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Temperature programming in macrocycle-based ion chromatography

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ABSTRACT

The well documented affinity of macrocyclic ligands for alkali and alkaline earth metal cations has been exploited in performing chromatographic separations of both cations and anions. It is shown that because the complexation reaction of metal ions with macrocycles is exothermic, the column capacity, and hence the retention of ions, is decreased as the column temperature is increased. While ion retention in classical ion exchange is also a function of temperature, the effect is less pronounced than in macrocycle-based systems. Temperature gradient elution of anions on macrocycle-based columns is performed based on the decrease of column capacity as the temperature is increased, showing improvements over separations performed under isothermal conditions.

INTRODUCTION

Since their discovery, macrocycles, such as crown ethers and cryptands, have been noted for their unique complexing abilities. This capability to selectively complex cations has led to their use in ion chromatography, as discussed in another paper by us at this symposium [1]. The selectivity of macrocycles for cations is mainly determined by the ability of the cation to fit into the central cavity of the macrocycle. Those cations that fit best into the cavity are often bound strongest, and can be separated from cations that are too small or too large to fit into the cavity.

Since most macrocycles are neutral molecules, the cations are complexed into the macrocycle in close association with an anion in order to maintain electrical neutrality. Since the macrocycle is generally hydrophobic in nature, the most hydrophobic anions allow greater interaction of the cation with the macrocycle in low dielectric media. Thus, not only cations with a common anion, but also anions with a common cation can be separated [2–6].

Gradient elution ion chromatography was generally considered incompatible with conductometric detection until recently. The changes in eluent composition or concentration resulted in significant baseline distortion, and impurities in eluents also resulted in baseline aberrations and spurious peaks. With improvements in column and suppressor technology, gradient separations of both anions and cations have become more feasible using salts of weak acids for anion separations and protonated cations of weak bases or amino acids as eluents for cation gradients [7,8]. Gradients in

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unsuppressed ion chromatography have been performed using a concentration gradient between two isoconductive eluents [9]. Anion gradients have also been performed by our group by altering the column capacity instead of the eluent ionic strength by using macrocyclic ligand–cation complexes as the exchange site. The column anion-exchange capacity is decreased during the course of the separation by switching from a mobile phase cation that is relatively strongly bound by the macrocycle to a cation with a lower affinity for the macrocycle, resulting in fewer macrocycle–cation complexes, and hence lower column anion exchange capacity [10,11].

While temperature gradient separation is a very common technique in gas chromatography, its use has been very limited in high-performance liquid chromatography (HPLC). It has been shown that increasing the column temperature causes the retention of some ions to increase, while decreasing the retention of others. Improved separations have been shown for specific analytes of interest by careful choice of operating temperature [12–15].

The dependence of cation retention on temperature with macrocycle-based systems was noted by Iwachido *et al.* [5], who described the changes in the retention of alkali metal cations between 15°C and 55°C. In our work the effect of temperature on the retention of both anions and cations on macrocycle-based stationary phases is more closely examined and compared to classical ion exchange. A novel gradient separation of anions is presented based on the decrease of column capacity with increasing temperature.

EXPERIMENTAL

Materials

Reagent grade cryptand *n*-decyl-2.2.2 (D-2.2.2), whose structure is shown in Fig. 1, was obtained from EM Science (Gibbstown, NJ, U.S.A.). All compounds used to make eluents and standards were reagent grade. Water used in making eluents was purified to 18 MΩ resistivity using a Milli-Q purification system (Millipore) and was degassed by sparging with helium.

Apparatus

A Dionex Series 4000i ion chromatograph was used in conjunction with Dionex anion micro membrane (AMMS) and cation micro membrane (CMMS) suppressors for eluent suppression prior to conductometric detection with a Dionex CDM-2 conductivity detector. The suppressant was 12.5 m*M* H_2SO_4 flowing at 3–5 ml/min for anion separations and 50 m*M* tetrabutylammonium hydroxide (TBAOH), flowing at

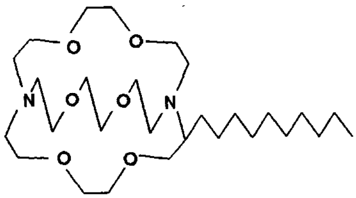

Fig. 1. Structure of cryptand *n*-decyl-2.2.2 (D-2.2.2).

3–5 ml/min for cation separations. Columns used were Dionex AG-4A and AS-4A anion separators, CG-3 and CS-3 cation separators, and NS-1 MPIC columns. Temperature was controlled either by coiling 1 m of tubing in a Dionex column heater just prior to the column or by placing the column and coiled tubing in a water jacket with a circulating water–ethylene glycol mixture from a constant temperature bath. The chromatograph was controlled and data captured with a personal computer using the Dionex AI400 software.

Column preparation

The macrocycle column was prepared as previously described by circulating a solution of the D-2.2.2 in a methanol–water (60:40) mixture through the MPIC column for 12 h [10].

RESULTS AND DISCUSSION

Macrocycle-based separation system

Macrocycles separate cations on the basis of a complexation–decomplexation reaction rather than an ion-exchange reaction. Thus separations with a pure water mobile phase are possible as no ionic species need to be added to the mobile phase in order to elute the cations from the column [2–6].

We have previously reported the use of macrocycles to separate anions through an ion-exchange mechanism rather than a ligand-exchange mechanism. This was accomplished by the addition to the mobile phase of the hydroxide of a cation that is known to bind to the macrocycle. The cation complexes with the macrocycle, forming an anion-exchange site on the stationary phase and anions are then eluted by the hydroxide eluent. The cation in the mobile phase has a large influence on the column capacity. With the cryptand D-2.2.2, lithium hydroxide eluent shows little retention of anions, while potassium hydroxide eluent of the same concentration shows a very high capacity. This effect of the mobile phase cation on column capacity has been exploited to perform gradient separations of anions by switching from a mobile phase cation that is strongly bound by the macrocycle to one that has a lower affinity for the macrocycle during the course of the separation [10,11].

Temperature effects

In ion chromatography of both cations and anions, the exchange reaction for an analyte ion, A, of charge y, and eluent ion, E, with charge x, can be written as:

$$yE^{x}R_{x} + xA^{y} \rightleftharpoons xA^{y}R_{y} + yE^{x} \qquad (1)$$

where R_x and R_y represent the resin exchange sites occupied by E and A ions respectively. The exchange reaction can be either exothermic or endothermic, depending on the ions involved. The ion-exchange equilibrium can thus be shifted in either direction as the temperature is increased. This results in either a positive or negative change in the capacity factor, k', for the analyte ion.

The complexation of cations with macrocycles can be written as:

$$M^{+} + L \rightleftharpoons ML^{+} \qquad (2)$$

where M^+ is the metal cation, L is the free macrocyclic ligand, and ML^+ is the cation–macrocycle complex. This is most often an exothermic reaction, favoring a lower amount of complexation as the temperature is increased. Thus the capacity factor for cations would be expected to decrease as the temperature is increased for this reason.

Our results illustrating the effect of temperature on retention for both cations and anions in classical ion-exchange and macrocycle-based systems are shown in Figs. 2–4. The retention of anions and cations, by both ion-exchange and ligand-exchange mechanisms, was measured as a function of temperature by placing the appropriate column in a jacket with circulating water controlled by a constant temperature bath around the column in order to control the temperature.

The cation exchange of alkali metal cations was measured using a Dionex CS-3 column with a 10 m*M* HCl eluent at 1.0 ml/min. The retention of alkali metal cations on the macrocycle-based column was measured using a Dionex MPIC NS-1 column, loaded with D-2.2.2 as described above, with a pure water eluent at 1.0 ml/min. Fig. 2a, plotting ln k' (capacity factor) *versus* $1/T$ (where T = temperature), shows that for the ion exchange of alkali metal cations the capacity factors of lithium and sodium increase slightly, while those of potassium, rubidium, and cesium decrease as the temperature is increased. These changes in retention are relatively small. On the other

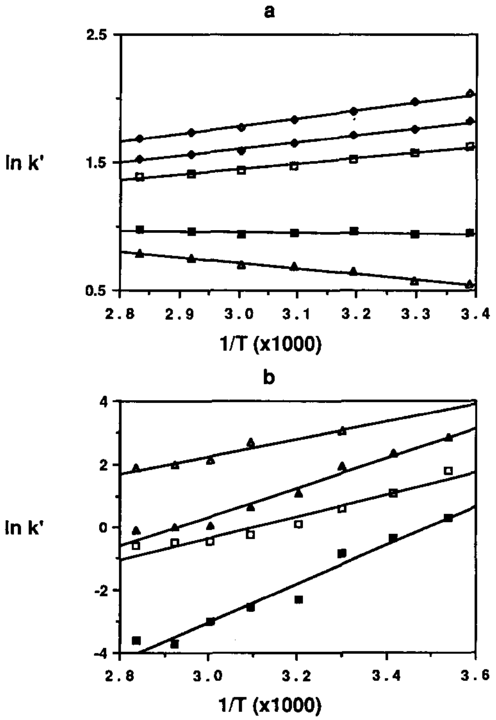

Fig. 2. (a) Variation in ln k' *versus* $1/T$ for alkali metal cations on ion-exchange column, Dionex CS-3 column, 10 m*M* HCl eluent. ◇ = Cs^+; ◆ = Rb^+; □ = K^+; ■ = Na^+; △ = Li^+. (b) Variation in ln k' *versus* $1/T$ for alkali metal cations on macrocycle-based column, pure water eluent. △ = K^+; ▲ = Rb^+; □ = Na^+; ■ = Li^+.

hand, for the macrocycle-based system (Fig. 2b), the capacity factors of all of the alkali metals decrease with increasing temperature. The magnitude of the change in retention with temperature is also much greater than that observed in ion exchange (Fig. 2a).

Similar experiments, using the same temperature controlling apparatus with the same columns, were performed to measure the retention of the alkaline earth cations. The experimental conditions were the same as those for the alkali metals, except that the eluent was 50 m*M* HCl–3.75 m*M* histidine–3.75 m*M* diaminoproprionic acid for the cation-exchange measurements. We observe that the behavior of the alkaline earth metal cations is similar to that of the alkali metals, with retention increasing for all of the cations in ion exchange (Fig. 3a), but decreasing in the macrocycle column (Fig. 3b).

The retention of anions on the ion-exchange and macrocycle columns is compared in Fig. 4a and b, respectively. The anion-exchange column was a Dionex AS-4A column and the eluent was 15 m*M* NaOH eluent flowing at 1.0 ml/min. The macrocycle-based separation was done using the MPIC column loaded with D-2.2.2 with a 20 m*M* NaOH eluent at 1.0 ml/min. Again the columns were placed in a water jacket, with the temperature controlled with a constant temperature bath. As with the cations, in classical ion exchange (Fig. 4a) the trend in capacity factors as a function of

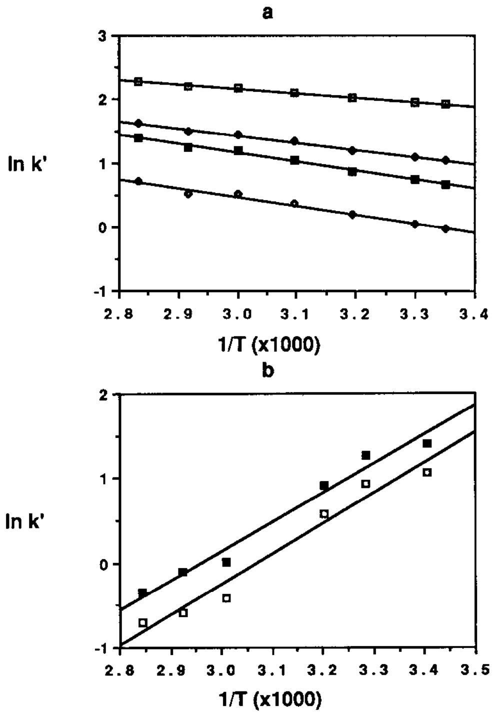

Fig. 3. (a) Variation in ln *k'* *versus* $1/T$ for alkaline earth cations on ion-exchange column, CS-3 column, 50 m*M* HCl–3.75 m*M* histidine–3.75 m*M* diaminopropionic acid eluent: □ = Ba^{2+}; ◆ = Sr^{2+}; ■ = Ca^{2+}; ◇ = Mg^{2+}. (b) Variation in ln *k'* *versus* $1/T$ for alkaline earth cations on macrocycle-based column, pure water eluent: □ = Mg; ■ = Ca.

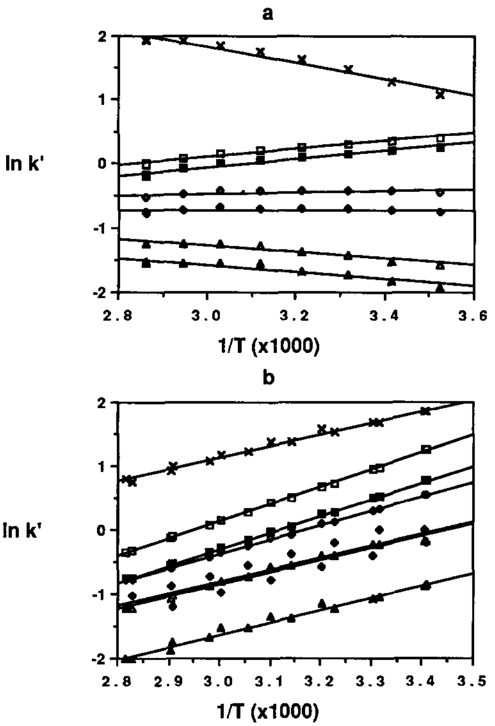

Fig. 4. (a) Variation in ln *k'* *versus* 1/*T* for seven common anions on anion-exchange column, Dionex AS-4A column, 15 m*M* NaOH eluent. (b) Variation in ln *k'* *versus* 1/*T* for same anions on macrocycle-based column, 20 m*M* NaOH eluent. Anions for (a) and (b): ▲ = fluoride; △ = acetate; ◆ = chloride; ◇ = nitrite; ■ = bromide; □ = nitrate; × = sulfate.

temperature varies from ion to ion. The capacity factors of chloride, nitrite, bromide, and nitrate decrease, while those of fluoride, acetate, and sulfate increase with temperature. Except for sulfate, the changes are relatively small. However, with the macrocycle column (Fig. 4b), the capacity factors of all of the anions decrease significantly as the temperature is raised.

The variation of the capacity factor as a function of temperature is described by the equation:

$$\ln k' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \qquad (3)$$

where φ is the phase ratio for the column, a constant for a given column [12] and ΔS is the entropy change. Plots of ln *k'* *versus* 1/*T* for the separations in Figs. 2–4 generate straight lines with a slope equal to $-\Delta H/R$ where ΔH is the enthalpy change for the retention reaction and *R* is the molar gas constant. The ΔH for the exchange (anion) or complexation (cation) retention reactions can thus be calculated. A comparison of the values thus obtained for the ΔH values for the exchange and complexation reactions is shown in Table I. The ΔH values for the complexation reactions are larger than those

TABLE I

MEASURED ΔH VALUES FOR BOTH ION-EXCHANGE AND MACROCYCLE-BASED COLUMNS FOR VARIOUS CATIONS AND ANIONS

Ion	Ion exchange ΔH (kcal/mol)[a]	Macrocycle ΔH (kcal/mol)[b]	Literature ΔH[c]
Li^+	0.837	−12.145	−1.401
Na^+	0.0066	−6.861	−7.620
K^+	−0.833	−5.423	−11.57
Rb^+	−1.081	−9.211	−11.77
Cs^+	−1.218	–	–
Mg^{2+}	2.774	−6.805	–
Ca^{2+}	2.742	−7.118	−0.800
Sr^{2+}	2.227	–	–
Ba^{2+}	1.363	–	–
F^-	1.073	−3.811	
Acetate	0.966	−3.750	
Cl^-	−0.017	−3.734	
NO_2^-	−0.256	−4.451	
Br^-	−1.328	−5.163	
NO_3^-	−1.251	−5.391	
SO_4^{2-}	2.548	−3.594	

[a] The ΔH for the ion-exchange reaction.

[b] For cations the ΔH for the cation–macrocycle complexation on the stationary phase. For anions the ΔH of the exchange reaction between analyte anions and hydroxide ions.

[c] The ΔH for the homogeneous solution cation–macrocycle complexation in water (from ref. 16).

for the exchange reaction, reflecting a much larger dependence on temperature than the ion-exchange reactions.

Column capacity as a function of temperature

The retention of anions on the macrocycle column is affected by two different factors with respect to temperature. The separation takes place mainly by an ion-exchange mechanism, with the cation–macrocycle complex acting as the ion-exchange site. If this mechanism were the only factor, as the temperature is raised, the behavior should resemble that of a classical ion-exchange column. However, Fig. 4b shows that the anion capacity factors decrease with a slope that is greater than that ascribed to the effect of temperature on the ion-exchange reaction (Fig. 4a). Indeed, there is decreased retention even for anions like sulfate that show increased retention with increasing temperature under classical ion-exchange conditions.

In order to better understand this phenomenon, we measured the column capacity at different temperatures by measuring the uptake of the sodium counterion on the column. The column was placed in the jacket connected to the constant temperature bath to carefully control the temperature. The 20 mM NaOH eluent was run through the column to reach equilibrium between the mobile phase and the stationary phase. The column was switched out of line and all of the connecting tubing was rinsed free of eluent by pure water. The column was then switched back on line and the sodium was eluted from the column by 0.1 M HNO_3 and the column effluent was

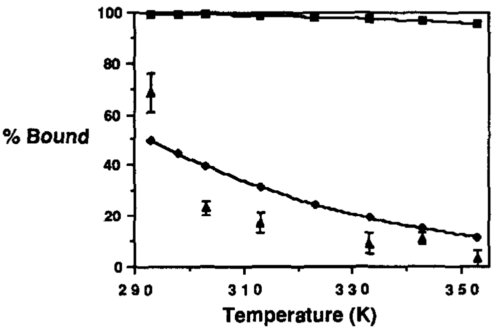

Fig. 5. Comparison of experimental *versus* calculated column anion-exchange capacity of macrocycle column expressed as the percent of macrocycle sites binding sodium cations. ■ = Theory, $K = 8000$; ◆ = theory, $K = 40$; ▲ = experimental.

collected. The sodium concentration in the effluent was measured, and by taking into account the column dead volume and the eluent sodium concentration, the amount of sodium uptake of the column was determined. Fig. 5 describes the drop of column capacity, shown as the percent of macrocyclic sites containing sodium ions.

The Van 't Hoff equation

$$\ln\frac{K_2}{K_1} = \frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{4}$$

relates the change in equilibrium constant (K) with temperature. Thus, the amount of macrocycle sites containing metal ions ($[ML^+]$) at any temperature can be calculated if the complexation constant at a given temperature, the ΔH for the complexation reaction, the total amount of ligand on the column, and the free metal ion concentration are known. In Fig. 5, the experimentally observed values for the percent of the macrocycle sites binding sodium ions are compared to the calculated values using two different possible complexation reaction mechanisms, each with its own values for the binding constant and the ΔH for the reaction. The first theoretical curve is generated using the literature value for the binding constant ($\log K = 3.9$ at 25°C) and the ΔH (-7.62 kcal/mol) for the homogeneous complexation reaction [16] in aqueous solution. This curve is much higher than the observed values, implying that under these conditions complexation does not occur in the aqueous phase. Indeed, in previous work we have directly measured the apparent binding constant ($K = 40$) for sodium with the macrocycle D-2.2.2 under the same chromatographic conditions [10]. This result agrees with those of Bourgoin *et al.* [17], who demonstrated that ion-pair formation leads to binding constants 300 or 400 times lower than for free cations. Using this value for the binding constant and the corresponding ΔH determined from the slope of the Na^+ line in Fig. 2b (using eqn. 3), the second theoretical curve in Fig. 5 was generated. This curve corresponds to a heterogeneous extraction complexation mechanism in which the sodium ion, together with its anion, leaves the aqueous environment upon complexation. The fact that the experimental curve lies close to this second theoretical curve implies that the heterogeneous rather than the homogeneous mechanism is prevalent. This result, in turn, implies that as anions are separated on this column, they undergo an extraction into the hydrophobic stationary phase environment. This conclusion reinforces our interpretation of previous results [10] and is the subject of ongoing investigation. The fact that the complexation constant is of moderate strength makes possible the large changes in column capacity necessary to achieve capacity gradients.

Temperature gradient separation of anions

According to theory, the retention of anions is directly proportional to the column ion-exchange capacity and inversely proportional to the eluent concentration. Most gradients are performed by increasing the eluent strength. However, the retention can also be modified by changing the column capacity. As seen previously, macrocycle-based columns display lowered anion capacity as the temperature is increased. Thus column capacity can be changed throughout the course of a separation by increasing the column temperature during the separation.

The capability to do temperature gradient separations is illustrated by Fig. 6. The column was heated by placing 1 m of tubing immediately preceding the column in a Dionex column heater. The temperature was controlled by the Dionex AI400 software. The eluent was 20 m*M* NaOH flowing at 1.0 ml/min. The separation of an

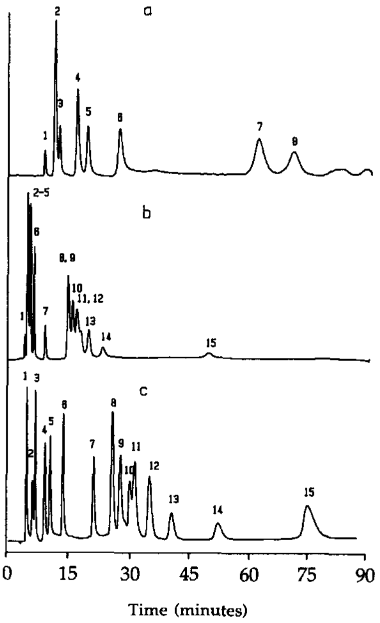

Fig. 6. Separation of 15 common anions: 1 = F$^-$, 1.5 ppm; 2 = acetate, 10 ppm; 3 = Cl$^-$, 2.5 ppm; 4 = NO$_2^-$, 10 ppm; 5 = Br$^-$, 10 ppm; 6 = NO$_3^-$, 10 ppm; 7 = I$^-$, 10 ppm; 8 = SO$_4^{2-}$, 10 ppm; 9 = oxalate, 10 ppm; 10 = CrO$_4^{2-}$, 10 ppm; 11 = MoO$_4^-$, 10 ppm; 12 = fumarate, 10 ppm; 13 = ClO$_4^-$, 10 ppm; 14 = PO$_4^{3-}$, 10 ppm; 15 = phthalate, 10 ppm; under three different chromatographic conditions: (a) at 30°C; (b) at 80°C; and (c) temperature gradient with program starting at 30°C and increasing to reach 80°C beginning at 5 min and ending at 30 min.

anion standard clearly shows the change in column capacity at different temperatures. In Fig. 6a, at 30°C, the first peaks are well resolved, but stronger retained anions do not elute from the column. In Fig. 6b, at 80°C, the stronger retained species elute, but resolution of the earlier peaks is lost. It should also be noted that the selectivity is different at higher temperatures, with iodide eluting before sulfate at 80°C. Fig. 6c displays the ability to perform temperature gradient separations on macrocycle-based columns. The temperature begins to increase at 5 min from 30°C to reach 80°C at 30 min. The resulting chromatogram shows good resolution of the earlier eluting peaks, while the stronger retained peaks elute much sooner than at ambient temperature, due to the decrease in column anion-exchange capacity at elevated temperatures.

To ensure that the loss of capacity is not due to loss of the adsorbed cryptand from the column, the column stability at elevated temperatures was explored on a freshly prepared column. Repeated injections of anions were made while the column was maintained at 80°C. Fig. 7 shows that the column showed a decrease in retention in the first three hours, probably due to the time required to fully reach thermal equilibrium. After equilibrium was reached, the retention showed no change over the duration of the experiment (50 h). Thus, it can be concluded that gradient retention is due to changes in the column capacity because of the decrease in the population of macrocycle–cation complexes, not from the loss of cryptand from the column.

One important consideration in gradient ion chromatography with conductometric detection is the stability of the baseline. With concentration gradients, baseline drift is caused by incomplete suppression of eluent ions, decreasing the quality of the chromatogram. In temperature programming, the baseline may be affected by the increase in temperature of the mobile phase during the chromatogram. Conductivity is a strong function of temperature, with the background conductivity increasing with increasing temperature. In order to maintain a stable baseline, the temperature of the eluent entering the detection cell was kept constant. With suppressed conductivity detection, temperature reequilibration with the room was accomplished in the suppressor. The heated eluent stream was cooled in the suppressor by the room temperature regenerant, which is flowing generally 3–5 times faster than the eluent stream, and which is separated from the eluent by only a thin membrane. This is sufficient to cool the eluent stream to near room temperature before entering the

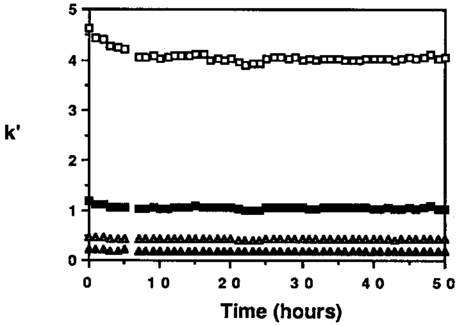

Fig. 7. Macrocycle column stability over time at elevated temperatures (80°C). □ = Sulfate; ■ = nitrate, △ = chloride; ▲ = fluoride.

detection cell, decreasing the amount of baseline perturbation that might otherwise be observed.

Temperature programming with macrocycle-based separation systems shows several possible advantages over other gradient techniques. The vaseline drift and other aberrations caused by increasing the eluent concentration and by eluent impurities are reduced, resulting in a stable baseline. Also, gradient elution can be performed with a single eluent reservoir, eliminating the need for expensive pumps and mixing systems.

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