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## RETENTION AND SEPARATION OF INORGANIC ANIONS BY REVERSED-PHASE ION-INTERACTION CHROMATOGRAPHY ON OCTADECYL SILICA

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### SUMMARY

The retention behaviour of chloride, nitrite, bromide, nitrate and sulphate was investigated using Partisil 10 ODS-3 as the stationary phase and a solution of tetrabutylammonium in aqueous phthalate or phosphate buffer as the eluent. The quaternary ammonium ion adsorption behaviour and the influence of the quaternary ammonium concentration, the pH and the buffer concentration on the retention of the inorganic anions is discussed. The major adsorption and ion-exchange equilibria in the retention process were studied and used to develop a retention model for the different analytes. This retention model was verified experimentally. Ion-interaction reversed-phase chromatography with an octadecyl-bonded silica stationary phase and tetrabutylammonium iodide-phthalate eluent was found to be a successful system for the separation and determination of inorganic anions and when conductivity detection is used.

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### INTRODUCTION

Inorganic anions have frequently been separated and determined by means of ion chromatography (IC) using an anion-exchange resin. Reflecting the rapid developments in recent years in the IC technique, a number of remarkable new anion exchangers and instrumentation have appeared on the market<sup>1–3</sup>, which allow the efficient separation of common anions such as  $F^-$ ,  $Cl^-$ ,  $NO_2^-$ ,  $Br^-$ ,  $NO_3^-$ ,  $PO_4^{3-}$  and  $SO_4^{2-}$  with detection limits at the sub-ppm level.

As an alternative to ion chromatography, several approaches based on reversed-phase chromatography (RPC) have been used to separate the common inorganic anions<sup>4–6</sup>. Among these, ion-pair chromatography (IPC) or ion-interaction chromatography (IIC) using an octadecyl-bonded silica as a nonpolar stationary phase has developed considerably. In this technique, a buffered<sup>7</sup> or unbuffered<sup>8</sup> aqueous eluent containing a hydrophobic positively charged ion is used. The major advantages of this technique are (a) the system is nearly completely nonspecific, (b) the separation system takes advantage of highly efficient reversed-phase columns and (c) it

can be readily modified to meet new analytical conditions and hence shows a much greater degree of flexibility than do conventional "fixed-site" ion exchangers. In addition, no special equipment is required to perform ion analyses by this technique; only conventional high-performance liquid chromatographic (HPLC) pumps and detectors are required<sup>9</sup>.

It is well known that retention and separation occur when a hydrophobic ion-interaction reagent is added to the mobile phase of a reversed-phase system. Several models of the retention mechanism of organic ionized solutes have been proposed for reversed-phase IPC, the most important being (a) ion-pair adsorption<sup>10,11</sup>, (b) dynamic ion-exchange and a similar ion-interaction model<sup>5,9,12-15</sup> and (c) the Stern-Gouy-Chapman theory of the electrical double layer and more recently a combination of the Gouy-Chapman theory and the Langmuir isotherm<sup>16,17</sup>.

The above retention models have been extensively discussed in the recent literature but their application in reversed-phase inorganic separations is still not clear. It was also often observed that the separation abilities of reversed-phase packings differ considerably. The frequently employed reversed-phase columns for the separation of inorganic or organic anions are  $\mu$ Bondapak C<sub>18</sub>, LiChrosorb RP-18, Zorbax ODS and ODS Hypersil. Skelly<sup>4</sup> used a Partisil 10 ODS-3 packing for the separation of a mixture of  $\text{IO}_3^-$ ,  $\text{Br}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{I}^-$ , and Cassidy and Elchuk<sup>6</sup> used it for the separation of  $\text{IO}_3^-$ ,  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{I}^-$ . In both investigations, a phosphate buffer and UV detection at low wavelength were used. Important anions such as  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were not included in the separation. In addition, degradation of the column performance much shorter column lifetimes occurred with a phosphate buffer at higher pH<sup>6</sup>.

We therefore studied the retention behaviour of common inorganic anions on a reversed-phase ion-interaction system with an octadecyl bonded stationary phase (Partisil 10 ODS-3) and a mobile phase consisting of a hydrophobic quaternary ammonium salt dissolved in a phosphate or phthalate buffer. The adsorption characteristics of the quaternary ammonium salt on the solid phase, the factors that control retention of sample anions and the differences in the column performance with different buffers present in the eluent are discussed. We found that using a phthalate buffer together with an ion-pair or ion-interaction reagent increases the column performance and its lifetime significantly in comparison with the conventional phosphate-buffered ion-pair chromatographic system. It also allows an interesting comparison with anion-exchange chromatography, where a simple phthalate eluent is used<sup>18-20</sup>. The similar separation characteristics with these two techniques are reflected in very close or identical anion separation sequences. This is useful for the understanding of the separation mechanism of ion-pair or ion-interaction reversed-phase chromatography, which may result in an important routine anion analysis method, more advantageous than the ion-exchange mode.

## EXPERIMENTAL

### *Apparatus and reagents*

The chromatographic system consisted of (1) a DuPont 870 HPLC pump; (2) a Valco injection valve with a 100- or 50- $\mu\text{l}$  loop volume; (3) a Partisil 10 ODS-3 RP column (250  $\times$  4.6 mm I.D.) (Whatman) acting as a separation column and protected

with a  $60 \times 2.1$  mm guard column with the same packing; (4) a Perkin-Elmer LC-21 conductivity detector and (5) an Omniscribe B5 217-5 strip-chart recorder. The separation column, guard column and detector cell were thermally isolated in a wooden box to minimize short-term temperature variations. All separations were carried out at room temperature and a mobile phase flow-rate of 2 ml/min unless stated otherwise.

Analytical-reagent grade chemicals were used unless stated otherwise. Water was deionized and passed through a Millipore (Bedford, MA, U.S.A.) Milli-Q water purification system. Tetrabutylammonium iodide (TBAI, 98% for synthesis), tetrabutylammonium hydroxide (TBAOH), potassium dihydrogen phosphate and phthalic acid ( $H_2P$ ) were obtained from Merck (Darmstadt, F.R.G.), potassium hydrogen phthalate (KHP), from RCB (Belgium) and sodium monohydrogenphosphate from Baker (Deventer, The Netherlands). Standard solutions (1000 ppm) of inorganic anions (chloride, nitrite, bromide, nitrate, sulphate and dichromate) were prepared by dissolving appropriate amounts of the corresponding potassium salt in pure water. These solutions were diluted daily to give the trace required.

#### *Mobile phases*

The eluents used contained (1) TBAI or TBAOH with KHP or  $H_2P$  at pH between 4 and 6.5 or (2) TBAI with phosphate buffer ( $KH_2PO_4$ - $Na_2HPO_4$ ) at pH between 5.8 and 7.3. The concentrations of TBAI ranged from 0.5 to 16 mM. The concentrations of KHP and phosphate buffer are stated for each individual experiment. The pH of all mobile phases was adjusted by adding to a solution, containing a weighed amount of the salt, potassium hydroxide or acetic acid followed by dilution to volume. The effect of these ions on the separation behaviour of anions (see Results and Discussion) is included in the global effect of ionic strength or buffer anion. These eluents were prepared daily, filtered through a  $0.45\text{-}\mu\text{m}$  porosity membrane filter (Millipore HSWP 04700) and degassed before use.

#### *Column preparation*

The columns were packed in the laboratory by a slurry technique using 2-propanol as a suspension medium under a pressure of 6000–6500 p.s.i. They were washed successively with 100 ml of methanol and 60 ml water before use. Column testing was carried out with the mobile phase. A sample containing a mixture of chloride, nitrite, bromide, nitrate and sulphate was prepared giving a capacity factor ( $k'$ ) of about 1.3–7. Columns that gave theoretical plate heights of more than 15 000 were accepted for use.

#### *Breakthrough method*

In order to determine the amount of TBAI adsorbed on the stationary phase, and the way it is affected by TBAI concentration, phthalate concentration and pH of the eluent, a series of experiments in which the latter variables were varied, was carried out. Each experiment was followed by column washing with methanol and water, equilibrating first the guard column then the analytical column (by disconnecting the analytical column from the guard column and reconnecting them later).

The amount of TBAI adsorbed by the stationary phase ( $Q_s$ , mol/g) was determined by use of the breakthrough volume ( $V_R$ ):

$$Q_s = (V_R - V_m)Q_m/W_s$$

where  $Q_m$  is the concentration of TBAI in the eluent (mol/l),  $W_s$  is the amount of solid phase in the column (g) and  $V_R - V_m$  is the net retention volume.

## RESULTS AND DISCUSSION

### *Choice of hydrophobic quaternary ammonium salt*

Initially, a number of hydrophobic quaternary ammonium salts such as tetrabutylammonium salt ( $C_{16}$ ), trioctylmethylammonium salt ( $C_{25}$ ) and tridodecylmethylammonium salt ( $C_{37}$ ) were used as ion-interaction reagents. Their adsorption behaviour on Partisil ODS-3 stationary phase differed.  $C_{25}$  and  $C_{37}$  were permanently coated on the stationary phase such that the aqueous mobile phase used did not contain any of the quaternary ammonium salt. The  $C_{16}$  salt, however, was not permanently adsorbed on the solid phase and a dynamic equilibrium existed between the solid phase and the aqueous mobile phase which must contain a certain amount of the salt. Because the reproducibility of the retention time and the peak height of the sample anion was not very good with the permanently coated  $C_{25}$  and  $C_{37}$  salts, the  $C_{16}$  salt (TBAI or TBAOH) was selected as the hydrophobic ion-interaction reagent and was used throughout.

### *Hydrophobic ion-adsorption behaviour on Partisil 10 ODS-3 stationary phase*

Tests have shown that, when no quaternary ammonium salt is precoated on the reversed-phase column packing or is present in the eluent, no anion retention will occur. The retention of inorganic anions depends primarily on the amount of quaternary ammonium ion adsorbed on the stationary phase.

The adsorption isotherms of TBAI are shown in Fig. 1. They were obtained by the breakthrough method with and without KHP at different pH values. An initial increase in TBA concentration results in an increase in the fraction of the TBA ion adsorbed on the column packing surface. The isotherms indicate that with a further increase in TBA concentration in the eluent, the amount of TBAI adsorbed approaches a plateau. In other words, the surface area of the modified silica gel that can be covered by TBA is limited. Further increases in the TBAI concentration in the mobile phase do not result in further increases in the TBA adsorbed.

Data resulting from the breakthrough method given in Table I and Fig. 2 indicate that the absence or presence of KHP and higher or lower pH values have little influence on the amount of TBA ion adsorbed. They suggest that the competing adsorption of KHP with the TBA adsorption on the stationary phase can be neglected.

This observation, however, is different from the results of other workers. Melin *et al.*<sup>10</sup> studied the retention behaviour of some acids and bases using  $\mu$ Bondapak  $C_{18}$  as the stationary phase and a solution of tetrabutylammonium in acetonitrile and phosphate buffer as the mobile phase. More recently, Sokolowski<sup>11</sup> studied the adsorption of organic ions on  $\mu$ Bondapak from a water-based mobile phase. They concluded that all ions in the eluent may affect the adsorption and retention of the quaternary ammonium ion. The adsorption of the quaternary ammonium ion increases when a second organic ion with opposite charge is added, or when the pH of the mobile phase is increased. It should be pointed out that under our chromatographic

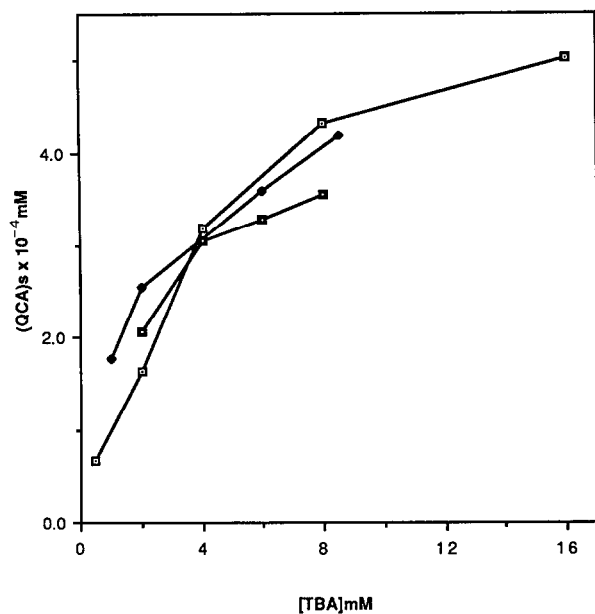


Fig. 1. Experimentally determined adsorption isotherms of tetrabutylammonium iodide on Partisil 10 ODS-3 column at 25°C from different eluent series. KHP concentration: (□) 0 mM, pH 4; (◆) 1.5 mM, pH 5; (■) 0.5 mM, pH 6.

TABLE I

INFLUENCE OF KHP CONCENTRATION AND pH ON THE AMOUNT OF TBAI ADSORBED ON ODS-3 STATIONARY PHASE

Eluent	KHP (mM)	Amount of TBAI adsorbed ( $\times 10^{-4}$ mM)	
		Per column	Per gram
4 mM, TBAI pH 6	0.00	3.25	1.12
	0.50	3.13	1.08
	1.00	3.41	1.18
	2.00	3.27	1.13
6 mM, TBAI pH 5	0.88	3.25	1.12
	1.50	3.35	1.16
	2.13	3.31	1.16
Eluent	pH	Amount of TBAI adsorbed ( $\text{mol} \times 10^{-4}$ )	
		Per column	Per gram
6 mM TBAI– 1.5 mM KHP	3.8	3.00	1.03
	5.0	3.35	1.16
	6.3	3.38	1.17

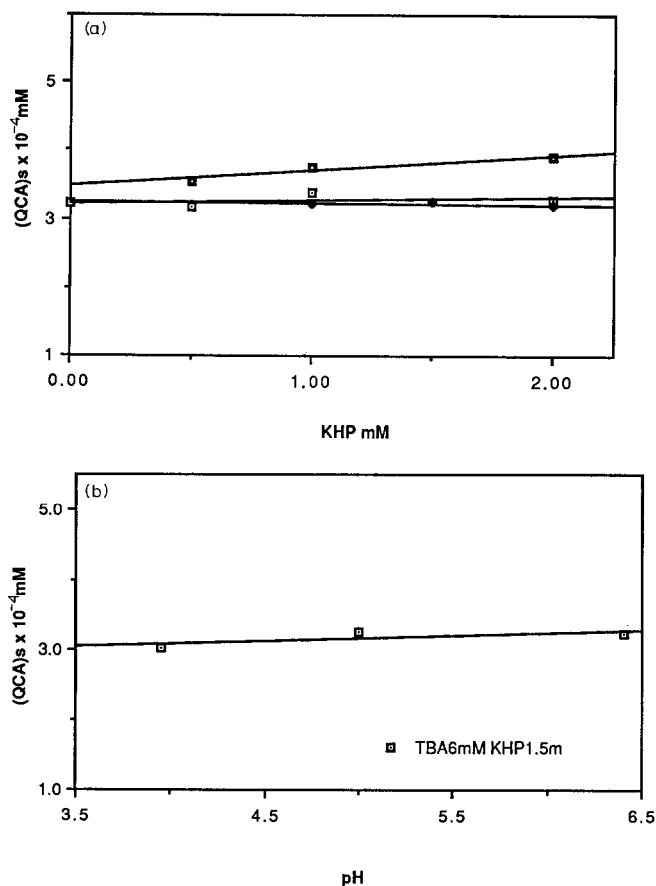


Fig. 2. (a) Influence of KHP concentration on the amount of TBAI adsorbed on Partisil 10 ODS-3 column. TBAI concentration: ( $\square$ ) 4 mM, pH 6; ( $\blacklozenge$ ) 6 mM, pH 5; ( $\blacksquare$ ) 8 mM, pH 6. (b) Influence of pH on the amount of TBAI adsorbed on Partisil 10 ODS-3 column. TBAI, 6 mM; KHP, 1.5 mM.

conditions, the amounts of the second organic ion ( $HP^-$ ) added are relatively small; in addition, the natures of the two organic compounds present are very different. Adsorption of TBA ion on the ODS stationary phase is due to a strong hydrophobic interaction force whereas KHP lacks such an interaction force with the stationary phase.

Our results are in agreement with those of Barber and Carr<sup>9</sup>, who studied the retention and UV detection of inorganic anions by reversed-phase ion-interaction chromatography. They observed that the buffer ions were unable to cause a significant change in the surface concentration of ion-interaction reagent. The buffer ions used in their work were either inorganic (phosphate) or small organic ions such as acetate, which are relatively hydrophilic and have relatively small extraction constants compared with long-chain organic sulphonates or sulphates. For example, higher "breakthrough" volumes are obtained on equilibration of a column when the eluent contains an alkanesulphonate.

### Factors that affect the inorganic anion retention

The discussion will focus on the three most important factors that influence the retention of sample anions: concentration of the quaternary ammonium salt, concentration of the buffer and pH of the eluent. In addition, no organic modifier must be added to the mobile phase, as we found that the stationary phase with a Partisil 10 ODS-3 packing is very weak, considering the retention of inorganic anions, even when quaternary ammonium salts are present.

**TBAI concentration.** Figs. 3 and 4 show the dependence of the capacity factor of five anions on the TBAI concentration in the eluent. In Fig. 3 the  $k'$  values of the anions increase with increasing TBAI concentration, reach a maximum and then decrease with further increase in TBAI concentration. Although all  $k'$  versus TBA concentration plots have a similar convex shape, the degree of convexity differs. The plot for  $\text{SO}_4^{2-}$  has the most pronounced convex shape and  $\text{Cl}^-$  has the smoothest curve. At low TBA ion concentrations, the magnitude of the retention increase with increasing TBA ion concentration follows the order  $\text{SO}_4^{2-} > \text{NO}_3^- > \text{Br}^- > \text{NO}_2^- > \text{Cl}^-$ . After each sample anion has passed the maximum  $k'$  value, the retention decreases with further increase in TBA ion concentration in the order  $\text{SO}_4^{2-} > \text{NO}_3^- > \text{Cl}^- \approx \text{NO}_2^- \approx \text{Br}^-$ . The relationship between  $k'$  and TBAI concentration in Fig. 4 is similar to that in Fig. 3. However, we observe only the initial part of the curve in Fig. 4, as the concentration range of TBAI tested was narrow.

**Phosphate or phthalate concentration and pH of the eluent.** Figs. 5–8 show partial linear relationships between  $1/k'$  and the mobile phase pH (except at high phosphate buffer concentration in Fig. 8) or ionic strength ( $\text{KH}_2\text{PO}_4$ – $\text{Na}_2\text{HPO}_4$  buffer or KHP

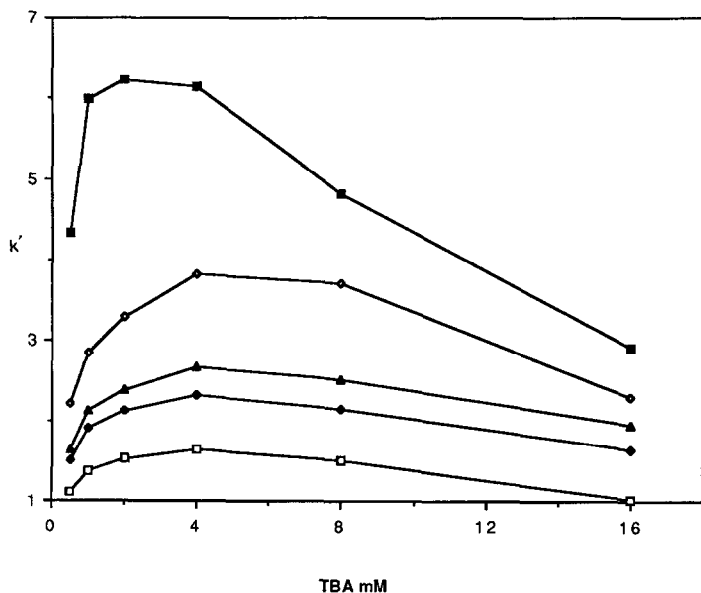


Fig. 3. Dependence of the capacity factor of inorganic anions on the concentration of TBAI in an aqueous phthalate eluent. Chromatographic conditions: flow-rate, 2 ml/min; Partisil 10 ODS-3 column; conductivity detector. Anions:  $\square$ ,  $\text{Cl}^-$ ;  $\blacklozenge$ ,  $\text{NO}_2^-$ ;  $\blacktriangle$ ,  $\text{Br}^-$ ;  $\diamond$ ,  $\text{NO}_3^-$ ;  $\blacksquare$ ,  $\text{SO}_4^{2-}$ .

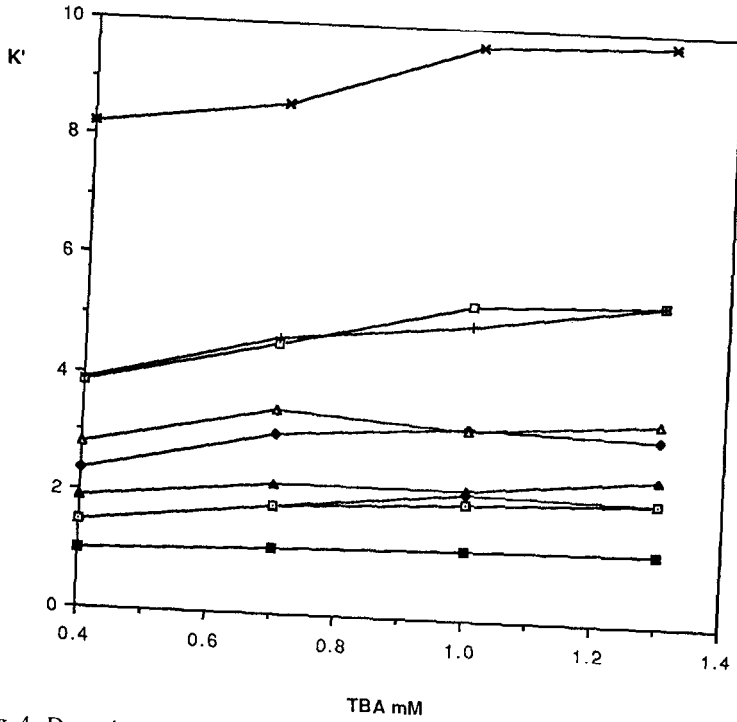


Fig. 4. Dependence of the capacity factor of inorganic anions on the concentration of TBAI in aqueous phosphate eluent of pH 6 and 7.15. Chromatographic conditions as in Fig. 3. pH 6:  $\diamond$ ,  $\text{Cl}^-$ ;  $\blacklozenge$ ,  $\text{NO}_2^-$ ,  $\text{Br}^-$ ;  $\square$ ,  $\text{NO}_3^-$ ;  $\times$ ,  $\text{SO}_4^{2-}$ . pH 7.15:  $\blacksquare$ ,  $\text{Cl}^-$ ;  $\square$ ,  $\text{NO}_2^-$ ;  $\blacktriangle$ ,  $\text{Br}^-$ ;  $\triangle$ ,  $\text{NO}_3^-$ ;  $+$ ,  $\text{SO}_4^{2-}$ .

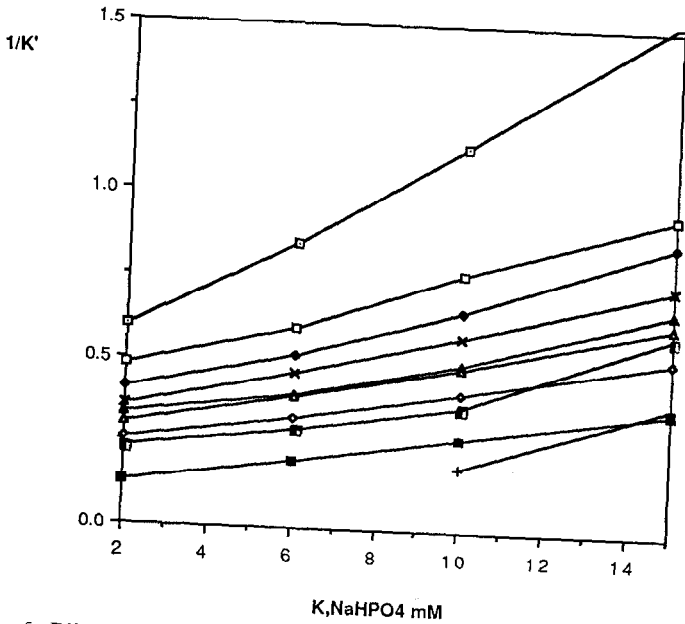


Fig. 5. Effect of phosphate concentration on anion retention. Eluent: 1 mM TBAI at pH 6.4 and 7.15. Chromatographic conditions as in Fig. 3. pH 6.4:  $\square$ ,  $\text{Cl}^-$ ;  $\blacktriangle$ ,  $\text{NO}_2^-$ ;  $\triangle$ ,  $\text{Br}^-$ ;  $\blacksquare$ ,  $\text{NO}_3^-$ ;  $+$ ,  $\text{SO}_4^{2-}$ . pH 7.15:  $\square$ ,  $\text{Cl}^-$ ;  $\blacklozenge$ ,  $\text{NO}_2^-$ ;  $\times$ ,  $\text{Br}^-$ ;  $\diamond$ ,  $\text{NO}_3^-$ ;  $\blacksquare$ ,  $\text{SO}_4^{2-}$ .



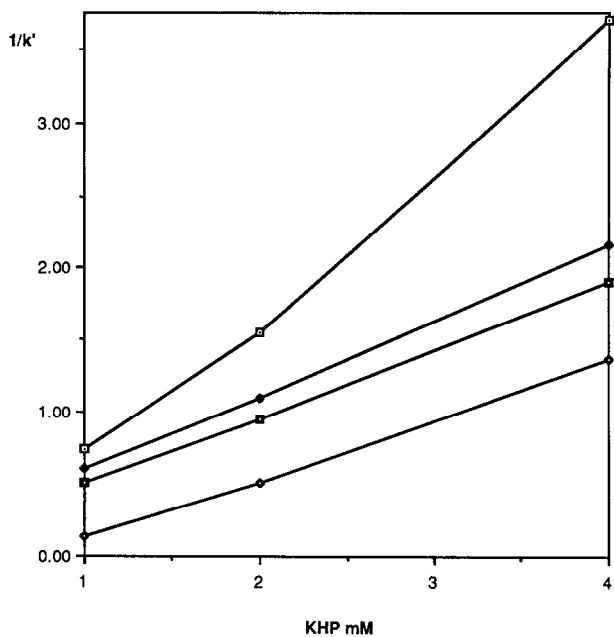


Fig. 6. Effect of phthalate concentration on anion retention. Eluent: 1 mM TBAI at pH 4.05. Chromatographic conditions as in Fig. 3. □, Cl<sup>-</sup>; ◆, NO<sub>2</sub><sup>-</sup>, Br<sup>-</sup>; ■, NO<sub>3</sub><sup>-</sup>; ◇, SO<sub>4</sub><sup>2-</sup>.

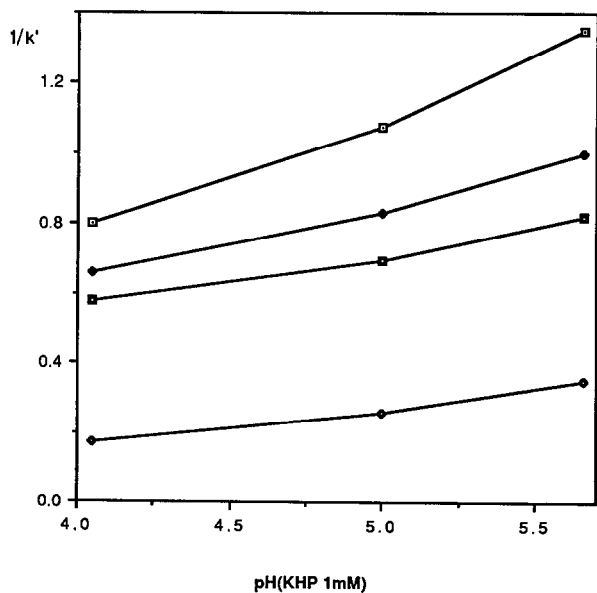


Fig. 7. Dependence of anion retention on pH with phthalate eluent containing 1 mM KHP and 1 mM TBAI. Chromatographic conditions as in Fig. 3. □, Cl<sup>-</sup>; ◆, NO<sub>2</sub><sup>-</sup>, Br<sup>-</sup>; ■, NO<sub>3</sub><sup>-</sup>; ◇, SO<sub>4</sub><sup>2-</sup>.

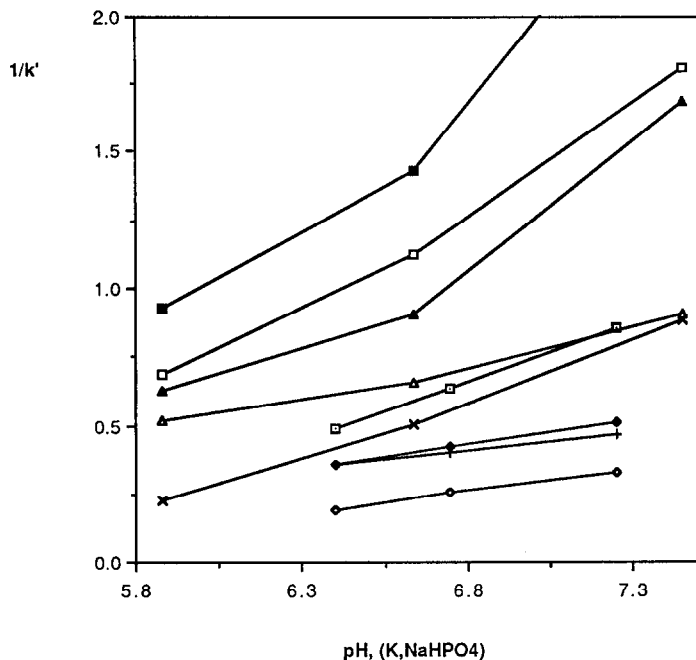


Fig. 8. Dependence of anion retention on pH with phosphate-buffered eluent. Eluent: TBAI concentration, 1.0 mM; phosphate buffer concentration, 6 and 22.5 mM. Chromatographic conditions as in Fig. 3. 6 mM: □, Cl<sup>-</sup>; ◆, NO<sub>2</sub><sup>-</sup>; +, Br<sup>-</sup>; ◇, NO<sub>3</sub><sup>-</sup>. 22 mM: ■, Cl<sup>-</sup>; □, NO<sub>2</sub><sup>-</sup>; ▲, Br<sup>-</sup>; △, NO<sub>3</sub><sup>-</sup>; ×, SO<sub>4</sub><sup>2-</sup>.

buffer). An increase in the buffer concentration results in a decrease in the capacity factor, as shown in Fig. 5 (phosphate buffer) and Fig. 6 (phthalate buffer). pH has a similar effect on the capacity factor, as an increase in pH shifts the equilibria of phthalate to HP<sup>-</sup> and P<sup>2-</sup>, of phosphate to H<sub>2</sub>P<sup>-</sup>, HP<sup>2-</sup> and P<sup>3-</sup> or, generally, to B<sup>-</sup> and B<sup>2-</sup>. A higher B<sup>-</sup> concentration yields a lower capacity factor, as shown in Fig. 7 (with phthalate buffer) and Fig. 8 (with phosphate buffer). The basis for this observation may be two-fold<sup>9</sup>. First, a higher buffer concentration means that the ionic strength of the eluent increases, which will tend to shield the sample ions from the ion-interaction reagent ions on the surface, thus decreasing its retention. Second, the buffer ions, which would normally be less strongly retained than the sample, will compete more effectively with the sample ions for retention on the surface at high buffer concentration.

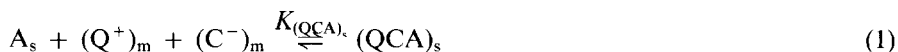
In order to understand these results better, we shall use a mathematical model describing the relationship between  $k'$  and those factors which influence the anion retention.

**Retention model.** Retention of inorganic anions on a Partisil octadecyl-bonded stationary phase can be considered as a result of two processes: (a) the formation of an electrical double layer at the boundary of adsorbed TBA and (b) an exchange process. The quaternary ammonium salt (Q<sup>+</sup>C<sup>-</sup>) was pre-adsorbed on the surface of the stationary phase based on hydrophobic interactions and led to a dynamic adsorption equilibrium of Q<sup>+</sup> between the solid phase and mobile phase. The co-anion C<sup>-</sup> of

$R_4N^+$  formed a secondary layer on the adsorbed  $Q^+$ ; it is here that the ion-exchange process takes place. In our system three significant ion-exchange equilibria have to be considered: ion exchange between (1) the co-anion of  $R_4N^+$  and the anion of additional ionic strength ( $B^-$ ) (hydrogenphosphate or hydrogenphthalate), (2) the co-anion of  $R_4N^+$  and the analyte anions ( $X^-$ ) and (3) the anion of ionic strength salt that replaced the co-anion of adsorbed  $R_4N^+$  and the analyte anion.

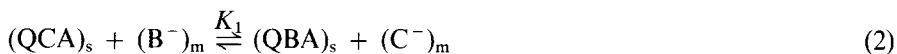
Concerning the buffer anions, the following predominant ionic forms are calculated. For phthalate buffer at pH 5 (a typical experimental condition), and using  $K_{a_1}$  and  $K_{a_2}$  values of  $1.3 \cdot 10^{-3}$  and  $3.9 \cdot 10^{-6}$ , respectively, the ratio  $HP^-/H_2P = 130$  and the ratio  $HP^-/P^{2-} = 2.6$ .  $HP^-$  is therefore the predominant form, but  $P^{2-}$  is not negligible. For phosphate buffer at pH 6 (also a typical experimental condition), and using  $K_{a_1}$ ,  $K_{a_2}$  and  $K_{a_3}$  values of  $7.52 \cdot 10^{-3}$ ,  $6.23 \cdot 10^{-8}$  and  $2.2 \cdot 10^{-13}$ , respectively, the ratio  $H_2PO_4^-/HPO_4^{2-} = 16$  and the ratio  $H_2PO_4^-/H_3PO_4 = 7520$ .  $H_2PO_4^-$  is therefore the predominant form. In the model we shall only consider monovalent buffer or additive anions. We also assume that the adsorption on the stationary phase of an organic ion other than quaternary ammonium ion is negligible. The amount of quaternary ammonium salt and the ionic strength salt (or buffer components) added to the mixed eluent are approximately equal. The major equilibria in the retention processes can be expressed as follows:

(a) The adsorption of quaternary ammonium by the stationary phase:



where  $A_s$  is the number of free sites on the stationary phase,  $(Q^+)_m$  and  $(C^-)_m$  are the quaternary ammonium ion and the co-anion in the mobile phase, respectively, and  $K_{(QCA)_s}$  is the equilibrium constant.

(b) The ion-exchange equilibria:



where  $(B^-)_m$  and  $(X^-)_m$  are the buffer anion and sample anion, respectively.  $K_1$  and  $K_2$  are the equilibria constants of eqns. 2 and 3, respectively.

(c) The sorption capacity ( $K_0$ ):

$$K_0 = A_s + (QXA)_s + (QCA)_s + (QBA)_s \quad (4)$$

where  $K_0$  is the sorption capacity of the stationary phase and is a measure of the total number of sites that can be occupied in the retention process (moles sorbed per gram of sorbent).

(d) The capacity factor  $k'_x$ , given by

$$k'_x = q(QXA)_s/(X^-)_m \quad (5)$$

where  $q$  is the phase ratio.

Combining eqns 1–4 with elimination of  $A_s$ ,  $(QCA)_s$  and  $(QBA)_s$  and substituting the solution of  $(QXA)_s$  into eqn. 5 gives

$$1/k'_x = 1/qK_0[(C^-)_m/K_1^{1/2}K_2^{1/2} + K_1^{1/2}(B^-)_m/K_2^{1/2} + 1/K_{(QCA)_s} K_1^{1/2}K_2^{1/2}(Q^+)_m + (X^-)_m] \quad (6)$$

$$1/k'_x = 1/qK_0K_1^{1/2}K_2^{1/2}[(C^-)_m + 1/K_{(QCA)_s}(Q^+)_m + K_1(B^-)_m + K_1^{1/2}K_2^{1/2}(X^-)_m] \quad (7)$$

*Interpretation of the experimental results.* Eqn. 7 describes a linearship between the reciprocal capacity factor,  $(k')^{-1}$ , and the concentrations in the mobile phase of the buffer anion or more generally the ionic strength of the mobile phase ( $B^-$ ), the anion of the quaternary ammonium salt ( $C^-$ ), the reciprocal quaternary ammonium ion ( $Q^+$ ) and the sample anion ( $X^-$ ). The linear relationship between the reciprocal capacity factor and the buffer anion concentration (eqn. 7) was demonstrated experimentally (Figs. 5 and 6). pH has a similar effect on the capacity factor. An increase in pH increases the concentration of the phthalate or phosphate ions, thus increasing  $B^-$  and  $B^{2-}$  (Figs. 7 and 8). As the ratio  $B^-/B^{2-}$  may change substantially when the pH is varied over a relatively broad range, the relationship between pH and  $(k')^{-1}$  may no longer be linear (see, for example, Fig. 8). The relationship between the capacity factor and the quaternary ammonium salt concentration is more complicated. Indeed, an increase in the latter results in an increase of both ions ( $C^-$  and  $Q^+$ ) in the mobile phase, but these ions, according to eqn. 7, have opposing effects on the capacity factor.

Bartha and Vigh<sup>21</sup> emphasized the competing effects on the capacity factor of sample ions of an increased surface concentration of the hydrophobic ion ( $Q^+$ ) and an increased mobile phase concentration of the counter ion ( $C^-$ ). We found that an increased concentration of TBA in the mobile phase results in increased adsorption on the hydrophobic surface, as indicated by the adsorption isotherm, and hence increased retention of the sample anions. Unless the counter ion,  $C^-$ , is a long-chain compound, which was not the case in our experiments, counter ions are not expected to have a large effect on the adsorption of the quaternary ammonium salt. The quaternary ammonium counter ion ( $C^-$ ) can, however, compete with the sample anions for retention on the surface, especially at high mobile phase concentrations of  $C^-$ . The retention of sample anions may then decrease.

To explain the results in Figs. 3 and 4 with our model, we have rewritten eqn. 7 using eqn. 1:

$$1/k'_x = 1/qK_0K_1^{1/2}K_2^{1/2}\{[(QCA)_s + A_s]/K_{(QCA)_s}(Q^+)_mA_s + K_1(B^-)_m + K_1^{1/2}K_2^{1/2}(X^-)_m\} \quad (8)$$

or

$$1/k'_x \propto [1/(Q^+)_m] \cdot [(QCA)_s + A_s]/A_s$$

where  $(Q^+)_m$  is the amount of quaternary ammonium ion not adsorbed at equilibrium,  $(QCA)_s$  the amount of quaternary ammonium salt QC adsorbed at equilibrium and  $A_s$  the number of free sites at equilibrium.

We shall now consider four situations to explain the experimental results shown in Figs. 3 and 4.

(1) At low quaternary ammonium salt concentration  $(Q^+)_m$  is low,  $A_s$  is high and  $(QCA)_s$  is small compared with  $A_s$ . This means that

$$1/k'_x \propto 1/(Q^+)_m$$

or

$$k'_x \propto (Q^+)_m$$

The initial part of the curve shows the greatest increase in  $k'_x$  for a given increase in TBA concentration.

(2) When half of the initially available adsorption sites on the resin are occupied,  $A_s = (QCA)_s$ . This means that

$$1/k'_x \propto 2/(Q^+)_m$$

or

$$k'_x \propto (Q^+)_m/2$$

and the increase in  $k'_x$  with increasing TBA concentration is lower than in the first instance; the curve has a slower slope.

(3) Still increasing the quaternary ammonium salt concentration leads to a maximum of the  $k'_x$  versus  $(Q^+)_m$  curve;  $A_s$  is very low and  $(QCA)_s \propto K_0$  is much larger than  $A_s$ . This means that

$$1/k'_x \propto [(QCA)_s/A_s] [1/(Q^+)_m] \propto (K_0/A_s) [1/(Q^+)_m]$$

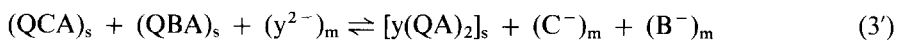
or

$$k'_x \propto (A_s/K_0) (Q^+)_m$$

As  $A_s/K_0$  is very small, the increase in  $k'_x$  with TBA also becomes very small or even zero.

(4) A further increase in the quaternary ammonium salt concentration reduces the capacity factor ( $A_s \rightarrow 0$ ) when all free sites are occupied. The term  $A_s/K_0$ , independently of the magnitude of  $(Q^+)_m$ , will tend to zero if  $A_s$  tends to zero. In this TBA concentration range,  $k'_x$  decreases for a given increase in TBA concentration. This mathematical result means in fact that the maximum amount of TBA that can be adsorbed is reached (saturation of the surface of the solid material), and that increasing amounts of TBA in the mobile phase result in increased competition between the counter ions and sample ions. Although under our experimental conditions the possibility of micelle formation is very small, this effect cannot be totally excluded as an alternative explanation for the reduction in the capacity factor at high TBA concentration.

Corresponding to eqn. 3, the ion exchange of a divalent anion  $y^{2-}$  becomes



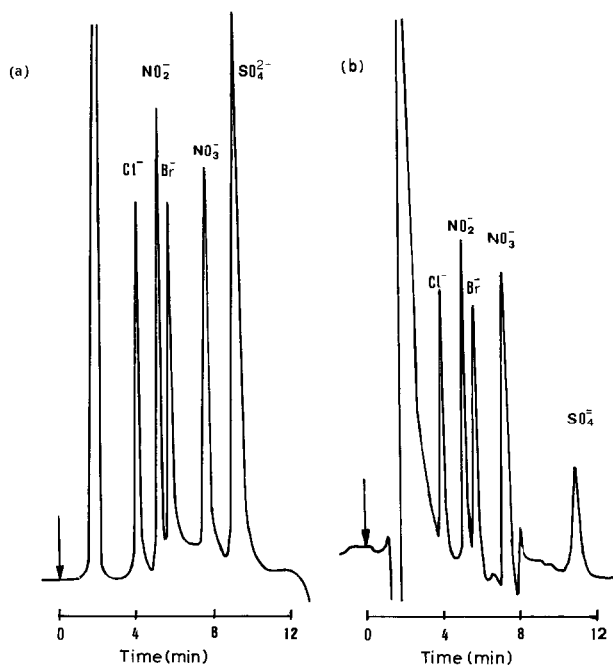


Fig. 9. Separation of sample anions with TBAI-phthalate and TBAI-phosphate eluents. Chromatographic conditions: Partisil 10 ODS-3 column ( $250 \times 4.6$  mm I.D.); flow-rate, 2 ml/min; sample injection volume, 25  $\mu$ l; conductivity detection at room temperature. Eluent: (a) TBAI concentration 8 mM, phthalate concentration 1.0 mM, pH 6; (b) TBAI concentration 0.5 mM, phosphate concentration 4 mM, pH 7.3.

TABLE II

PERFORMANCE CHARACTERISTICS OF COLUMNS FOR THE SEPARATION OF A STANDARD ANION MIXTURE

Column	Eluent	pH	Retention time (s)				
			Cl <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	Br <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>
ODS-3	TBAI (0.5 mM)- K <sub>2</sub> NaHPO <sub>4</sub> (4 mM)	7.3	240	315	338	425	645
ODS-3	TBAI (0.5 mM)- KHP (1 mM)	5.0	210	250	268	324	540
ODS-3	TBAI (8 mM)- KHP (1 mM)	6.0	229	302	342	462	528
Hamilton PRP-X100	KHP (1 mM)	5.5	128	148	169	195	490
Hamilton PRP-X100	<i>p</i> -Hydroxybenzoic acid (4 mM)	8.5	160	186	242	300	750
Vydac 302 IC*	KHP (2 M)	5.0	—	—	—	—	—

\* Efficiency data supplied by manufacturer.

Using a similar treatment as above to eqn. 3' results in a quadratic equation. The solution of this equation is too complex a function of ( $B^-$ ), ( $C^-$ ), ( $X^-$ ) and ( $Q^+$ ) to be useful for interpretation of the experimental results. The plot of  $SO_4^{2-}$  has a more pronounced convex shape than the plots for monovalent anions (Fig. 3).

#### *Partisil 10 ODS-3 column performance characteristics*

The efficiency of nonpolar columns is usually assessed by chromatographing aromatic solutes with methanol-water as the mobile phase. However, such an evaluation of the performance of a nonpolar packing material has no meaning when it is used in an ion-pair or ion-interaction chromatographic rather than in a reversed-phase system<sup>8</sup>. Moreover, a realistic test for an IPC or IIC system must be carried out. The ODS-3 columns used for this performance study were all freshly slurry packed in the laboratory. A standard mixture containing 20 ppm of chloride, 40 ppm of nitrite and sulphate and 50 ppm of bromide and nitrate was used for the evaluation of the column performance. Sample injection volumes were 25  $\mu$ l. The chromatograms obtained are shown in Fig. 9. The chromatograms obtained with the above standard mixture using TBAI phthalate (pH 6) and TBAI phosphate (pH 7.3) were used to calculate the column efficiency and the resolution of a critical peak pair. The results are given in Table II and show that an eluent containing tetrabutylammonium iodide and phthalate buffer has more advantages than using a phosphate buffer. The ODS-3 column gives the highest theoretical plate number when an eluent containing 0.5 mM TBAI and 1 mM KHP was used. Under these conditions, the resolution of the nitrite-bromide peak pair and the peak heights of chloride, nitrate and sulphate, however, were low. When a more concentrated TBAI (8 mM) eluent was used at a higher pH (6), the column gave a lower theoretical plate number, but an adequate resolution for the nitrite-bromide peak pair. In addition, we obtained the highest conductance (peak heights) for chloride, nitrate and sulphate, and also the shortest overall retention time.

Efficiency, theoretical plates ( $k' = 4$ )		Resolution of critical peak pair		Peak height (cm) (conductance measurement)		
Per column	Per metre	$NO_2^- - Br^-$	$NO_3^- - SO_4^{2-}$	$Cl^-$	$NO_3^-$	$SO_4^{2-}$
3688	14 752	1.14	6.00	6.4	8.1	3.2
4996	19 984	0.95	7.20	6.8	6.4	4.1
3780	15 120	1.38	1.52	10.2	11.7	19.4
2158 ( $Br^-$ ) (150 cm column length)	14 387	1.40	1.33 ( $Cl^- - NO_2^-$ )	6.7	2.9	1.2
1442 (150 cm column length)	9613	1.70	0.60 ( $F^- - CO_3^{2-}$ )	9.2	2.0	2.5
4936 ( $NO_3^-$ )	19 744	—	—	—	—	—

TABLE III  
COMPARISON OF COLUMN PERFORMANCE DEGRADATION WITH TBAL-KHP AND TBAL-PHOSPHATE ELUENTS

Eluent	Retention time (s)				Resolution ( $R_s$ ), $\text{NO}_2^-$ - $\text{Br}^-$	Theoretical plates per column	Peak height (cm)			Time period	No. of injections
	$\text{Cl}^-$	$\text{NO}_2^-$	$\text{Br}^-$	$\text{NO}_3^-$			$\text{SO}_4^{2-}$	$\text{Cl}^-$	$\text{NO}_3^-$		
8 mM TBAL-	230	295	328	440	1.20	4190	8.80	8.20	12.5	3-8/1/86	56
1 mM KHP, pH 5	220	290	325	445	1.06	2943	8.30	9.00	17.0	9-16/1/86	38
	235	298	325	429	0.90	2824	11.1	12.0	19.9	16/1-4/2/86	100
	238	305	325	425	0.59	2772	11.0	10.2	21.0	5/2-6/5/86	85
0.5 mM TBAL-				440	1.54		5.3	6.5	3.6	12/10/85-12/11/85	80
4 mM $\text{K}_2\text{NaHPO}_4$ ,				325	0.93		5.1	4.7	5.9		
pH 7.3				320	0.73		5.9	5.2	6.1		
8 mM TBAL-	184	238	269	378	0.94	2594	8.0	7.9	14.4	Repacked column	-
1 mM KHP, pH 6.0				434							



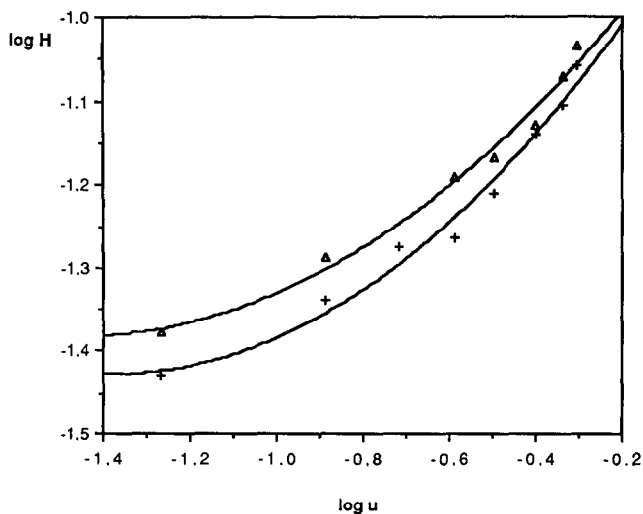


Fig. 10. Plate height *versus* mobile phase flow-rate plots for a laboratory-packed Partisil 10 ODS-3 column. Eluent: TBAI concentration 0.5 mM, KHP concentration 1 mM, pH 5. Chromatographic conditions as in Fig. 3. +, Nitrate;  $\Delta$ , sulphate.

The performance of the ODS-3 column was also compared with those of Hamilton PRP-X100 and Vydac 302 IC low-capacity pre-packed ion-exchange columns. The separation of anions with these ion-exchange columns was carried out with a simple phthalate eluent. The plate number and resolution values given in Table II show that the efficiency of the ODS-3 column when used with a TBAI phthalate eluent is as good as that of the commercially pre-packed columns. The peak heights of nitrate and sulphate obtained with the ODS-3 column using TBAI-phthalate eluent were several times higher than those obtained with the PRP-X100 column. This indicates that the TBAI-phthalate eluent together with an ODS-3 column provides a sensitive means of separation and detection of common inorganic anions. In addition, the same order of elution of the analytes as with the different ion-exchange columns listed in Table II was obtained, indicating that an ion-exchange mechanism is involved in the separation of anions when using a reversed-phase ODS-3 packing.

The plate height ( $H$ ) is also a measure of the column packing according to Snyder and Kirkland<sup>22</sup>. The best packed column will give a value of  $H$  between 0.01 and 0.03 mm. The relationship between plate height and mobile phase velocity was studied (Fig. 10). The plate heights for nitrate were 0.037 mm at a mobile phase flow-rate of 0.4 ml/min and 0.053 mm at 2 ml/min and those for sulphate were 0.042 mm at 0.4 ml/min and 0.064 mm at 2 ml/min. These values are close to those given by Snyder and Kirkland<sup>22</sup>.

#### *pH limitation and column degradation*

The basic material of the Partisil ODS-3 packing consisted of silica gel. This material provides excellent rigidity of the packing and hence tolerance to high back-pressures and flow-rates, but creates problems when eluents of high or low pH are used. In addition, the tendency to adsorb fluoride makes it difficult to determine this ion.

The pH limitation towards alkaline eluents is imposed by the solubility of the silica material and represents a serious drawback to the use of the ODS-3 column. In addition, phosphate eluents are relatively aggressive and attack the silica packing<sup>22</sup>, especially at higher pH. Therefore, it was necessary to seek other eluents to replace the TBAI-phosphate eluent.

Phthalate is usually a powerful eluent, often used in low-capacity single-column anion-exchange chromatography<sup>19</sup>. The equivalent conductance of phthalate is relatively low compared with other inorganic ions, *e.g.*, the limiting equivalent conductance of phthalate is 38 whereas those of phosphate, potassium and sodium ions are 67, 74 and 50, respectively. A low conductance is an important factor when using a conductivity detector. We found that for anion separations using a reversed-phase ODS-3 packing, phthalate is the most suitable additive when a quaternary ammonium salt is present. The advantages of using phthalate as an additive to the TBAI eluent are (1) better anion separations can take place even when the pH is below 6, (2) there is a wider pH range (4–6.5) in which the variation of the chromatograms is relatively small, (3) a relatively low background conductance increased the detection response when using conductivity detection and (4) reduced column degradation and longer column lifetime were obtained compared with the use of a TBAI-phosphate eluent.

Table III shows the degradation data when TBAI-phosphate and TBAI-phthalate eluents were used. The lifetime of the ODS-3 column was approximately three times longer when using the TBAI-phthalate eluent. For further prolongation of the column lifetime a regeneration technique (repacking the column) can be used<sup>23</sup>. In our work, we tested a simple repacking procedure in which no fresh column material was added, *i.e.*, the packing was washed with methanol and then suspended in de-gassed 2-propanol. Using the common slurry method for repacking, gave the results shown in Table III. The repacked columns are good enough for a new series of analyses.

## CONCLUSION

Ion-interaction or ion-pair reversed-phase chromatography carried out with an aqueous eluent containing a quaternary ammonium salt and a phthalate buffer was found to be an efficient system for the separation and determination of inorganic anions. The presence of phthalate and variation of the pH in the eluent have a minor influence on the adsorption of tetrabutylammonium iodide on an octadecyl-bonded stationary phase. A model developed to explain the retention of inorganic anions has been verified by the experimental results. Ion-exchange-based anion retention is primarily decided by the adsorption of a quaternary ammonium salt on the stationary phase and its amount in the mobile phase; second, it is influenced by the type and amount of buffer and the pH of the eluent.

## REFERENCES

- 1 H. Small, *Anal. Chem.*, 55 (1983) 235A–240A.
- 2 J. A. Mosko, *Anal. Chem.*, 56 (1984) 629–633.
- 3 T. H. Jupille and D. T. Gjerde, *J. Chromatogr. Sci.*, 24 (1986) 427–433.
- 4 N. E. Skelly, *Anal. Chem.*, 54 (1982) 712–715.

- 5 W. E. Barber and P. W. Carr, *J. Chromatogr.*, 316 (1984) 211–215.
- 6 R. M. Cassidy and S. Elchuk, *Anal. Chem.*, 54 (1982) 1558–1563.
- 7 I. Molnár, H. Knauer and D. Wilk, *J. Chromatogr.*, 201 (1980) 225–240.
- 8 M. Dreux, M. Lafosse, P. Agbo-Hazoume, B. Chaabane-Doumandji, M. Gibert and Y. Levi, *J. Chromatogr.*, 354 (1986) 119–128.
- 9 W. E. Barber and P. W. Carr, *J. Chromatogr.*, 301 (1984) 25–38.
- 10 A. T. Melin, M. Ljungcrantz and G. Schill, *J. Chromatogr.*, 185 (1979) 225–239.
- 11 A. Sokolowski, *Chromatographia*, 22 (1986) 168–176.
- 12 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 202 (1980) 333–345.
- 13 Z. Iskandarani and D. J. Pietrzyk, *Anal. Chem.*, 54 (1982) 1065–1071.
- 14 R. L. Smith, Z. Iskandarani and D. J. Pietrzyk, *J. Liq. Chromatogr.*, 7 (1984) 1935–1959.
- 15 B. A. Bidlingmeyer and F. V. Warren, Jr., *Anal. Chem.*, 54 (1982) 2351–2356.
- 16 R. S. Deelder and J. H. M. Van den Berg, *J. Chromatogr.*, 218 (1981) 327–339.
- 17 J. Ståhlberg, *J. Chromatogr.*, 356 (1986) 231–245.
- 18 R. J. Dennis and G. K. Pagenkopf, *Anal. Chem.*, 56 (1984) 85–88.
- 19 P. R. Haddad, P. E. Jackson and A. L. Heckenberg, *J. Chromatogr.*, 346 (1985) 139–148.
- 20 V. K. Jones, S. A. Frost and J. G. Tarter, *J. Chromatogr. Sci.*, 23 (1985) 442–445.
- 21 A. Bartha and G. Vigh, *J. Chromatogr.*, 395 (1987) 503–509.
- 22 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, pp. 30, 170–172 and 296.
- 23 N. C. Avery and N. Light, *J. Chromatogr.*, 328 (1985) 347–352.