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Journal of Chromatography A, 884 (2000) 61–74

JOURNAL OF CHROMATOGRAPHY A

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Retention behaviour of strong acid anions in ion-exclusion chromatography on sulfonate and carboxylate stationary phases

John O'Reilly[a], Philip Doble[a], Kazuhiko Tanaka[b], Paul R. Haddad[a,*,1]

[a]*School of Chemistry, University of Tasmania, GPO Box 252-75, Hobart, Tasmania 7001, Australia*

[b]*National Industrial Research Institute of Nagoya, Nagoya 462-8510, Japan*

Abstract

Some factors influencing the retention of strong-acid anions on ion-exclusion columns were investigated using columns with sulfonate and carboxylate functional groups. The nature of the functional group on the resin, the eluent pH and the eluent ionic strength all significantly affected the retention and separation of these analytes. Retention was observed for all strong-acid anions over the eluent pH range 2.2–5.7 and increased with both decreasing eluent pH and increasing eluent ionic strength. Some separation of strong-acid anions was possible when using a resin with carboxylate functional groups. It has also been demonstrated that strong-acid anions are poor markers of column void volume for ion-exclusion chromatography. A more accurate value was obtained using the neutral polymeric material dextran blue. When using eluents of low ionic strength, poor or fronted peak shapes were observed. A mechanism for these observations is proposed that relates the shape to ionic strength changes across the peak. A system peak was encountered under most experimental conditions. The properties of this peak are discussed and a cause for the system peak postulated.

Keywords: Retention mechanisms; Inorganic anions; Organic acids; Strong acids

1. Introduction

Ion-exclusion chromatography is used widely for the determination of weak acids (usually carboxylic acids) [1,2] and separation is achieved using ion-exchange resins carrying functional groups with the same charge as the analytes. The mechanism of this separation is based on the proportion of the analyte acid present in the neutral form. Neutral molecules can permeate into the pores of the resin and are therefore retained, while ionic species are excluded from this phase by electrostatic repulsion from the functional groups on the resin. Therefore, the separation selectivity is governed primarily by the pK_a of the acid [2]. Tanaka et al. [3] have recently shown that some strong acids can be separated on a carboxylate ion-exclusion resin using a solution of a weak carboxylic acid (tartaric acid) as eluent. This result is somewhat contradictory to the above separation mechanism of ion-exclusion chromatography in which all strong-acid anions should be totally excluded from the resin and should be eluted together at the void (unretained) volume of the column [2,4].

The primary aim of the present study was to investigate the retention behaviour of strong-acid anions on ion-exclusion chromatography columns under conditions where the functional group on the resin, the nature of the eluent acid anion, eluent pH

*Corresponding author. Tel.: +61-3-6226-2179; fax: +61-3-6226-2858.

E-mail address: paul.haddad@utas.edu.au (P.R. Haddad)

[1]http://www-ssg.chem.utas.edu.au/

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01312-6

and eluent ionic strength were all varied. Secondary aims were to investigate the properties of a system peak observed throughout the study, and explain the cause of fronted and poor peak shapes observed when using low ionic strength eluents.

2. Experimental

2.1. Instrumentation

The pump used was a Waters Model 590 HPLC pump (Waters, Milford, MA, USA) which was set at 1.00 ml/min throughout this work. Sample injection was performed using a Waters 717 Plus Autosampler. A 100-μl injection volume was used unless otherwise specified. The column heater was a Waters temperature control module (TCM) set at 35°C. The detectors used were a Waters Model 484 UV tunable absorbance detector set at 210 nm for all UV-absorbing anions and 190 nm for methanol and a Waters Model 430 conductivity detector, connected in series. In order that chromatograms recorded on both UV and conductivity detectors could be compared directly, the dead volume between them was calculated and an appropriate correction introduced to account for the time lag. All chromatographic data were collected using a Waters Maxima 820 chromatographic workstation.

2.2. Columns

Two different columns were used: a Tosoh TSKguardgel SP-5PW polymethacrylate column with sulfonate functional groups and a Tosoh TSKguardgel CM-5PW polymethacrylate column with carboxylate functional groups (Tosoh, Tokyo, Japan). Both columns were 300 mm long and had an internal diameter of 7.8 mm, ion-exchange capacity of 0.1 mequiv./ml, a particle size of 5 μm, and pore size of 1000 Å. The CM-5PW column is functionalised with methoxy acetic acid groups which have a pK_a of 3.57. The H^+ form of the columns was used unless stated otherwise. Column void volume was determined from the retention volume of dextran blue from *Leucouostoc* spp. (Fluka, Buchs, Switzerland).

2.3. Reagents

All solutions and eluents were prepared in water purified using a Milli-Q (Millipore, Bedford, MA, USA) water purification system. The working standard solutions were diluted from stock solutions using the eluent unless stated otherwise. The acid, sodium or potassium salt of the anions under investigation were used and reagents were of analytical grade unless stated otherwise. Acetic acid, methanol, nitric acid, potassium fluoride, sodium chloride, sodium dihydrogenphosphate dihydrate, sodium iodide, sodium thiosulfate pentahydrate and tartaric acid were obtained from BDH (Kilsyth, Australia). Perchloric acid, sodium formate, sodium nitrate, sodium nitrite, sodium perchlorate (LR grade only) and sulfuric acid were obtained from Ajax (Auburn, Australia). Ethanesulfonate sodium salt (98%), hydrobromic acid, sodium arsenate dibasic heptahydrate and sodium thiocyanate (98%) were obtained from Aldrich (Milwaukee, WI, USA). Methane sulfonic acid and sodium bromide were obtained from Sigma (St. Louis, MO, USA). Dextran blue from *Leucouostoc* spp. was obtained from Fluka and sodium sulfate from Prolabo (Paris, France).

2.4. Eluents

All eluents were filtered using Whatman 0.45-μm cellulose nitrate membrane filters (Whatman, Maidstone, UK) or Waters 0.45-μm membrane filters of type HA and degassed under vacuum prior to use. Filtering/degassing was repeated every 24 h. Eluent pH was measured using an Activon Model 210 pH meter with AEP336 semi-micro pH probe (Activon, Thornleigh, Australia).

3. Results and discussion

3.1. Preliminary investigations on retention behaviour

The chromatographic behaviour of a mixture of strong-acid anions, weak-acid anions and methanol (listed in Table 1) was determined on two polymethacrylate columns which were identical apart from the functional group (sulfonate and carboxylate)

Table 1
Anions investigated in the preliminary studies and their corresponding pK_a values

Compound	pK_a of acid
Acetate (CH_3COO^-)	4.75 [5]
Arsenate (AsO_4^{3-})	2.24
	6.96
	11.50 [6]
Bromide (Br^-)	−9 [7]
Chloride (Cl^-)	−6.1 [8]
Ethanesulfonate ($CH_3CH_2SO_3^-$)	−2 [9]
Fluoride (F^-)	3.45 [5]
Formate ($HCOO^-$)	3.75 [5]
Iodide (I^-)	−10 [7]
Methanesulfonate ($CH_3SO_3^-$)	−2 [9]
Methanol	17 [10]
Nitrate (NO_3^-)	−1.4 [8]
Nitrite (NO_2^-)	3.15 [6]
Perchlorate (ClO_4^-)	−10 [8]
Phosphate (PO_4^{3-})	2.12
	7.21 [5]
	12.15 [11]
Sulfate (SO_4^{2-})	−3 [8]
	1.99 [6]
Sulfite (SO_3^{2-})	1.91
	7.18 [6]
Tartrate $[(CHOHCOO)_2]^{2-}$	2.98
	4.34 [5]
Thiocyanate (SCN^-)	0.9 [6]
Thiosulfate ($S_2O_3^{2-}$)	0.6
	1.6 [6]

bound to the resin. Methanol was included to determine the volume of total permeation for each column, which is the sum of the void volume and the volume of the pores in the resin. The retention times were determined using tartaric and sulfuric acid eluents at two pH values and the results are listed in Table 2.

Fig. 1 shows typical plots of pK_{a1} versus retention factor obtained when 6 m*M* tartaric acid (pH 2.67) was used as the eluent on the carboxylate and the sulfonate columns. The shapes of pK_{a1} versus retention factor graphs for the six other conditions were similar except the retention and separation of the anions decreased with increasing pH. On both columns at pH 2.67 each of the weak acids (acetate, fluoride, formate and nitrite) was retained longer than methanol, regardless of the nature of the eluent anion. This was also the case for acetate and nitrite at pH 3.63. This behaviour indicated the presence of retention mechanisms other than ion exclusion for these anions, and for formate and acetate the increased retention may be attributable to hydrophobic interactions with the resin. Retention was observed for all the strong-acid anions on both columns, regardless of the eluent, with greater retention being observed for the carboxylated resin. The nature of the resin functional group exerted a significant effect on the extent of retention and separation of strong acids (pK_a<1). On the sulfonated resin, co-elution of monovalent strong-acid anions occurred and the divalent strong-acid anions thiosulfate and sulfate were eluted earlier than the monovalent species. On the carboxylated resin, partial separation of the strong acids was observed, especially at the lower pH (2.67). In general, retention was higher on both columns when the eluent pH was decreased. The nature of the eluent anion exerted only a comparatively small effect on retention.

Throughout the preliminary study a system peak was observed regardless of the eluent composition whenever strong-acid anions were injected. For sulfuric acid eluents the presence of this peak often made it difficult to determine the exact retention time of the injected anion and conductivity traces typically had the form shown in Fig. 2. Comparison of the UV and conductivity traces for the UV-absorbing anions on the sulfonate column showed that the dip in the conductivity trace corresponded to the retention factor of the analyte anion. This behaviour was assumed to be the same for the non-UV-absorbing anions and the retention factors were determined accordingly. A different relationship was observed for the carboxylate column and the retention factors of the non-UV-absorbing strong- and moderately strong-acid anions (perchlorate, methanesulfonate, ethanesulfonate, chloride and phosphate) were estimated as the time at which the conductivity trace crossed the baseline between the dip and the peak.

With tartaric acid eluents all analyte peaks on the conductivity detector were positive rather than negative and the system peak was eluted later than with sulfuric acid of the same pH. With this eluent the system peak appeared in both the conductivity and UV chromatograms, because tartaric acid eluent is UV absorbing. Fig. 3 shows typical conductivity and UV chromatograms obtained when using tartaric acid eluents. When injecting non-UV-absorbing anions, a

Table 2
Retention factors when injecting the 19 test solutes on each of the columns using the eluents indicated

Solute	k'							
	Column: TSKguardgel SP-5PW polymethacrylate column with sulfonate functional groups				Column: TSKguardgel CM-5PW polymethacrylate column with carboxylate functional groups			
	Eluent:				Eluent:			
	1 m*M* Sulfuric acid pH 2.67 (±0.05)	0.1 m*M* Sulfuric acid pH 3.63 (±0.05)	6 m*M* Tartaric acid pH 2.67 (±0.05)	0.24 m*M* Tartaric acid pH 3.63 (±0.05)	1 m*M* Sulfuric acid pH 2.67 (±0.05)	0.1 m*M* Sulfuric acid pH 3.63 (±0.05)	6 m*M* Tartaric acid pH 2.67 (±0.05)	0.24 m*M* Tartaric acid pH 3.63 (±0.05)
Acetate	2.54	2.42	2.54	2.43	2.01	1.91	2.02	1.94
Arsenate	1.57	0.90	1.56	0.80	1.43	0.87	1.43	0.75
Bromide	1.21	0.79	1.21	0.71	1.35	0.67	1.35	0.71
Chloride	1.21	0.82	1.21	0.71	1.41[a]	0.78[a]	1.31	0.71
Ethanesulfonate	1.22	0.81	1.21	0.71	1.41[a]	0.78[a]	1.29	0.70
Fluoride	2.59	1.65	2.56	1.66	1.81	1.30	1.83	1.35
Formate	2.46	1.95	2.47	1.96	1.94	1.56	1.95	1.59
Iodide	1.22	0.80	1.22	0.71	1.55	0.87	1.56	0.75
Methanesulfonate	1.21	0.81	1.21	0.71	1.41[a]	0.78[a]	1.29	0.70
Methanol	2.29	2.29	2.30	2.31	1.77	1.77	1.75	1.79
Nitrate	1.21	0.80	1.22	0.71	1.39	0.77	1.39	0.72
Nitrite	9.03	3.85	9.07	3.89	5.31	2.21	5.28	2.25
Perchlorate	1.23	0.81	1.24	0.71	UN[b]	0.85[a]	2.15	0.79
Phosphate	1.48	0.86	1.45	0.76	1.44[a]	0.80[a]	1.36	0.72
Sulfate	UN	UN	1.09	0.61	UN	UN	1.25	0.57
Sulfite	1.61	0.87	1.61	0.78	1.57	0.87	1.29	0.75
Tartrate	1.88	1.25	UN	UN	1.58	1.15	UN	UN
Thiocyanate	1.24	0.81	1.24	0.72	1.87	0.91	1.90	0.79
Thiosulfate	1.07	0.56	1.07	0.61	1.25	0.57	1.35	0.57

[a] Uncertainty in retention factor was ±0.12.
[b] UN = Unknown.

dip appeared in the UV trace corresponding to the retention volume of the anion (Fig. 3c). Peaks in the UV traces for monovalent UV-absorbing strong-acid anions when using 0.1 m*M* sulfuric acid were of poor shape for both columns.

3.2. Effect of eluent pH

The improvement in separation of the strong-acid anions on reducing the eluent pH from 3.63 to 2.67 when using the carboxylated resin was consistent with results observed in a previous study [3] for sulfate, nitrate and chloride. In order to examine this factor more extensively, retention data of some strong-acid anions were collected at further eluent pH values on the carboxylated resin and using tartaric acid as eluent. The pH conditions for this experiment are given in Table 3. The sulfonated resin was not considered further because separation of the strong-acid anions was not observed. The retention of all the strong-acid anions increased with decreasing pH and that of methanol was constant (Fig. 4). In general the elution order for the strong acids was:

$$ClO_4^- \geq SCN^- \geq I^- \geq NO_3^- \geq Br^- \geq Cl^- \approx CH_3SO_3^- \geq SO_4^{2-} \approx S_2O_3^{2-}$$

The exceptions to this elution order occurred at low pH, as can be seen from Fig. 4. The elution order of the monovalent anions is identical to that typically observed when using a strong-base anion exchanger [2].

With 0.012 m*M* tartaric acid eluent (pH 4.69) poor peak shapes made it difficult to identify the exact retention factor of the anions. The results

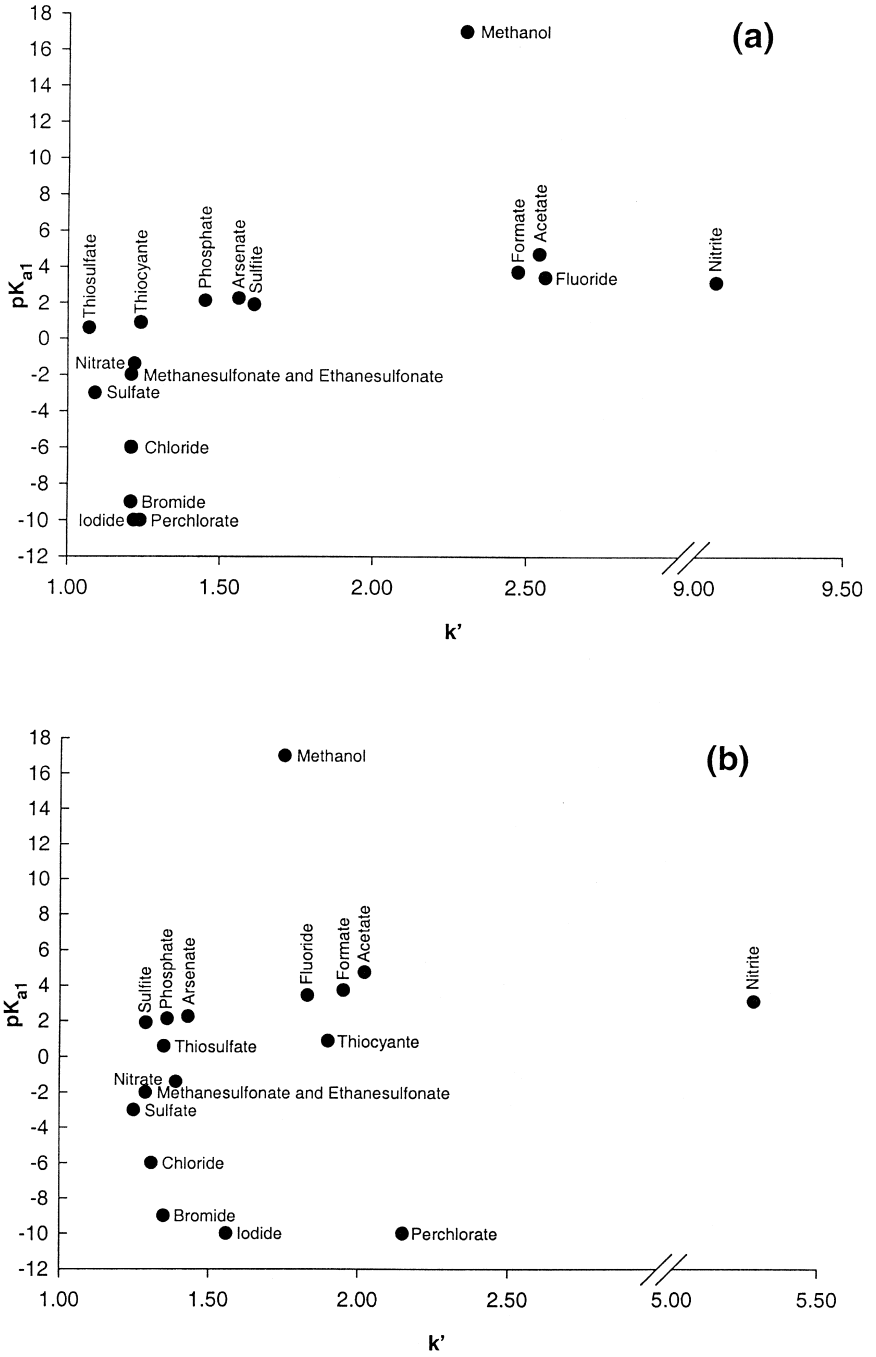

Fig. 1. Plot of retention factor versus pK_{a1} for 18 test solutes using 6 m*M* tartaric acid eluent on the sulfonated (a) and carboxylated (b) resin.

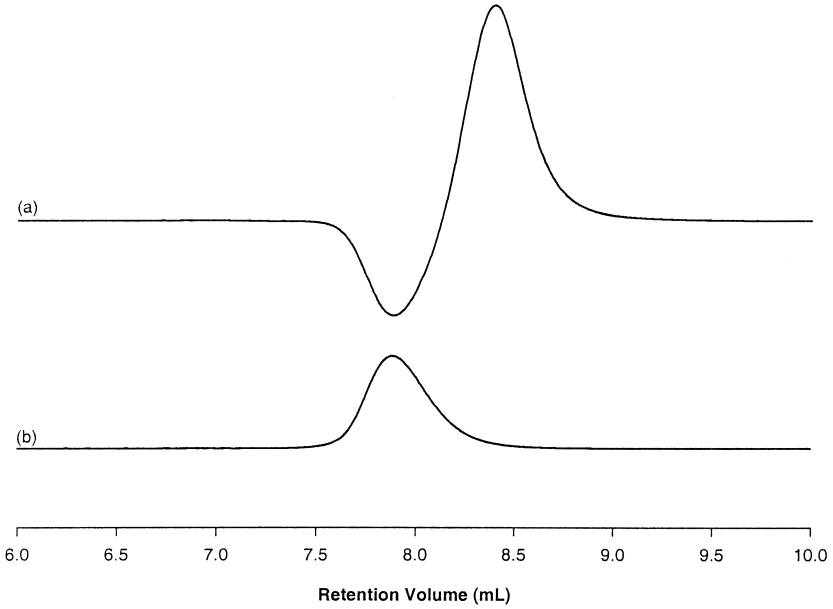

Fig. 2. Conductivity (a) and UV (b) chromatograms obtained for 0.2 m*M* NaBr solution on the sulfonated resin when using 1 m*M* sulfuric acid eluent.

quoted are therefore estimates (±0.05). All anion peaks were fronted when using 1 μ*M* tartaric acid and water eluents.

3.3. Effect of ionic strength of the eluent

The above results demonstrated that retention of

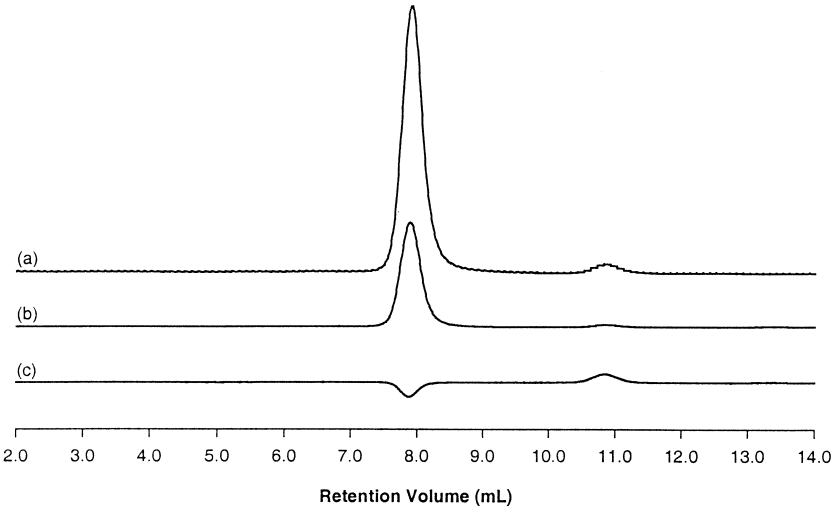

Fig. 3. Conductivity (a) and corresponding UV (b) chromatograms for 0.2 m*M* NaBr when using the sulfonate column and 6 m*M* tartaric acid eluent. The UV chromatogram for 0.2 m*M* NaCl under the same conditions is shown in (c). The small peak at approximately 11 ml is the system peak.

Table 3
Eluent conditions used to assess the effect of pH on retention of strong-acid anions

Tartaric acid concentration of eluent (m*M*)	pH of eluent (±0.05[a] unless otherwise stated)	Comments
44.3	2.27	Working standards were prepared so that sample and eluent were matrix-matched
6.00	2.71	Data obtained from previous experiment
0.240	3.63	Data obtained from previous experiment
0.012	4.69	Working standards prepared in Milli-Q water not the eluent
0.001	5.5 (±0.1)	Working standards prepared in Milli-Q water not the eluent
0 (water)	5.7 (±0.2)	

[a] Estimate of uncertainty in pH measurements only.

strong-acid anions increased with decreasing eluent pH. However, reducing the pH resulted in a concomitant increase in the ionic strength of the eluents, so further studies were conducted to determine whether the ionic strength, pH or a combination of both, influenced the retention of strong-acid anions.

Mixed solutions of sulfuric acid and sodium sulfate were used as eluents since this enabled the pH to be varied while maintaining a constant ionic strength. UV-absorbing analytes were used namely; thiocyanate, thiosulfate, iodide, nitrate and bromide, in order to achieve unequivocal identification of retention times. Results are given in Table 4 which shows that at each pH the retention factor and separation increased with increasing ionic strength. The most marked change in retention occurred when using low-ionic-strength eluents. Fig. 5 shows the results at pH 5.7 and plots for the other two pH values investigated were of similar shape. The effect of pH was examined at two different ionic strengths, 63 m*M* and 9.45 m*M* and the results are given in Table 5. Retention factors and separation of the strong-acid anions increased with decreasing pH when using eluents of identical ionic strength. Fig. 6 shows this behaviour for 63 m*M* ionic strength eluents. Best separation occurred at low pH (2.22) and high ionic strength (63 m*M*). These studies demonstrated that both ionic strength and pH affected the retention of strong-acid anions. Under all the conditions the elution order was identical to that observed in the previous study with tartaric acid eluents.

Fig. 7 shows the chromatogram obtained after injecting a mixture containing 0.2 m*M* thiosulfate, bromide, nitrate, iodide and thiocyanate using the optimum conditions. Separation was still quite poor for thiosulfate, bromide and nitrate and better separations are achievable using ion-exchange or ion-interaction chromatography. However, the point of interest is that some separation occurs, in contradiction to predictions from established ion-exclusion theory.

For all the eluents with ionic strength $\geq$9.45 m*M*, peaks for the analyte anions were reasonably symmetrical, regardless of the pH. However, fronted or ill-defined peak shapes were observed when ionic strength was low. This behaviour may be explained as follows. When an analyte is injected into an eluent of low ionic strength the initial peak shape (at time t_0) can be assumed to be Gaussian (see Fig. 8). The ionic strength of the sample band is much higher than the surrounding eluent. As the sample passes through the column dilution of the sample band in the eluent will occur (t_1), with this dilution being more pronounced at the extremities of the band. This results in the ionic strength of the solution being less at the edges of the band than at the centre. Analyte at the leading edge of the band will move progressively further away from the centre of the band because retention is reduced at lower ionic strength, whilst analyte at the trailing edge will ''catch up'' to the centre of the band by the same mechanism. This effect continues as the analyte band moves through the column so that when the band is eluted from the column (t_2) the peak shape is fronted. This mechanism is analogous to that proposed for weak acids in ion-exclusion chromatography using poorly buffered eluents [12] where fronted peaks are attributed to the

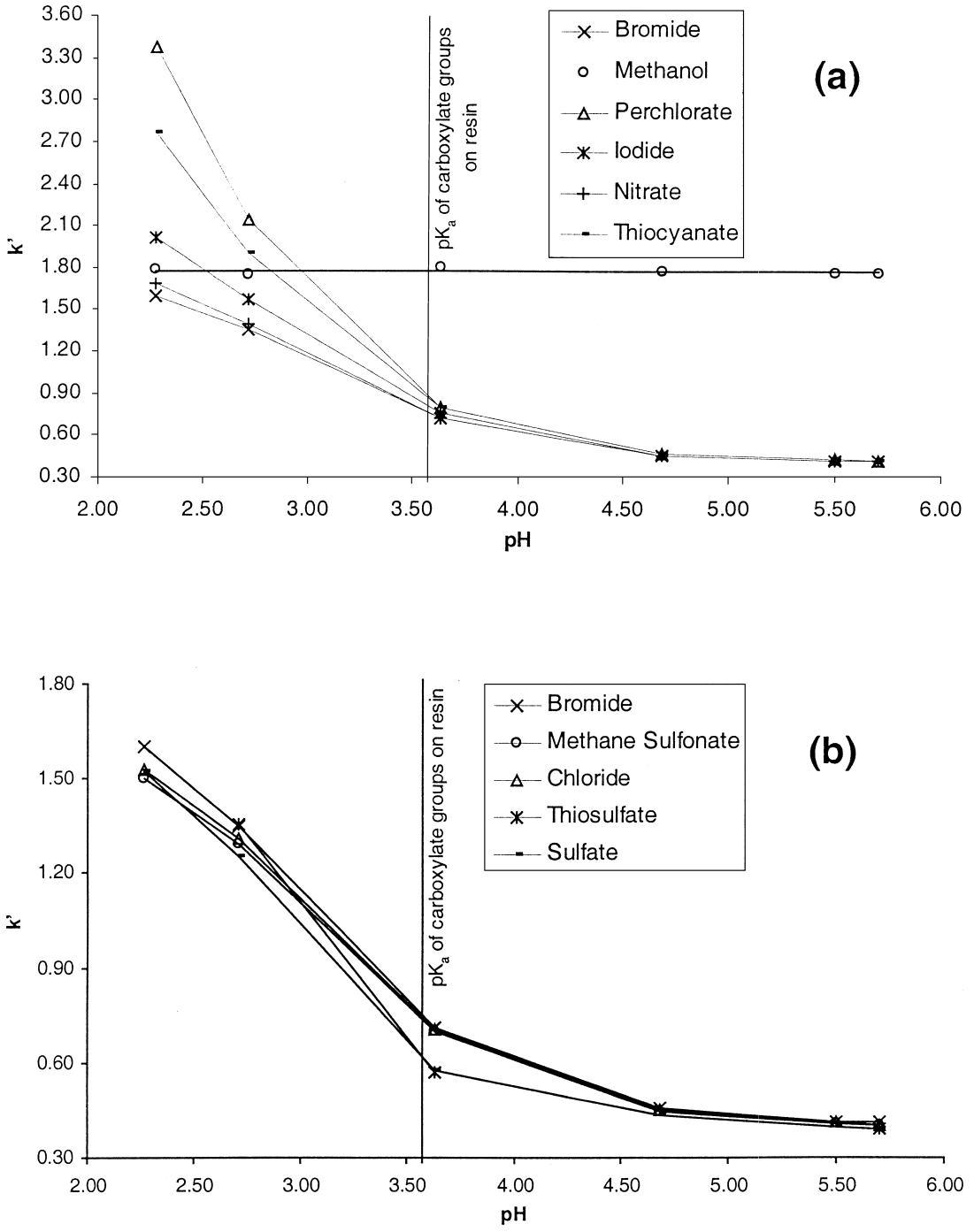

Fig. 4. Plot of pH versus retention factor for the ions that were retained longer than bromide (a) and less than bromide (b) on the carboxylate column using tartaric acid eluents. The retention factor of thiosulfate was not determined when using 0.012 m*M*, 1.0 μ*M*, or 0.0443 *M* tartaric acid eluents.

Table 4
Retention factors obtained on the carboxylate column using sulfuric acid–sodium sulfate eluents, used to determine the effect of ionic strength at constant pH

pH (±0.05)[a]	Ionic strength (m*M*)	*k'*				
		Bromide	Iodide	Nitrate	Thiosulfate	Thiocyanate
2.65	3.00	1.35	1.55	1.39	1.25	1.87
	6.30	1.43	1.63	1.47	1.33	1.97
	9.45	1.50	1.74	1.54	1.40	2.15
	63.0	1.61	1.96	1.67	1.49	2.64
3.63	0.300	0.67	0.87	0.77	0.57	0.91
	9.45	1.32	1.40	1.33	1.21	1.53
	36.3	1.45	1.57	1.47	1.36	1.78
	63.0	1.48	1.62	1.51	1.39	1.88
5.7 (±0.2)	10^{-4}[b]	0.32	0.32	0.32	0.30	0.32
	9.45	1.16	1.17	1.16	1.05	1.19
	63.0	1.39	1.43	1.40	1.32	1.51

[a] Estimate of uncertainty in measurements.
[b] Column in Na^+ form.

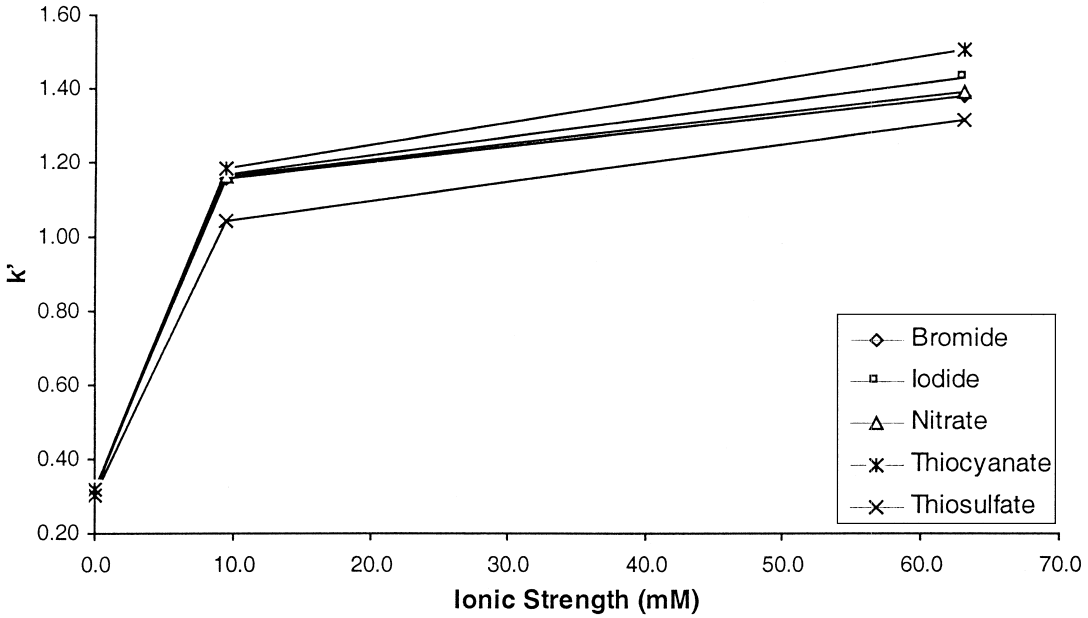

Fig. 5. Plot of ionic strength versus retention factor for UV-absorbing anions at pH 5.7 using the carboxylate column.

Table 5
Retention factors obtained on the carboxylate column using sulfuric acid–sodium sulfate eluents, used to determine the effect of pH at constant ionic strength

Ionic strength (m*M*)	pH (±0.05)[a]	*k'*				
		Bromide	Iodide	Nitrate	Thiosulfate	Thiocyanate
9.45	2.31	1.58	1.95	1.64	1.51	2.63
	2.65	1.50	1.74	1.54	1.40	2.15
	3.63	1.32	1.40	1.33	1.21	1.53
	5.7 (±0.2)	1.16	1.17	1.16	1.05	1.19
63.0	2.22	1.67	2.16	1.75	1.54	3.10
	2.38	1.64	2.07	1.71	1.52	2.89
	2.65	1.61	1.96	1.67	1.49	2.64
	2.89	1.57	1.85	1.62	1.46	2.36
	3.63	1.48	1.62	1.51	1.39	1.88
	5.7 (±0.2)	1.39	1.43	1.40	1.32	1.51

[a] Estimate of uncertainty in measurements.

inability of the eluent to stabilise the degree of dissociation of the analyte acid across the chromatographic peak.

3.4. System peaks in ion-exclusion chromatography

For all eluents having significant background conductance a negative system peak was observed in the conductivity trace when injecting solutions that were prepared in water or in the eluent acid at a lower concentration than that of the eluent itself. This corresponded closely to the retention factor of the system peak observed when injecting the eluent acid anion at a concentration higher than the eluent. This peak has been called an ''eluent dip'' in a previous study [3]. The use of matrix-matching

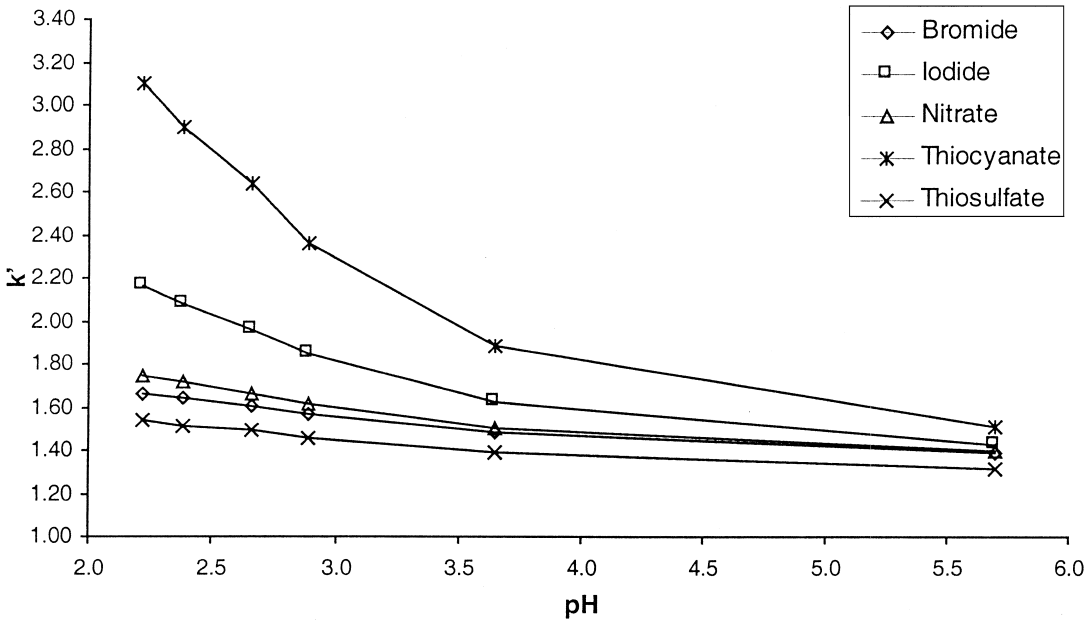

Fig. 6. Plot of pH versus retention factor for UV-absorbing anions at ionic strength 63 m*M* using the carboxylate column.

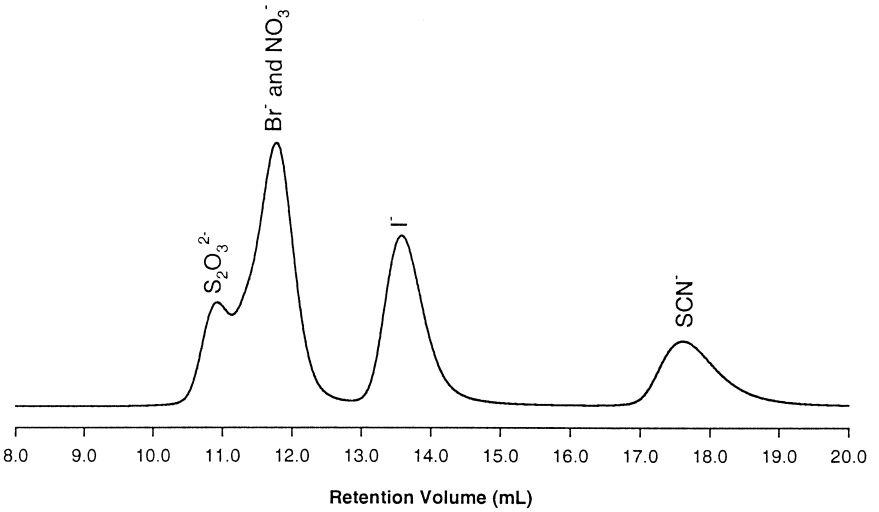

Fig. 7. UV chromatogram of a solution containing 0.2 m*M* $S_2O_3^{2-}$, Br^-, NO_3^-, I^- and SCN^- using the carboxylated resin and a H_2SO_4–Na_2SO_4 mixed eluent of pH 2.25 (±0.05) and ionic strength 63 m*M*.

methods by making up standards in the eluent did not remove this problem when injecting strong or moderately strong-acid anions ($pK_a < 3$) because the negative system peak was replaced with a positive system peak with the same retention factor. For the purposes of discussion the system peak will be described as a negative system peak if it corresponded to a decrease in detector response, and as a

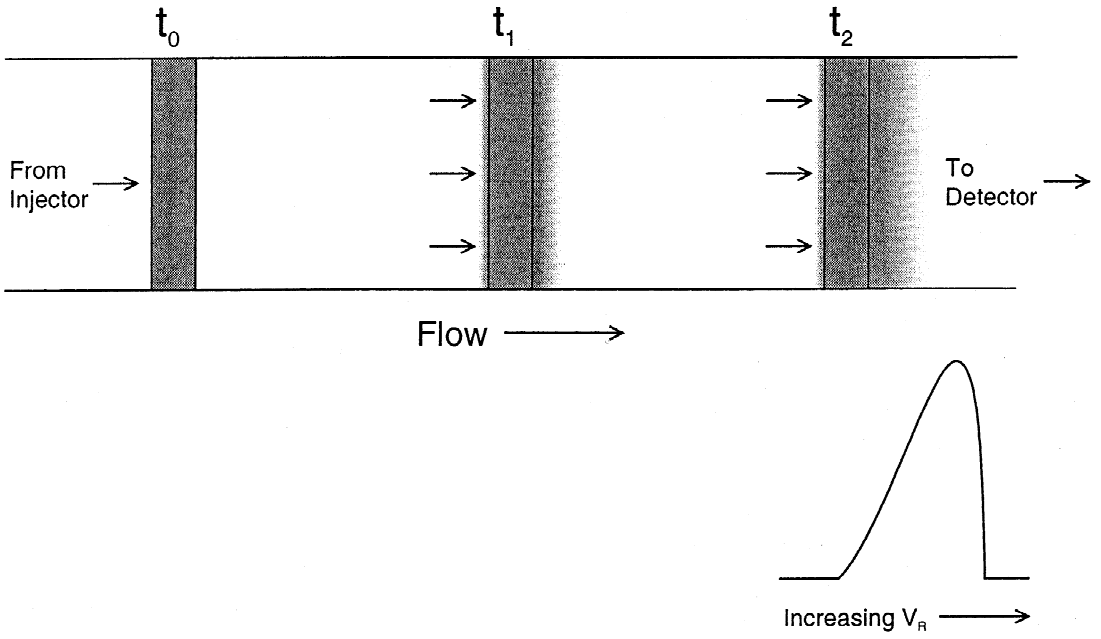

Fig. 8. Schematic illustration of the proposed mechanism by which peak fronting for strong-acid anions occurs in low-ionic-strength eluents. The peak at bottom of diagram illustrates the peak shape that would be observed if sample band was eluted at t_2.

positive system peak if it corresponded to an increase in detector response. Some properties of the system peak with sulfuric and tartaric acids when injecting strong-acid anions prepared in the eluent have already been discussed in Section 3.1. Other properties were also noted. For all eluents, changing from the carboxylate to the sulfonate column altered the retention factor of both the analyte anion and system peak, and changing the eluent pH had a similar effect. On both columns when using sulfuric acid eluents the areas of the dip and the system peak were proportional to the amount of analyte anion injected, as shown in Fig. 9 for bromide on the sulfonate column. Smaller system peaks were observed for eluents with some buffering capacity (for example 6 m*M* tartaric acid) than for the others examined (for example sulfuric acid and 0.24 m*M* tartaric acid), which are poor buffers. This indicated the buffering capacity of the eluent may exert an effect on the size of the system peak.

The results suggest that there is a portion of eluent displaced during the chromatographic run that is caused by the injection of strong-acid anions. There are two observations that provide evidence for this. The first is the appearance of a dip in the UV trace at the retention factor of non-UV-absorbing anions when using tartaric acid eluent (Fig. 3c). If tartaric acid had not been displaced then the dip would be absent. The second is the appearance of the dip in the conductivity trace at the retention factor of the injected anion when using sulfuric acid eluents. If the eluent concentration had not decreased where the anion was eluted, the conductivity would have to increase due to the presence of the injected anion.

A suggested mechanism for the formation of these system peaks is as follows. Consider injection of an anion, A^-, onto a column equilibrated with eluent acid, E^-. At the instant of sample injection (t_0, in Fig. 10), there is a single sample band of different composition to the eluent in the column. However, at some time (t_1) an equilibrium disturbance occurs forming two bands. The earlier eluting band contains the injected anion, whilst the second (later eluting) band contains the eluent acid at a greater or lesser concentration than the bulk solution, depending on the injected sample. There will be no system peak observed in the UV trace when using a non-UV-absorbing eluent, but a system peak will appear in

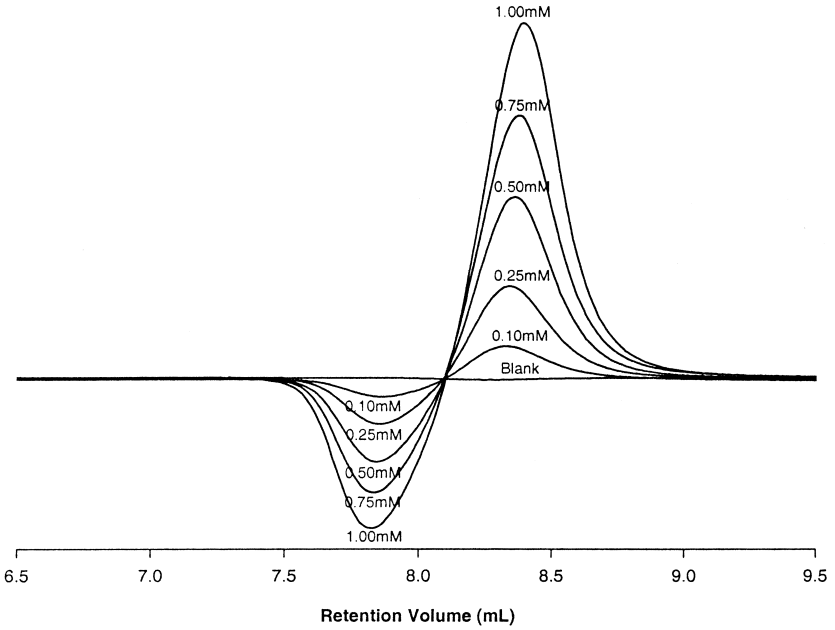

Fig. 9. Conductivity chromatograms for NaBr solutions of the indicated concentrations when using the sulfonate column and 1 m*M* sulfuric acid eluent. Injection volume was 40 μl.

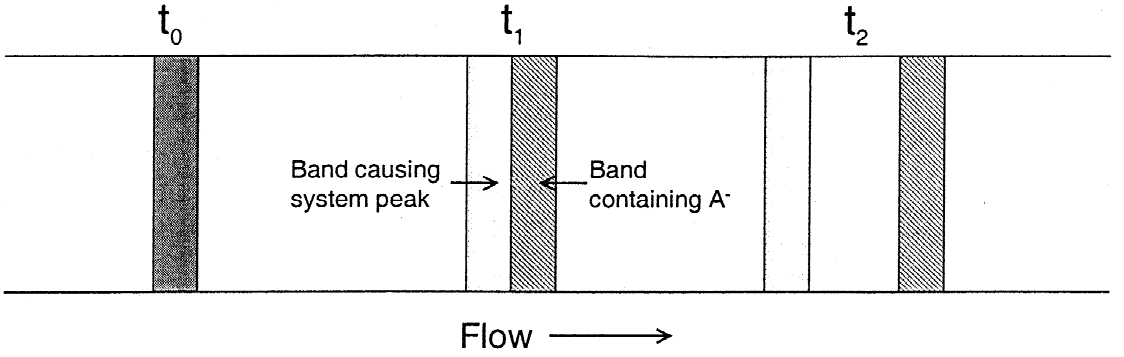

Fig. 10. Schematic illustration of the formation of the system peak.

the UV trace if a UV-absorbing eluent is used. These trends were observed in practice. The two bands move down the column (t_2) and elute with different retention factors. This results in chromatograms of the form shown in Figs. 2 and 3.

3.5. Accuracy of strong acids as void volume markers in ion-exclusion chromatography

Strong-acid anions are often used as void volume markers for anion-exclusion columns. The separation and retention of strong-acid anions on such columns shown in this paper implies that this practice is questionable. One study using an unmodified silica column [13] has already noted that the void marker nitrate shifted with eluent pH. Dextran blue was used as an alternative compound for determining the void volume. This molecule has a high-molecular-mass (~1 000 000 g/mol) and is therefore excluded from the pores of the resin on the basis of its size. Retention of this molecule via a hydrophobic mechanism is unlikely since it is a sugar polymer and is hence quite hydrophilic. The compound was injected and detected at 210 nm using water as the eluent. Unfortunately a fronted peak was obtained and hence the retention volume increased with increasing concentration of the injected sample. The void volume of the columns was therefore measured by determining the retention volume of a 10-μl injection of a 17 μg/ml solution, which was the lowest concentration that gave a reasonable peak size. The minimum retention volume observed for any of the strong-acid anions was at least 39% higher than for dextran blue, so the use of strong-acid anions can provide erroneous values for the void volume determination of ion-exclusion columns.

4. Conclusions

The functional group on the resin, eluent pH and eluent ionic strength are all important parameters in determining the retention of strong-acid anions in ion-exclusion chromatography. Separation of some strong-acid anions was possible when using a carboxylated resin but not with a sulfonated resin that was otherwise identical. The retention factor of the strong-acid anions increased with decreasing pH on the sulfonate resin, but the monovalent species co-eluted. Both the retention factor and the separation of the strong-acid anions increased on the carboxylated resin with decreasing pH. Retention factor of strong-acid anions also increased with increasing ionic strength and this effect was particularly strong in the low-ionic-strength region. The elution order of monovalent strong-acid anions was identical to that observed in ion-exchange chromatography when using a strong-base anion exchanger. When using eluents of low ionic strength fronted or poorly-shaped peaks were obtained. These observations have been explained in terms of variations in ionic strength (and hence retention) across the chromatographic peak.

A system peak was observed during this investigation when injecting strong-acid anions. When the strong-acid anions were dissolved in the eluent matrix and injected this peak was positive and corresponded to a local increase in the concentration

of the eluent acid anion. There was also a reduction in the concentration of the eluent anion in the region where the anion was eluted. The retention factor of the system peak was affected by the nature of the resin functional group, eluent acid anion and eluent pH. Its size was proportional to the amount of the injected strong-acid anion. It is proposed that the system peak is caused by an equilibrium disturbance but why this disturbance occurs is still unclear.

Strong-acid anions were retained under all conditions investigated. These species are therefore unsuitable as void volume markers of ion-exclusion columns. The use of a large hydrophilic polymer such as dextran blue that is excluded from the pores of the resin on the basis of size gave a more accurate value for this parameter.

Acknowledgements

Support from the Australian Research Council and Dionex Corporation is gratefully acknowledged.

References

[1] J. Weiss, in: Ion Chromatography, 2nd Edition, VCH, New York, 1995, Chapter 4.
[2] P.R. Haddad, P.E. Jackson, Ion Chromatography – Principles and Applications, Elsevier, Amsterdam, 1991.
[3] K. Tanaka, K. Ohta, J.S. Fritz, S. Matsushita, A. Miyanaga, J. Chromatogr. A 671 (1994) 239.
[4] K. Tanaka, T. Ishizuka, H. Sunahara, J. Chromatogr. 174 (1979) 153.
[5] D.R. Lide (Ed.), CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1993.
[6] A.E. Martell, R.M. Smith, Critical Stability Constants, Plenum Press, New York, 1974.
[7] R.P. Bell, The Proton in Chemistry, 2nd Edition, Chapman and Hall, London, 1973.
[8] B.K. Glod, W. Kemula, J. Chromatogr. 366 (1986) 39.
[9] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, J. Akiyama, J. Chromatogr. 271 (1983) D1.
[10] H.H. Landolt, R. Bornstein, in: 6th Edition, Landholt–Bornstein Zahlenwerte und Funkionen, Vol. 2, Part 7, Springer-Verlag, Berlin, 1960, p. 847.
[11] A.G. Miller, J.W. Macklin, Anal. Chem. 55 (1983) 684.
[12] B.K. Glod, Neurochem. Res. 22 (1997) 1237.
[13] K. Ohta, K. Tanaka, P.R. Haddad, J. Chromatogr. A 739 (1996) 359.