Fluoride, chloride, bromide and iodide represented the halides, nitrite and nitrate monovalent oxoanions, sulphate and thiosulphate divalent ions and sulphite and phosphate ions with charge dependent on pH.

Analytical-reagent grade salts were used. Stock standard solutions containing 1000 mg/l of anion were prepared and appropriate working standard solutions were prepared by dilution with distilled, deionized water. Sodium hydrogensulphite was dissolved in 0.2% formaldehyde solution owing to its instability in aqueous solution [22].

RESULTS AND DISCUSSION

Two types of selectivity can be distinguished in IC [10]: selectivity for anions of the same charge and that for anions with different charges. The selectivity of a sorbent for the separation of anions with the same charge can be compared on the basis of the separation factor (proportional to the selectivity coefficient). The elution sequence of monovalent anions $F^- < Cl^- < NO_2^- < Br^- < NO_3^- < I^-$ does not vary, with only slight shifts in the relative retentions of the halides and oxoanions. The sorbents studied did not show the problems with the resolution of the NO_2^-–Br^- and Br^-–NO_3^- pairs that sometimes occur on agglomerated sorbents. The various sorbents are compared on basis of their separation factors for nitrate–chloride in Fig. 1.

For the separation of anions with different charges, the separation factor is a function of retention and therefore it could not be directly compared. The graphs of dependences according to eqn. 4 can serve for an illuminating comparison of the selectivities of mono- and divalent ions.

Five commercially available and one research sample sorbent (Table I) were compared for selectivity in phthalate eluent, based on measured [HEMA-S 1000 Q-L (Table II) and HEMA-BIO 1000 Q-L (Table III)] and literature [12,20] data. Relative retentions at pH 6.0 are plotted in Fig. 2 against the capacity factor of chloride to provide a correction for different sorbent capacities. At pH 6.0 with 65% phthalate as divalent anion [20] the silica-based Vydac 302 IC shows very low selectivity for
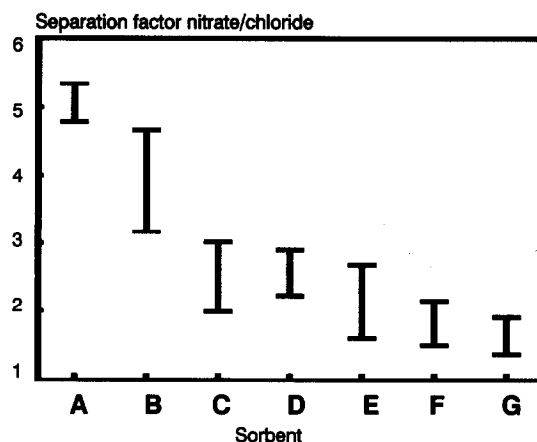

Fig. 1. Comparison of separation factors of nitrate and chloride on different sorbents. The data based on refs. 12 and 20 and this work were measured in phthalate eluents at concentrations from 1 to 4 mmol/l and pH 4.0–6.0 (for the Dionex column pH 8.5 was adjusted with 4 mM borate). (A) Dionex AS-1 agglomerated sorbent; (B) HEMA-BIO 1000 Q-L; (C) HEMA-S 1000 Q-L; (D) Waters IC Pak A; (E) Hamilton PRP-X-100; (F) Wescan 269-001 silica-based sorbent; (G) Vydac 302 IC.

monovalent and a high affinity for divalent anions. The sulphate is eluted after iodide even at very low capacity factors of chloride, where fluoride is incompatible with the silica matrix. The polystyrene-based PRP-X 100 resin has a similar character, but the selectivity for monovalent ions is higher and sulphate is usually eluted between nitrate and iodide. The IC Pak A and HEMA-S 1000 Q-L hydroxyethyl methacrylate-based sorbents behave in an almost identical manner with high selectivity for monovalent anions and sulphate eluting before or after nitrate. HEMA-BIO 1000 Q-L shows an extremely high selectivity for monovalent anions and a low retention for sulphate. The agglomerated sorbent AS-1 shows a high selectivity for nitrate, but sulphate is eluted before or after nitrate. Limited data were available for this column; the pH was adjusted by addition of 4 mM borate. The published hydrogensulphite retention times [12] are almost identical with those reported for sulphate, and so are not labelled in Fig. 2. Extreme care must be used to avoid the oxidation of hydrogensulphite to sulphate.

The character of the matrix exhibited a larger effect than different substituents on the amino functional group. When an additionally hydrophilized HEMA-BIO matrix was used, the retention of

TABLE I

COMPARISON OF SORBENT PROPERTIES

Sorbent	Matrix	Capacity
Hamilton PRP-X 100	Styrene–divinylbenzene	0.19 mmol/g
Vydac 302 IC	Silica	0.1 mmol/g
Waters IC Pak A	Methacrylate	0.03 mmol/ml
HEMA-S 1000 Q-L	Hydroxyethyl methacrylate	0.15 mmol/g
HEMA-BIO 1000 Q-L	Hydroxyethyl methacrylate	0.1 mmol/g
Dionex AS-1	Styrene–divinylbenzene (agglomerated)	0.05 mmol/g

TABLE II

DEPENDENCE OF RETENTION TIMES (min) ON ELUENT CONCENTRATION AND pH

Column, HEMA-S 1000 Q-L (10 μm) (70 × 3.3 mm I.D.); eluent, hydrogenphthalate; flow-rate, 0.5 ml/min; indirect UV detection.

Ion	Eluent concentration (mmol/l)								
	1 (pH 4)	2 (pH 4)	4 (pH 4)	1 (pH 5)	2 (pH 5)	4 (pH 5)	1 (pH 6)	2 (pH 6)	4 (pH 6)
Fluoride	3.76	2.76	2.00	2.45	1.80	1.48	2.05	1.75	1.28
Chloride	7.58	5.14	3.45	4.75	3.35	2.46	3.81	3.21	2.18
Bromide	13.36	8.68	5.53	8.51	5.78	4.06	6.78	5.65	3.63
Iodide	41.06	26.01	15.16	27.76	18.48	12.45	21.52	18.97	10.86
Nitrite	10.19	6.65	4.04	7.03	4.76	3.38	5.76	4.57	3.25
Nitrate	17.13	11.01	6.85	11.23	7.51	5.27	9.09	7.30	4.91
Sulphite	5.38	3.71	2.52	3.31	2.31	1.81	2.74	2.18	1.64
Sulphate	79.91	30.16	13.76	23.30	10.49	5.43	12.77	7.22	3.71
Thiosulphate	148.00	64.26	24.87	43.32	20.10	9.70	25.36	14.42	6.75
Phosphate	3.85	2.78	1.94	2.44	1.76		2.54	1.98	1.36
Void	0.66	0.69	0.68	0.66	0.72	0.74	0.68	0.79	0.75
System	52.32	29.76	16.05	43.06	26.16	15.54			19.66

TABLE III

DEPENDENCE OF RETENTION TIMES (min) ON ELUENT CONCENTRATION AND pH

Column, HEMA-BIO 1000 Q-L (10 μm) (70 × 3.3 mm I.D.); eluent, hydrogenphthalate; flow-rate, 0.5 ml/min; indirect UV detection.

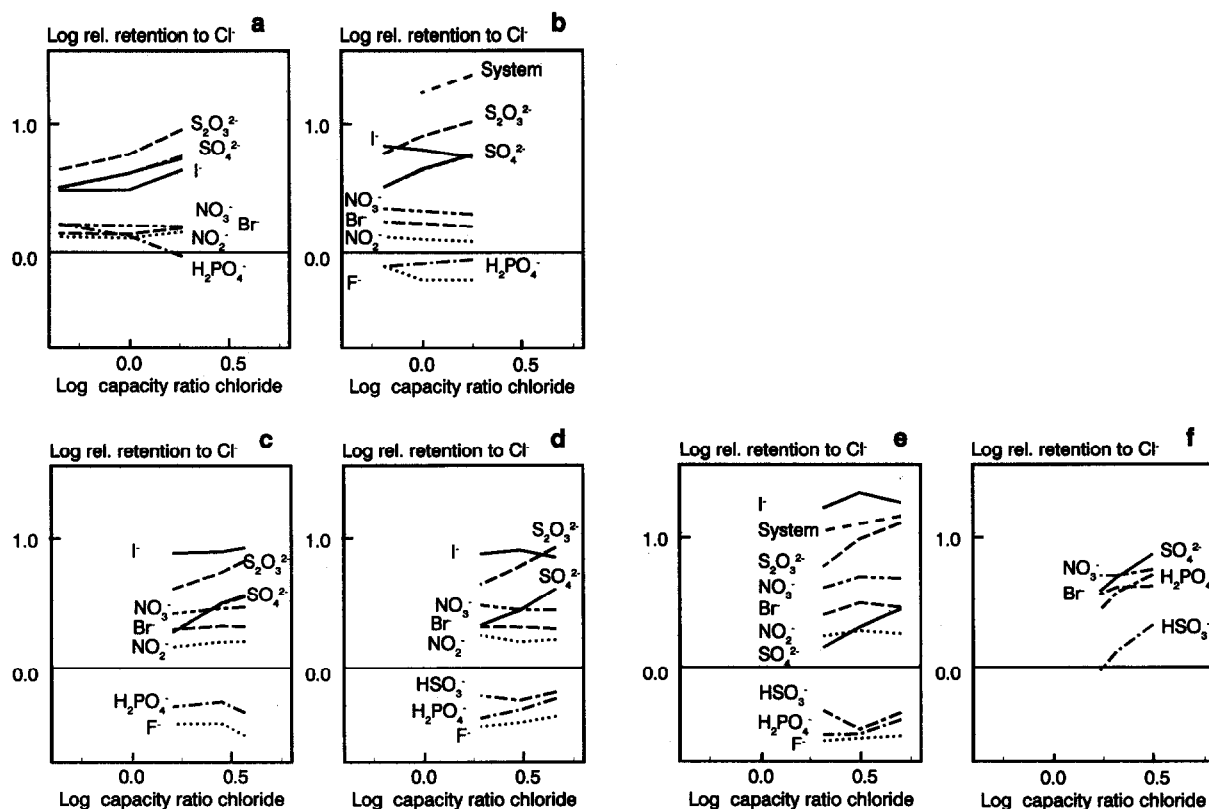| Ion	Eluent concentration (mmol/l)								
	1 (pH 4)	2 (pH 4)	4 (pH 4)	1 (pH 5)	2 (pH 5)	4 (pH 5)	1 (pH 6)	2 (pH 6)	4 (pH 6)
Fluoride	2.83	2.07	1.50	1.88	1.48	1.23	1.71	1.43	1.18
Chloride	6.60	4.41	2.84	4.35	3.03	2.24	4.03	3.03	2.27
Bromide	14.70	9.53	5.66	10.37	6.72	4.61	10.02	7.71	4.46
Iodide	70.99	46.43	25.41	58.30	33.60	22.75	57.36	46.85	24.21
Nitrite	9.43	6.06	3.98	6.93	4.54	3.20	6.59	5.04	3.34
Nitrate	21.09	13.01	7.53	15.40	9.52	6.40	16.00	11.59	6.68
Sulphite	3.98	2.76	1.88	2.50	1.91	1.50	2.22	1.55	1.47
Sulphate	55.11	24.84	8.77	15.61	7.55	4.02	9.74	5.32	2.88
Thiosulphate		92.05	24.98	60.10	27.53		40.48	21.59	9.31
Phosphate	2.97	2.12	1.53	1.93	1.51	1.21	2.05	1.48	1.23
Void	0.67	0.69	0.68	0.65	0.72	0.73	0.67	0.74	0.74
System	31.36	17.73	10.39		17.80	9.88	45.72		16.60

Fig. 2. Relative retention of anions to chloride plotted against capacity factor of chloride in phthalate eluent (pH 6.0) (concentrations 1.0, 2.0 and 4.0 mmol/l). (a) Vydac 302 IC; (b) Hamilton PRP-X-100; (c) Waters IC Pak A; (d) HEMA-S 1000 Q-L; (e) HEMA-BIO 1000 Q-L; (f) Dionex AS-1 (pH 8.5, adjusted with 4 mM borate).

divalent (sulphate) anions decreased dramatically, so sulphate eluted before nitrate and sometimes even with chloride. Both the HEMA Q-L have the same functional group and differ only in the matrix treatment. The HEMA-S matrix is additionally cross-linked with monomers, whereas HEMA-BIO is treated with epichlorohydrin and hydrolysed. HEMA-BIO exhibits a higher hydrophilicity towards proteins, but the increase in polarity for small molecules such as toluene (in methanol–water) or benzyl alcohol (in water) is very small when compared with the difference from hydrophilized silica sorbents. Also, one would expect doubling of the content of hydroxyl groups due to the hydrophilization, but in reality there is a decrease of about 30%. The explanation probably lies in the polymer structure of HEMA surface: epichlorohydrin apparently causes some cross-linking of the polymer chains, resulting in more hydroxyl groups oriented to the

solvent (increasing the hydrophilicity for proteins) but not changing significantly the polarity for small molecules, which penetrate into the polymer matrix.

The influence of pH is demonstrated in Fig. 3, where the separation factors of nitrate–chloride and sulphate–chloride are plotted against the capacity factor of chloride. Similar pictures are obtained with all the other sorbents compared. The selectivity for monovalent ions increases and the retention of divalent anions decreases in all instances with increasing pH (increase in the ratio of divalent phthalate to monovalent hydrogenphthalate). It can be seen from Fig. 3 that at the same k_{Cl^-} the retention of sulphate will decrease with increase in pH and thus in proportion to the divalent phthalate in the eluent. The divalent phthalate is therefore more efficient eluent for divalent sulphate than for monovalent ions.

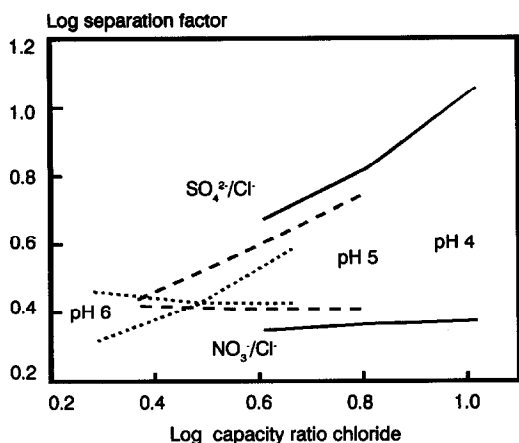This can be confirmed by the difference in selec-

Log separation factor

Fig. 3. Separation factor of nitrate and sulphate to chloride plotted against capacity factor of chloride in phthalate eluent at different pH values on a HEMA-S 1000 Q-L (10 μm) column (70 × 3.3 mm I.D.).

tivity observed when using mono- and divalent eluents of comparable eluting power, e.g., with the same retention for the nitrate anion. The retention of divalent anions increased substantially with the use of monovalent eluents, so the resulting selectivity approached that commonly encountered with silica-based sorbents, where the monovalent anions are either not resolved or sulphate elutes very late (Fig. 4).

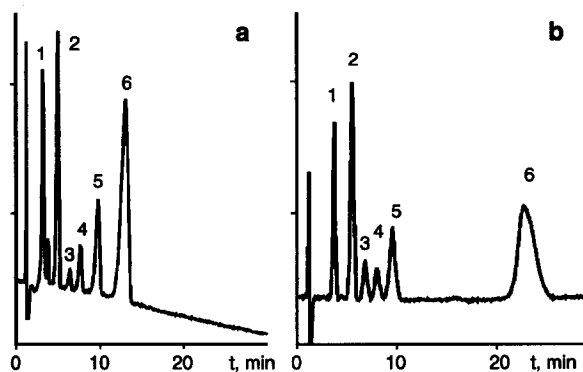Two factors are considered to influence the selec-

tivity: loss of free energy on the formation of a functional group–analyte anion pair and an electroselectivity, where the ion with higher charge is more strongly bound. The electroselectivity, however, needs two sites on the sorbent to interact with divalent ions simultaneously. The electroselectivity theory [23] supposes that the lower affinity of some sorbents to divalent anions is caused by hindered access to the formation of an ion pair with two sites simultaneously. From the electroselectivity theory in IC one could expect that the selectivities for ions with the same and with different charges would be independent, so a sorbent can show a high selectivity both for monovalent and for mono- and divalent anions. With the exception of the Dionex sorbent, the results compared here do not support this conclusion, however. The reason may be that this sorbent has a high density of ion-exchange groups concentrated in small latex particles, attached to an inert core, whereas the other sorbents have a low content of functional groups distributed on a large surface.

The rules of the influence of pH are complicated, as the pH influences the dissociation of the eluent anion, sorbent functional group and analyte anion. The effective concentration and charge of these anions change with pH in different manners according to their dissociation constants. In addition to the known shift in the retention of phosphate from a position before chloride at pH < 6 to a position after
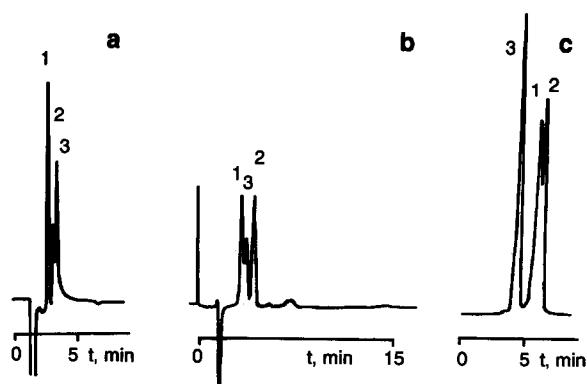

Fig. 4. Comparison of selectivity in divalent and monovalent eluents. Column, HEMA-S 1000 Q-L (10 μm), PEEK (150 × 4.6 mm I.D.); flow-rate, 1 ml/min; detection, indirect spectrophotometry at 254 nm. Eluent: (a) 0.5 mM sulphosalicylate (pH 5.4); (b) 3.0 mM salicylate (pH 5.8). Peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = bromide; 5 = nitrate; 6 = sulphate.

Fig. 5. Separation of fluoride in the presence of acetate and formate. Column, HEMA-S 1000 Q-L (10 μm) (150 × 3.3 mm I.D.); flow-rate, 0.5 ml/min. (a, b) Indirect spectrophotometry detection at 254 nm, eluent 0.5 mM sulphosalicylate–5% MeOH at (a) pH 3.1 and (b) pH 4.5; (c) suppressed conductivity detection, eluent 1.5 mM sodium carbonate. Peaks: 1 = acetate; 2 = formate; 3 = fluoride.
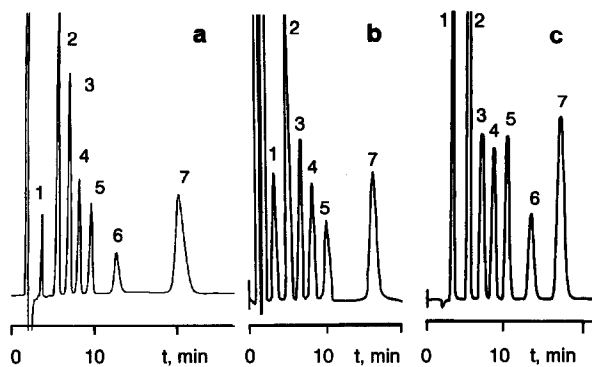
Fig. 6. Comparison of commonly used eluents on an Alltech universal anion PEEK column (150 × 4.6 mm I.D.). Flow-rate, 1.0 ml/min; conductivity detection. Eluent: (a) 5 mM p-hydroxybenzoate (pH 7.9); (b) 4 mM phthalate (pH 4.5); (c) 2.8 mM hydrogencarbonate–2.2 mM carbonate (suppressed conductivity detection). Peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = bromide; 5 = nitrate; 6 = phosphate; 7 = sulphate.

Fig. 7. Separations of late-eluting anions. (a) Column, Alltech universal anion PEEK (150 × 4.6 mm I.D.); eluent, 8 mM ethylenediaminetetraacetic acid, disodium salt; flow-rate, 0.5 ml/min; conductivity detection. Peaks: 1 = phosphate; 2 = pyrophosphate; 3 = tripolyphosphate. (b) Column, Alltech universal anion PEEK (150 × 2.1 mm I.D.); eluent, 1.5 mM sodium octanesulphonate; flow-rate, 0.5 ml/min; conductivity detection. Peaks: 1 = chloride; 2 = nitrate; 3 = iodide; 4 = thiocyanate; 5 = sulphate; 6 = thiosulphate.

nitrate at pH > 7.5, caused by its transition from mono- to divalent form, as another example Fig. 5 shows the separation of fluoride from formate and acetate peaks. At high pH fluoride is eluted before acetate and formate, whereas on decreasing the pH to 4.5 fluoride elutes between them and at pH 3.1 elutes after them, as the dissociation of weaker organic acids is suppressed.

The selectivity of HEMA-S 1000 Q-L sorbent was found to be acceptable in all commonly used IC systems [14], as is shown in Fig. 6. With the selection of a suitable eluent, the selectivity can be influenced to separate even difficult mixtures such as late-eluting anions or polyphosphates. The divalent ethylenediaminetetraacetate disodium salt eluent proved to be an efficient eluent for polyphosphate in food additives (Fig. 7a), whereas monovalent octanesulphonate permitted the elution of late-eluting monovalent iodide and thiocyanate before sulphate and thiosulphate (Fig. 7b).

CONCLUSIONS

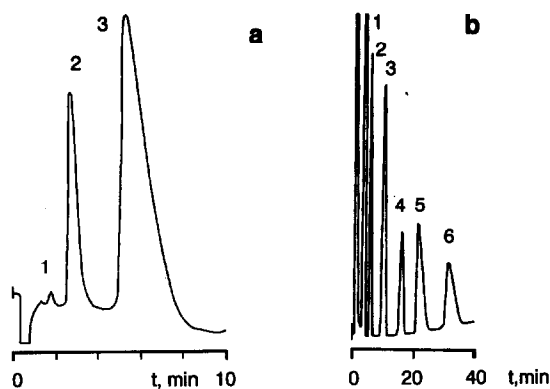Some dependences of retention on chromatographic conditions have been evaluated and the matrix character and eluent charge were shown to play significant roles in the selectivity of IC separations. The selectivities of commonly used sorbents were compared for the separations of mono- and divalent anions on the basis of the separation factor–capacity factor dependence. The selectivity for monovalent ions increases from silica-based through non-polar and polar polymers to agglomerated sorbents. A higher selectivity for monovalent ions seems to be connected with a lower affinity for divalent anions. Monovalent eluents seem to give a lower selectivity for monovalent and a higher retention for divalent anions. The influence of pH can be estimated from dissociation constants of eluent and analyte anions.

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