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Anion chromatography with a crown ether-based stationary phase and an organic modifier in the eluent

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ABSTRACT

The unique ability of macrocyclic ligands, such as the crown ethers and cryptands, to selectively complex alkali metal cations can be used as the basis for chromatographic separations of anions. Specifically, macrocycles which are adsorbed onto a reversed-phase column, form positively charged anion-exchange sites when they combine with eluent cations. Previously we have demonstrated gradient anion separations based on changing the column capacity during the course of the separation by altering the eluent cation, temperature, or organic modifier content using cryptand-based columns. Herein we report that excellent separations can also be achieved using 18-crown-6 based columns. In this column, anion retention increases with increasing eluent strength and organic modifier content. This observation is in keeping with the relatively moderate affinity of crown ethers for alkali metals when compared to cryptands. The separation of anions achieved by optimizing mobile phase variables shows that isocratic separations of anions on the crown-based column are almost as good as separations achieved only under gradient conditions on cryptand-based columns. Cation gradients provide additional improvements on the separations using the crown-based column.

INTRODUCTION

Since their discovery, macrocyclic ligands such as the crown ethers and cryptands have been noted for their ability to selectively complex cations [1,2]. This unique selectivity has been used to perform chromatographic and other types of separations [3–8]. The selectivity of macrocycles for cations is often based on the ability of the cation to fit into the central cavity of the macrocycle, *i.e.*, those cations that fit most closely into the macrocyclic cavity are bound more strongly than those cations that are too small or too large to fit into the cavity. In chromatographic separations, cations that are bound most tightly by the macrocycle are retained longer than the more loosely bound cations [3,4].

Macrocycle-based columns have also been used to separate anions chromatographically with the macrocycle–cation complex serving as the anion-exchange site. This is possible because most macrocycles are uncharged, and thus an anion must be associated with the complex to maintain electrical neutrality. Since the macrocycle is also generally hydrophobic and is associated with the hydrophobic environment of the column, those anions which are more hydrophobic are retained longer. Thus, anions with a common cation can be separated, as well as cations with common anion.

Most macrocycle-based separations have involved a ligand-exchange mechanism. We have explored the use of macrocycle-based anion separations which are based rather on an ion-exchange mechanism. An alkali metal hydroxide solution is used as eluent. The eluent metal ions complex with the stationary phase macrocycles, creating positively charged anion-

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exchange sites. The hydroxide ion elutes sample anions from the column [9].

Using columns of the type described above, gradient separations can be achieved by changing the column capacity during the course of the separation. The column anion-exchange capacity can be varied in several ways, including changing (i) the eluent cation, (ii) the temperature, or (iii) the organic modifier concentration [10]. Gradients of the first type are performed by changing the eluent cation from one which has a high affinity for the macrocycle to one which has a lower affinity. This change results in fewer macrocycle–cation complexes on the column, decreasing the ion-exchange capacity [11, 12]. Gradient separations of the second type are performed by increasing the column temperature during the separation. Since the complexation reaction between cations and macrocycles is generally quite exothermic, increasing the temperature results in fewer column complexes, decreasing the column capacity [12,13]. Gradients of the third type have been used in separations of nucleosides and nucleotides [10]. These capacity gradients enjoy some advantages over conventional gradients in ion chromatography which are carried out by increasing eluent strength, resulting in baseline drift when using conductivity detection. Capacity gradients are carried out with very little or no change in the eluent ionic strength, yielding a stable baseline.

We have previously reported capacity gradient separations on columns based on cryptand-type macrocycles [9–13]. In this work we report anion separations on chromatography columns based on crown ether macrocycles and introduce capacity gradients based on organic modifier concentration.

EXPERIMENTAL

Materials

Reagent-grade cryptands *n*-decyl-2.2.2 (D-2.2.2) and *n*-decyl-2.2.1 (D-2.2.1) (Fig. 1) were obtained from EM Science (Gibbstown, NJ, USA). The *n*-decyl-18-crown-6 and *n*-tetradecyl-18-crown-6 crown ethers were synthesized by the procedure reported by Ikeda *et al.* [14]. Reagent-grade compounds were used in making all standards and eluents. HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Water used in making eluents and standards was purified to 18 MΩ resistivity with a Milli-Q water purification system (Millipore) and all eluents were degassed by sparging with helium.

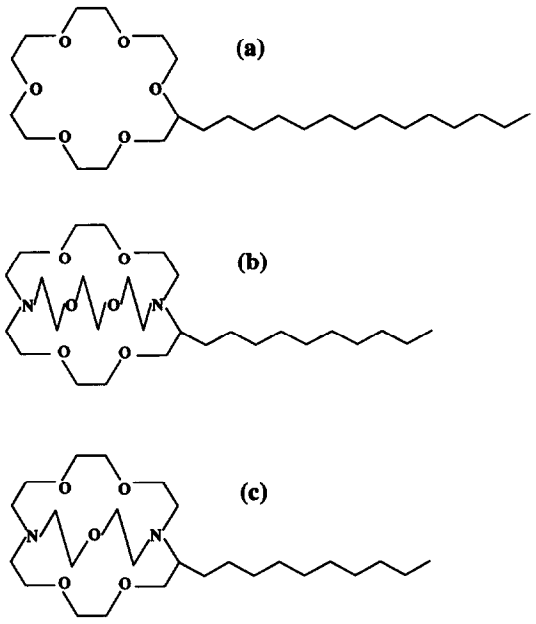

Fig. 1. Structures of (a) *n*-tetradecyl-18-crown-6 (TD-18-crown-6), (b) *n*-decyl-2.2.2 (D-2.2.2) and (c) *n*-decyl-2.2.1 (D-2.2.1).

Instrumentation

A Dionex 4000i series ion chromatograph was used to perform all chromatographic separations. Dionex anion micromembrane (AMMS) suppressors were used for eluent suppression prior to conductometric detection with a Dionex conductivity detector. Suppressant was 25 m*M* H_2SO_4 flowing at 3–5 ml/min. Macrocycle columns were prepared from Dionex MPIC NS-1 columns. The chromatograph was controlled and the data collected on a personal computer using the Dionex AI400 control software.

Column preparation

Macrocycle-based columns were prepared as previously described [8] by recirculating a solution of the macrocycle in a methanol–water (60:40) solution through the column for a period of 12 h.

RESULTS AND DISCUSSION

Factors affecting anion retention

In traditional ion chromatography, the anion retention is mainly a function of the eluent strength, with other factors playing a lesser role. With macrocycle-based chromatography the retention is determined not only by the eluent strength but also by the column capacity, a factor that is usually fixed in traditional ion chromatography. The column capacity in macrocycle-based chromatography is not only a function of the amount of ligand on the column, but also the number of cation–macrocycle complexes formed. The number of complexes formed can be affected by the identity of the eluent cation, the cation concentration, and the amount of organic modifier added to the eluent. The effect of each of these factors will be discussed.

Eluent cation

The ability of the macrocycle to bind cations is expressed by the binding constant for the reaction between the macrocycle adsorbed on the column and the cation in the eluent. In our previous work we used the cryptands D-2.2.1 and D-2.2.2 as the basis for columns to separate anions. In Fig. 2, the log of the binding constants for these macrocycles with the alkali metal cations are compared to those of 18-crown-6, the type of macrocycle used in this study. The 18-crown-6 and 2.2.2 ligands show similar selectivities, each binding K^+ strongest, followed by Rb^+, Na^+, Cs^+ an Li^+. One significant difference between these two ligands is that the crown has a higher affinity for Cs^+ than Na^+ while D-2.2.2 binds Na^+ better than Cs^+. On the other hand, D-2.2.1 shows the highest affinity for Na^+, followed by K^+, Rb^+, Li^+ and Cs^+.

The dependence of anion retention on eluent cation is demonstrated by measuring anion retention with different cations in the eluent. The effect of eluent cation on the retention of chloride is demonstrated in Fig. 3. In these experiments, approximately the same amount of macrocycle was loaded on each column and the eluents are all 20 m*M* alkali metal hydroxides. The amount of retention is clearly related to the binding constant of the cation in the eluent with the macrocycle on the column. For all three ligands the order of retention directly mirrors the order of the binding constants of the ligands for the eluent cations shown in Fig. 2. The 18-crown-6 and D-2.2.2 columns show the most retention with the KOH eluent, while the D-2.2.1 columns retains anions longest with the NaOH eluent.

The most significant difference between cryp-

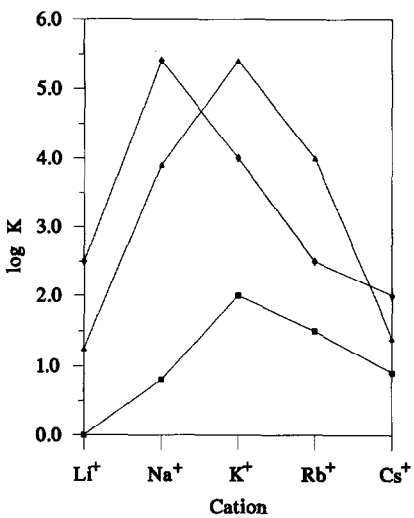

Fig. 2. Variation in log *K*, the stability constant for alkali metal ion complexes, in water for the cryptands D-2.2.2 (▲) and D-2.2.1 (◆) and the crown ether 18-crown-6 (■) at 25°C. From ref. 1.

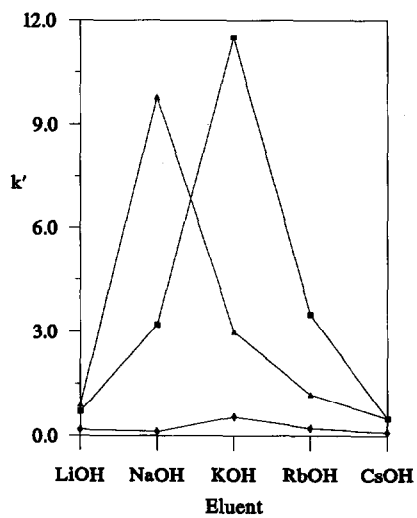

Fig. 3. Variation in Cl^- capacity factor, *k'*, with eluent alkali metal cation (20 m*M*) on D-2.2.2 (■), D-2.2.1 (▲) and TD-18-crown-6 (◆) columns.

tands and crown ethers is that the cryptands show a much higher affinity for all of the alkali metals [1,2]. This implies that for a given amount of macrocycle loaded on the column, cryptand columns should show higher column capacities than crown columns for a given cation in the eluent. This expectation is indeed realized, as shown by Fig. 3. The retention of chloride is much greater for the cryptand columns with all of the eluents.

Eluent concentration

In traditional ion chromatography the retention of anions decreases with increasing eluent strength. With macrocycle-based chromatography, there are two separate and opposing effects of changing eluent concentration. As the eluent concentration is increased: (i) the eluting power of the eluent is increased and (ii) the column capacity increases. In order to understand these two opposing effects we can consider the reactions responsible for anion retention taking place in the column. The first reaction is the formation of the cation–macrocycle exchange site:

$$M_m^+ + OH_m^- + L_s \rightleftarrows (ML^+OH^-)_s$$

where ML^+ is the cation–macrocycle complex, OH^- is the hydroxide ion, and L is the macrocyclic ligand, the subscript m refers to the species in the mobile phase and the subscript s refers to the species in the stationary phase. The associated formation constant is:

$$K_C = \frac{[(ML^+OH^-)_s]}{[M_m^+][OH_m^-][L_s - (ML^+OH^-)_s]}$$

The second reaction is the ion-exchange reaction described by the equation:

$$(ML^+OH^-)_s + A_m^- \rightleftarrows ML^+A_s^- + OH_m^-$$

where M^+, OH^-, and L have the same meaning as above and A^- is the analyte anion. The equilibrium constant for this reaction can be written:

$$K_A = \frac{[(ML^+A^-)_s][OH_m^-]}{[(ML^+OH^-)_s][A_m^-]}$$

We can rearrange the expression for K_C to solve for $[(ML^+OH^-)_s]$ to find the result:

$$[(ML^+OH^-)_s] = \frac{K_A[L_s][M_m^+]^2}{1 + K_A[M_m^+]^2}$$

This result can then be sustituted in the equation for K_A and solved to yield:

$$\frac{[(ML^+A^-)_s]}{[A_m^-]} = \frac{\dfrac{K_A K_C[L_s][M_m^+]^2}{1 + K_C[M_m^+]^2}}{[M_m^+]} = K_D$$

The factor on the left side of the equation is the distribution coefficient, K_D, for the anion between the stationary and mobile phases, which is directly proportional to retention as expressed in the capacity factor k'.

If the product $K_C[M_m^+]^2$ is much less than 1, then the distribution coefficient K_D approaches being directly proportional to the eluent concentration, $[M_m^+]$, and analyte retention should increase with increasing eluent concentration. On the other hand, if the product is much greater than 1 then K_D is inversely proportional to the eluent concentration $[M_m^+]$ and retention should decrease as the eluent concentration is increased. Thus, if we perform separations at a variety of eluent MOH concentrations, we should observe that retention increases with MOH concentration in the low concentration region, reaches a maximum, then decreases with increasing [MOH] at high concentration. The experimental results that we have previously reported with the cryptand columns [9] agree with this

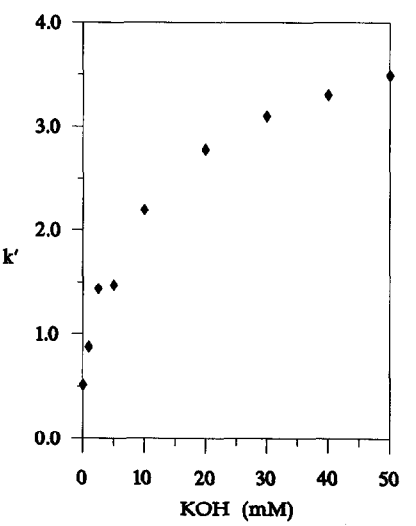

Fig. 4. Variation in NO_3^- capacity factor with eluent KOH concentration in 20% (v/v) aqueous acetonitrile on D-18-crown-6 column.

prediction. With the D-2.2.2 column we observed that anion retention increased with eluent KOH concentrations below 0.75 m*M*, where retention reached a maximum, with retention decreasing at eluent KOH concentrations greater than 0.75 m*M*.

Fig. 4 shows the effect of eluent concentration on the retention of nitrate ion using the crown-based column. As KOH concentration is increased, the capacity factor of nitrate is increased. In this case, the effect of the increasing the population of ion-exchange sites outweighs the effect of increasing the eluting power of the eluent. This is to be expected because the binding constants for the alkali metal cations with the crown are significantly lower than for the cryptands.

Organic modifier

The use of organic modifiers on ion chromatography has been limited until the recent development of solvent compatible columns. For common inorganic anions, the use of organic solvents in the eluent has a limited effect. However, the effect of solvent on the strength of binding of cations by crown ethers has been well documented, with the binding constants being several orders of magnitude higher in solvents such as methanol and acetonitrile than in pure aqueous systems [1,2]. Thus, it was anticipated that the column capacity of macrocycle columns would be increased by incorporation of an organic modifier in the eluent system. This effect is demonstrated in Fig. 5, which summarizes experiments in which the retention of nitrate ion on the crown column was measured *versus* the acetonitrile content of the mobile phase. The eluent was 50 m*M* KOH and the acetonitrile content was varied from 0 to 20% (v/v). The retention of nitrate is affected only slightly until the acetonitrile content reaches 10%, above which retention increases rapidly as the content of acetonitrile is increased. Column capacity, and thus anion retention can be significantly increased by increasing the amount of organic modifier in the eluent.

One concern with the use of organic solvents in the eluent in macrocycle-based chromatography of the type described herein is column stability. In our studies, the crown was adsorbed onto the reversed-phase column by hydrophobic interactions between the hydrophobic tail of the crown ether and the polystyrene resin. The addition of an organic solvent can affect the column stability since it can serve to elute the crown from the column. It was therefore necessary to determine the effect of organic solvent on column stability. We first studied the use of the C_{10} derivative of 18-crown-6 (Fig. 1). Column stability was checked first with a purely aqueous eluent by repeatedly injecting a 250 μM nitrate standard onto a column equilibrated with 20 m*M*

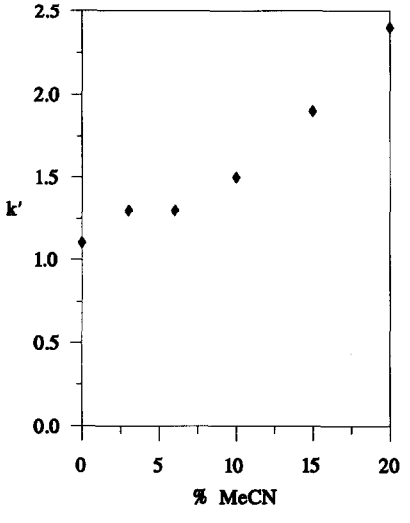

Fig. 5. Variation in NO_3^- capacity factor with acetonitrile content in 20 m*M* KOH eluent using TD-18-crown-6 column.

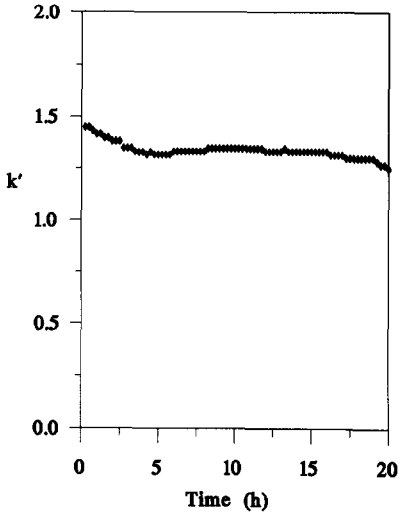

Fig. 6. Retention of NO_3^- on TD-18-crown-6 column *versus* time with 20 m*M* KOH eluent.

KOH eluent. A plot of the nitrate capacity factor over 20 h (Fig. 6) shows that crown was slowly eluted from the column by the purely aqueous eluent. It was concluded that the decyl derivative was insufficiently hydrophobic to provide a stable column even with water eluent. We therefore incorporated a longer aliphatic sidechain onto the crown ether functionality to provide better column stability. Aromatic substitution of the crown ether to increase hydrophobicity was avoided because the addition of an aromatic moiety to the macrocyclic ring results in lower binding stability and selectivity. The C_{14} derivative of 18-crown-6 provided a much more stable column than the C_{10} substituted crown. When a similar experiment was carried out with the tetradecyl crown column, no macrocycle loss was observed. The tetradecyl-18-crown-6 column was then evaluated by a similar experiment, but this time using an eluent containing 20% acetonitrile. The column was stable over 15 h even with 20% organic modifier in the eluent, as shown in Fig. 7. However, at higher concentrations of acetonitrile the crown was stripped from the column, as indicated by loss of column capacity.

Anion separations

The factors that affect anion retention in macrocycle-based chromatography can be combined to perform successful separations. In the past we have demonstrated successful gradient separations using cryptand-based columns. The crown ether columns useful characteristics as compared to the cryptand and traditional ion chromatography columns.

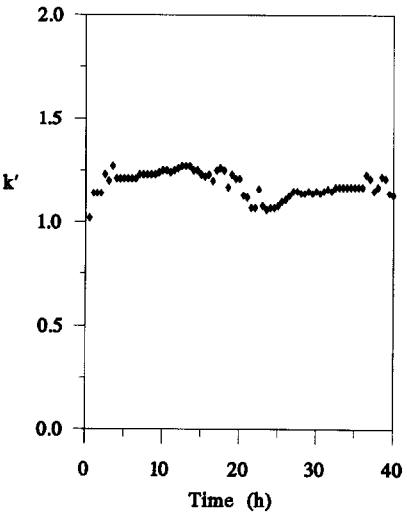

Fig. 7. Retention of NO_3^- on TD-18-crown-6 column *versus* time with 20 m*M* aqueous KOH–acetonitrile (80:20, v/v) eluent.

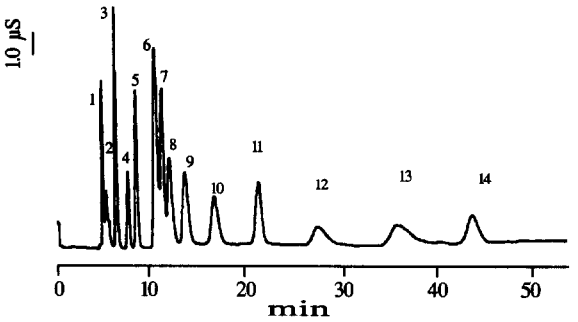

Fig. 8. Separation of 14 anions: 1 = F^-, 1.5 ppm (w/w); 2 = acetate, 10 ppm; 3 = Cl^-, 2.5 ppm; 4 = NO_2^-, 10 ppm; 5 = Br^-, 10 ppm; 6 = SO_4^{2-}, 10 ppm; 7 = NO_3^-, 10 ppm; 8 = oxalate, 10 ppm; 9 = CrO_4^{2-}, 10 ppm; 10 = phthalate, 10 ppm; 11 = I^-, 10 ppm; 12 = PO_4^{3-}, 10 ppm; 13 = citrate, 10 ppm; 14 = SCN^-, 10 ppm; on TD-18-crown-6 column with 50 m*M* aqueous KOH–acetonitrile (80:20) eluent.

The separation of a 14-anion standard on two tetradecyl-18-crown-6 columns joined in series with an eluent consisting of 50 m*M* aqueous KOH–acetonitrile (80:20) is shown in Fig. 8. Two columns were used because a single column showed insufficient capacity to effectively separate the anions. All 14 anions were well separated in less than 45 min. This separation shows several interesting differences from the separations of the same anions carried out using cryptand-based columns. Specifically, on the cryptand columns weakly retained species such as fluoride and chloride and strongly retained anions such as phthalate and thiocyanate were effectively separated only by performing capacity gradients, as shown in Fig. 9. The crown column on the other hand achieves similar separations in approximately the same time period under isocratic conditions. The column shows a lower selectivity for divalent anions as compared to the cryptand-based and traditional ion-exchange columns. This effect may be due to a lower density of exchange sites on the crown column, permitting interaction with only one monovalent exchange site at a time. The effect results in changes in elution order of the anions, with sulphate eluting before nitrate, a reversal from the usual

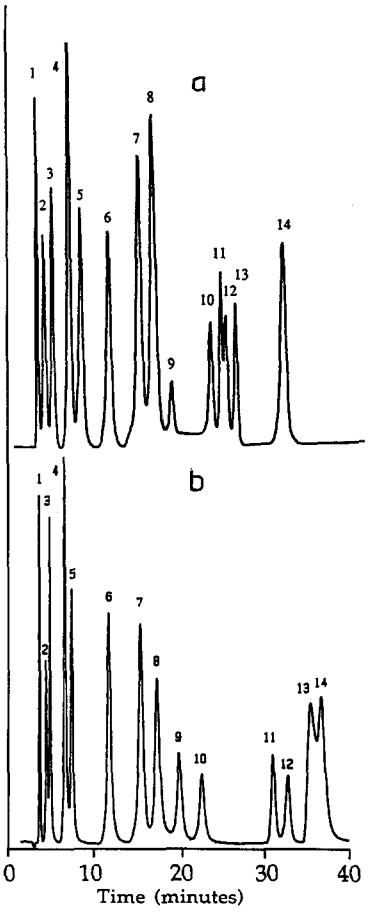

Fig. 9. Capacity gradient separation of 14 anions: 1 = F^-, 1.5 ppm (w/w); 2 = acetate, 10 ppm; 3 = Cl^-, 2.5 ppm; 4 = NO_2^-, 10 ppm; 5 = Br^-, 10 ppm; 6 = NO_3^-, 10 ppm; 7 = SO_4^{2-}, 10 ppm; 8 = oxalate, 10 ppm; 9 = CrO_4^{2-}, 10 ppm; 10 = I^-, 10 ppm; 11 = PO_4^{3-}, 10 ppm; 12 = phthalate, 10 ppm; 13 = citrate, 10 ppm; 14 = SCN^-, 10 ppm; on (a) D-2.2.2 column, with 30 m*M* NaOH–30 m*M* LiOH linear gradient for 20 min; (b) D-2.2.1 column, with 30 m*M* KOH–30 m*M* LiOH linear gradient for 20 min. From ref. 11.

elution order on the cryptand column and on traditional ion-exchange columns. The retention of the more hydrophobic anions such as thiocyanate is lower on the crown column than on the cryptand column due to the presence of acetonitrile in the eluent of the former.

Cation gradient separations are possible with the crown-based column, as with the cryptand-based columns. Such gradients, as shown in Fig. 10, result

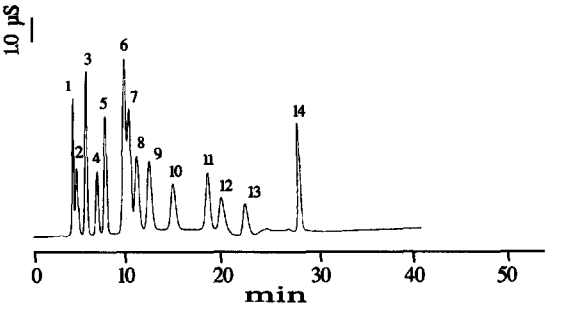

Fig. 10. Capacity gradient separation of 14 anions: 1 = F^-, 1.5 ppm (w/w); 2 = acetate, 10 ppm; 3 = Cl^-, 2.5 ppm; 4 = NO_2^-, 10 ppm; 5 = Br^-, 10 ppm; 6 = SO_4^{2-}, 10 ppm; 7 = NO_3^-, 10 ppm; 8 = oxalate, 10 ppm; 9 = CrO_4^{2-}, 10 ppm; 10 = phthalate, 10 ppm; 11 = I^-, 10 ppm; 12 = PO_4^{3-}, 10 ppm; 13 = citrate, 10 ppm; 14 = SCN^-, 10 ppm; on TD-18-crown-6 column with 50 m*M* KOH–50 m*M* NaOH (each in 20% aqueous acetonitrile) 20-min linear gradient starting at 5 min.

in faster separations than can be achieved under isocratic conditions. In this case, the eluent was changed from 50 m*M* KOH to 50 m*M* NaOH during the separation, decreasing the column capacity and speeding the elution of the anions from the column.

CONCLUSIONS

We have demonstrated the ability of an anion separator column, formed by the adsorption of a hydrophobic crown ether onto a reversed-phase column, to separate anions with widely varying anion-exchange affinities under isocratic conditions. This is possible because of the lower density of exchange sites, resulting in lower selectivity for divalent anions, and the use of an organic modifier in the eluent, speeding the elution of hydrophobic anions from the column. This system has some possible advantages over gradient systems. Gradient separations require pumps with gradient capabilities, making separations more complicated and expensive. Also, most gradients involve a change in eluent conditions, resulting in some baseline disturbance. Eluent gradients speed elution of strongly retained species from the column. However, some of this speed of elution is wasted because of the time required to re-equilibrate the column with the original eluent in preparation for the next separation. The separation on the crown column suffers none of these disadvantages, making the separations

of anions from fluoride to thiocyanate possible under isocratic conditions.

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