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CHEMICALLY SUPPRESSED ANION CHROMATOGRAPHY BASED ON MACROCYCLE–CATION COMPLEXATION

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SUMMARY

A novel ion chromatographic technique is described using macrocyclic ligand–cation complexes as anion-exchange sites, yielding columns of variable capacity and chromatograms independent of sample cation. Hydrophobic macrocycles are coated onto commercially available C_{18}-derivatized silica or polystyrene columns. The aqueous eluent contains cations that bind dynamically to the macrocycle, forming positively charged exchange sites. The nature of the ion-exchange sites, and thus the column characteristics, can be altered simply by changing the eluent cation.

INTRODUCTION

Macrocyclic ligands, such as crown ethers and cryptands, are well known for their size-selective binding of metal and other cations[1,2]. This selectivity has been successfully exploited for the separation of cations by solvent-extraction, liquid-membrane and chromatographic methods for analytical and other purposes. Further analytical applications of these compounds have been reported in ion-selective electrode and spectrophotometric techniques[3,4].

Column chromatographic applications of macrocyclic ligands have focused primarily on cation separations, although other species have also been separated. Blasius and co-workers[4–6] prepared a large number of polymeric crown ethers which serve as anchor groups for chromatographic separation of alkali, alkaline earth and precious metals and also some organic compounds and water. These relatively soft polymers are not suited to high-performance liquid chromatography (HPLC). Further, Dotsevi *et al.*[7] used chiral macrocycles covalently bound to polystyrene for the chromatographic separation of the optical isomers of amino acids. More recently, Bradshaw *et al.*[8] and Dudler *et al.*[9] reported the preparation of silica gel-bound crown ethers, such as 18-crown-6, which provide excellent preparative-scale metal ion separations in acidic or neutral solution.

The HPLC separation of cations using macrocycles was pioneered by Kimura's group using silica supports. The crown ether or cryptand exchange sites were held in place in one of two ways: (1) by covalent bonding to the silica support[10–14] or (2) by dynamic coating of lipophilic macrocycles on octadecylsilanized silica (ODS)[15,16]. In the former instance, the synthetic procedure required the use of benzo-substituted

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crowns, which are less selective than the parent compounds and demonstrate relatively weak cation binding[1]. Nonetheless, good separations of alkali metals were achieved when dimeric or polymeric crowns were used. Dynamic coating of lipophilic crown ethers also yielded excellent separations of alkali and alkaline earth metal ions. However, the lipophilically substituted cryptand 2.2.2 bound alkali metals and other cations too strongly to yield a practical elution profile. Highly stable coatings were obtained even after exposure to large volumes of eluents containing 40% methanol. Macrocycles hav also been successfully employed as mobile phase modifiers in the separation of amino acids[17] and β-lactam antibiotics[18] on cation-exchange columns.

Using column chromatography, Blasius *et al.*[6] showed that anions may be separated on polymeric crown ether resins containing bound metal cations[6]. Igawa *et al.*[19], Nakajima *et al.*[11,12] and Blasius and co-workers[20–22] reported how this principle can be succesfully applied to HPLC using bis- and polymeric crown ethers covalently bonded or polymerically coated on silica. In these studies, non-ionic eluents (water or water–methanol) were used. Sample cations and anions eluted in bands as ion pairs at rates dictated by the interaction of sample cations with the neutral macrocyclic exchange sites. The retention times of sample anions depended strongly on the sample cation present.

We report here a novel method for making use of macrocyclic ligands as exchange sites in the analysis of anions by chemically suppressed ion chromatography. The column preparation is based on the principle described by Cassidy and co-workers[23–26] and by Kimura and co-workers[15,16] of dynamic coating of hydrophobic exchangers on commercially available C_{18}-derivatized silica or polystyrene columns. The aqueous eluent contains a cation that has an affinity for the macrocycle coating, causing the formation of positively charged cation–macrocycle complex exchange sites. The effect on anion retention of sample cations is minimized by an anion-exchange mechanism in the presence of an overwhelming concentration of eluent cations. Of special value in this method is the capability of altering the nature of the ion-exchange sites simply by changing the eluent cation. This capability makes it possible to customize the ion-exchange stationary phase to yield the desired separation. The use of new polystyrene-based C_{18} columns eliminates problems of support degradation that are common with silica-based columns when basic anion eluents are used.

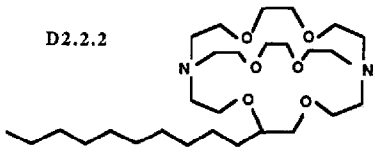
EXPERIMENTAL

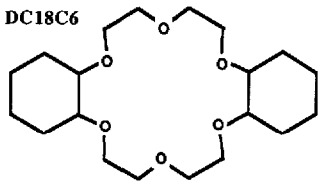
Materials

Analytical-reagent grade macrocyclic ligands cryptand *n*-decyl-2.2.2 (D2.2.2), 18-crown-6 (18C6) and dicyclohexano-18-crown-6 (DC18C6) (mixture of isomers) were obtained from Parish Chemical. HPLC-grade methanol was obtained from Fisher Scientific. Eluent water was purified to 18 MΩ cm using a Milli-Q purification system (Millipore). Eluents were degassed by helium purging or sonication. All chemicals used to prepare eluent and standard solutions were of analytical-reagent grade.

Equipment and columns

All chromatograms were obtained using Dionex 2000i (isocratic) or 4000i





(gradient) ion chromatographs, which have non-metal pumps and valves. Eluent suppression for conductometric detection was provided by a Dionex Anion Micromembrane Suppressor using 12.5 m*M* sulfuric acid at 3 ml/min. Chromatograms were collected using the Spectraphysics Labnet computer system.

Four kinds of reversed-phase columns were used: Dionex MPIC NS1 (25 cm × 4.6 mm I.D.), polystyrene–divinylbenzene; Spherisorb 10-μm ODS-2 (25 cm × 4.6 mm I.D.), C_{18} on silica; Spherisorb 5-μm S5 ODS-1 (25 cm × 4.6 mm I.D.), C_{18} on silica; and Interaction ACT-1 10-μm average particle size (15 cm × 4.6 mm I.D.), C_{18} on polystyrene–divinylbenzene.

Column coating procedure

D2.2.2 was supplied as a 50 wt.-% solution in toluene; 50 μl of this solution were added to 100 ml of methanol–water (60:40, v/v) which was degassed by sonication for 10 min. After the column had been rinsed with methanol–water (60:40, v/v) solution, the D2.2.2 solution was pumped in recycle fashion through the column for 16 h at 0.5 ml/min.

RESULTS AND DISCUSSION

Macrocycles as mobile phase modifiers

Our early experiments using macrocycles in ion chromatography (IC) involved the incorporation of water-soluble crown ethers, such as 18C6 and DC18C6, into the ion chromatographic mobile phase. In this instance a Dionex MPIC (mobile phase ion chromatography) column was used. It was expected that some sample cations would complex with the crown ether in the mobile phase and thereby develop sufficient hydrophobic character to be retained on the non-polar stationary phase as in pairs with eluent anions. The eluent contained 10 m*M* nitric acid, which was chemically suppressed for conductivity detection. Indeed, the alkali metals were retained in the same order as that of their 18C6 complex stability constants, $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$, eluting as nitrate salts. Typical chromatograms using 18C6 and DC18C6 are shown in Fig. 1a and b, respectively.

A series of experiments was performed using various amounts of DC18C6 in the mobile phase. The results (Fig. 2) show a maximum capacity factor (k') for K^+ at a DC18C6 concentration of approximately 5 m*M*. Below this level there is insufficient

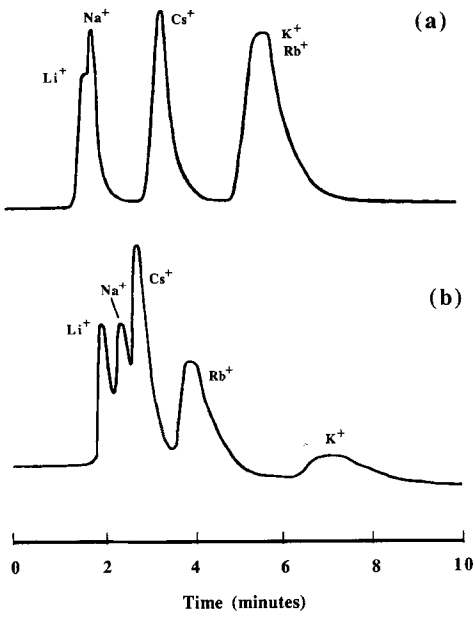


Fig. 1. MPIC of cation nitrate salts (Li^+, 132 μM; Na^+, 124 μM; Cs^+, 248 μM; Rb^+, 260 μM; K^+, 193 μM) using crown ethers in the mobile phase. Flow-rate, 1.0 ml/min. (a) 25 mM 18C6 in 500 μM HNO_3; (b) 20 mM DC18C6 in 500 μM HNO_3.

crown ether for effective retention and above it there is competition for adsorption on the stationary phase by cation-free over cation-bound macrocycle. In these experiments, before each point could be measured, it was necessary to allow sufficient time for equilibration of the reaction $[DC18C6]_S = [DC18C6]_M$ to occur, where S represents stationary phase and M mobile phase. The establishment of equilibrium was indicated

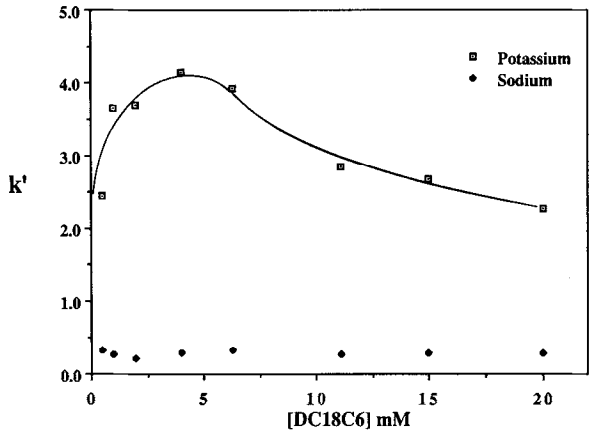


Fig. 2. Variation of capacity factor, k', of K^+ and Na^+ with [DC18C6] in MPIC system. The eluent also contans 500 μM HNO_3.

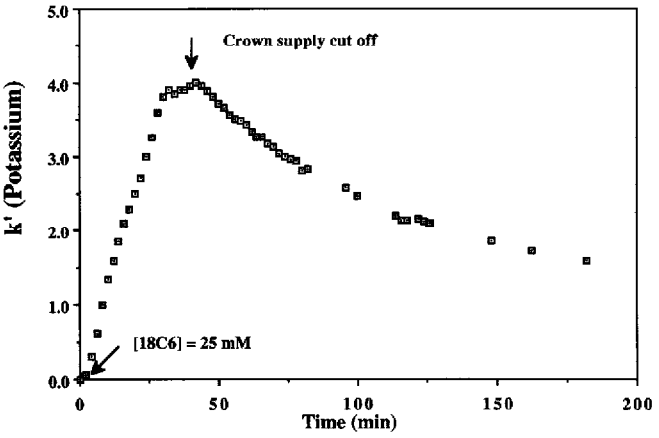


Fig. 3. Variation of k' (K^+) with time after introduction and subsequent removal of 25 mM 18C6 as eluent in MPIC system. The eluent also contains 500 μM HNO_3.

by no further change in k' as a sequence of sample injections was performed at each DC18C6 concentration.

The results of the above experiments made it clear that the MPIC column was sorbing unbound macrocycle. Fig. 3 shows that even the hydrophilic 18C6, which is soluble in water to concentrations over 2.0 M, has a considerable affinity for the reversed-phase column. When 25 mM 18C6 eluent was first pumped through the MPIC column, the capacity factor for K^+ increased gradually over a period of about 40 min, after which a steady state was reached. When the crown supply was cut off, the capacity factor decreased slowly with time. Clearly, even this hydrophilic crown was not acting in the mobile phase as expected, but was adsorbing on the non-polar stationary phase, where it served to retain the K^+ ions by a complexation–sorption mechanism. Hence, in the early portion of the curve (Fig. 2) the column is being saturated with sorbed macrocycle, and in the latter portion K^+ retention persists even though no crown is present in the mobile phase. Therefore, it appears more accurate to describe this system as involving "dynamically bound" exchange sites than ion pairing in the mobile phase.

Cation macrocycle complexes as exchange sites for anion separations

The ready immobilization of macrocycles onto non-polar stationary phases offers a novel approach to anion chromatography using chemical suppression and conductivity detection. As noted in the Introduction, others have used dynamically bound macrocycles for the HPLC determination of cations. The ODS columns on which the macrocycles were adsorbed are sensitive to base, which makes difficult the achievement of anion separation by such systems using basic eluents which are amenable to chemical suppression. However, we have found that by careful control of the eluent pH, macrocycles coated on ODS columns can be used for the chemically suppressed IC of anions. Further, the recent development of C_{18} reversed-phase columns based on polystyrene that are not susceptible to base degradation offer a new alternative for applying macrocycles to anion chromatography in this way.

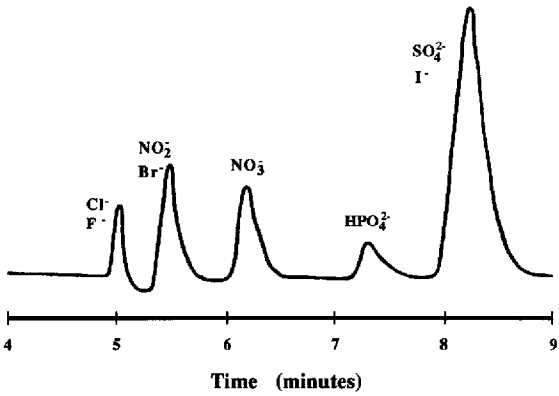


Fig. 4. Chromatogram of eight anions (F^-, 15 μM; Cl^-, 17 μM; Br^-, 29 μM; NO_2^-, 25 μM; NO_3^-, 39 μM; HPO_4^{2-}, 36 μM; I^-, 30 μM; SO_4^{2-}, 42 μM; all as K^+ salts) using an ODS column (5 μm) loaded with D2.2.2. Eluent, 5.6 mM $KHCO_3$–29.3 mM H_3BO_3 in water; flow-rate, 0.7 ml/min.

When a sample containing 50 μM KCl, KNO_3, KF, KI, K_2SO_4 or KSCN was injected onto the ODS column which had not been coated with D2.2.2, no retention was observed, but when a sample containing eight anions (F^-, Cl^-, Br^-, NO_2^-, NO_3^-, HPO_4^{2-}, I^-, SO_4^{2-}) was injected onto an ODS column that had been treated with D2.2.2, the chromatogram shown in Fig. 4 was obtained. In this instance, the eluent was an aqueous buffer (pH 7.62) consisting of 5.6 mM $KHCO_3$ and 29.3 mM H_3BO_3 at a flow-rate of 0.7 ml/min. Excellent peak resolution is observed with good Gaussian peak shapes.

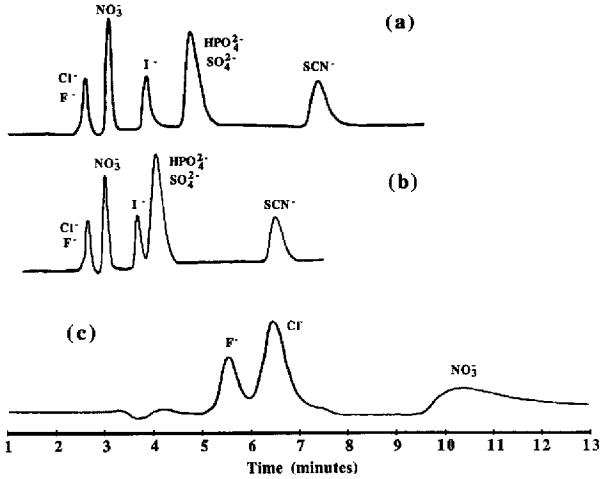


Fig. 5. Chromatograms of seven anions (F^-, 15 μM; Cl^-, 17 μM; NO_3^-, 39 μM; I^-, 30 μM; HPO_4^{2-}, 36 μM; SO_4^{2-}, 42 μM; SCN^-, 47 μM; all as K^+ salts) using an ODS column (10 μM) and eluent of pH 9.3 (5 mM $K_2B_4O_7$–5 mM H_3BO_3) at a flow-rate of 0.9 ml/min, (a) during first hour and (b) after 5 h. (c) Chromatogram of three sample anions (F^-, 34 μM; Cl^-, 39 μM; NO_3^-, 90 μM) using an ACT column loaded with D2.2.2. Same eluent as for (a) and (b), except in methanol–water (5:95, v/v) and flow-rate 0.5 ml/min.

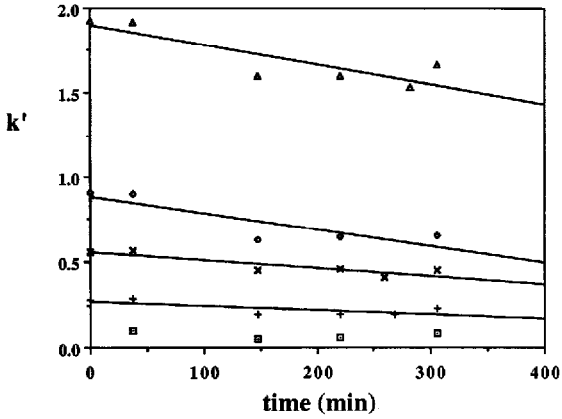


Fig. 6. Decrease in k' with time for five anions: (□) Cl^-; (+) NO_3^-; (×) I^-; (◇) SO_4^{2-}; (△) SCN^-. Experimental conditions as in Fig. 5a and b.

Column stability

The principle obstacle to using macrocycle-coated ODS columns for anion separations in this fashion is the sensitivity of these columns to base. It was expected that the chromatogram in Fig. 4 could be improved by increasing the pH of the eluent. In an attempt to extend the limits of the pH tolerance specifications of the silica column, a buffer of pH 9.3 was used, consisting of 5 m*M* H_3BO_3 and 5 m*M* $K_2B_4O_7$ in water. When a sample containing potassium salts of F^-, Cl^-, NO_3^-, I^-, SO_4^{2-} and SCN^- was injected at a flow-rate of 0.9 ml/min, the chromatogram shown in Fig. 5a was obtained. It was clear from the chromatogram in Fig. 5b, however, that after 5 h of eluent flow the capacity had decreased significantly. The change in capacity factor for the five ion peaks (F^- and Cl^- eluted together) with time is shown in Fig. 6. The

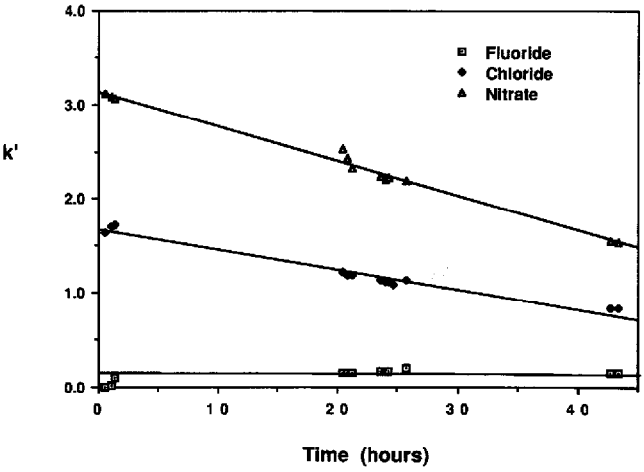


Fig. 7. Decrease in k' with time for three anions using the ACT column and methanol–water (40:60, v/v) as eluent.

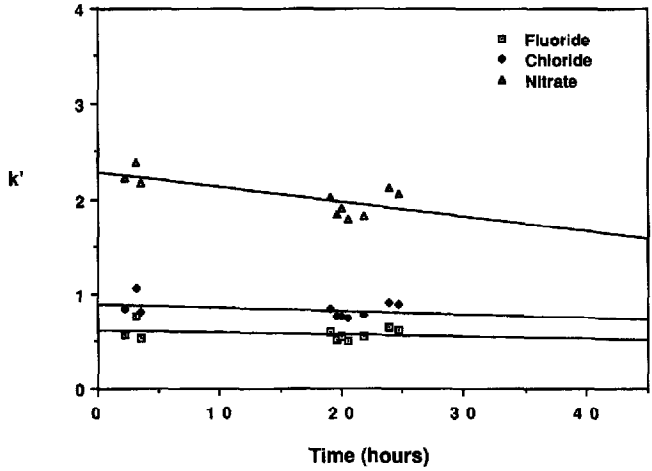


Fig. 8. Decrease in k' with time for three anions using the ACT column and methanol–water (15:85, v/v) as eluent.

sensitivity of the ODS column to base makes it difficult to determine whether or not the loss of D2.2.2, and hence capacity, was due at least in part to loss of the C_{18} coating. It is possible to use ODS columns for the application described here, but only if the eluent pH is kept below 8, as specified by the manufacturer.

The sensitivity of ODS columns to base as described above imposes serious constraints on the eluent composition for anion chromatography. A clear solution to this problem is to adopt a chemically inert polymer-based C_{18} column, such as the ACT-1. However, the gain in chemical stability with this column is partially offset by the higher cost and less uniform particle size, with a concomitant decrease in column efficiency. This column, loaded with D2.2.2, was used in conjunction with an eluent identical with that of pH 9.3 described above, except that the solvent was a methanol–water mixture. The relatively lower efficiency of this column is illustrated in Fig. 5c. Calculations based on the NO_3^- peak give an efficiency of $N \approx 600$ for the ACT column compared with $N \approx 1800$ for the 10-μm ODS column.

An eluent comparable to that described above was prepared with various amounts of methanol to determine the stability of the D2.2.2-coated ACT to loss of macrocycle. At 90% methanol, the cryptand was stripped immediately from the column. At 40% methanol, the anion capacity factors decreased sigificantly over a 44-h period (representing approximately 1.3 l of eluent flow), as illustrated in Fig. 7. At 15% methanol (Fig. 8), the decrease is less rapid (the slope of the NO_3^- line is $-0.016\ h^{-1}$, compared with $-0.037\ h^{-1}$ for the 40% methanol plot). At 5% methanol, no decrease in k' was observed even after 60 h. The long-term stability of these dynamically coated columns against loss of macrocycle at low methanol concentrations corresponds to the stability observations by Kimura *et al.*[16] and Cassidy and Elchuk[23] under dynamic conditions.

Effect of eluent cation concentration

Fig. 9. illustrates the effect on anion capacity factor of variations in the concentration of eluent K^+ ion. In this instance, a simple potassium hydroxide eluent

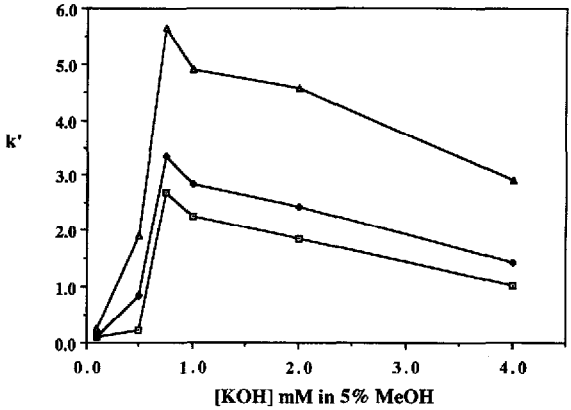


Fig. 9. Change in k' for three anions with [KOH] in methanol (MeOH)–water (5:95, v/v) as eluent using the ACT column loaded with D2.2.2. (□) F^-; (◆) Cl^-; (△) NO_3^-.

in methanol–water (5:95, v/v) was used with the ACT column. At very low concentrations of K^+, the anions were not significantly retained and were not separated from one another. This observation conforms to the proposed mechanism of retention, *i.e.*, the formation of anion-exchange sites via the complexation of eluent metal cations. As the eluent [K^+] increases, the column capacity increases quickly and anion separation begins. Capacity factors reach a maximum at a K^+ eluent concentration of approximately 0.75 m*M* and then fall gradually. The probable explanation for this behavior is that at the curve maximum, the cryptand sites are fully occupied by K^+ ions and further increases in [K^+] have no favorable effect. Beyond the maximum, the effect of increasing [OH^-] comes into play, as eluent anions compete with sample anions for the cryptand complex exchange sites.

The position of the curve maxima in Fig. 9 helps to elucidate the nature of the stationary phase in these dynamically bound systems. There are at least two possible ways in which D2.2.2 might be sorbed on the C_{18} stationary phase coating: (1) with the hydrophobic tail immersed in the C_{18} coating and the cryptand head protruding into the mobile phase; or (2) with the entire cryptand molecule buried in the coating. If the former mechanism predominates the reaction for K^+ uptake by the macrocycle should be very similar to the complexation reaction in water:

$$K^+_{aq} + 2.2.2_{aq} \rightleftharpoons (K2.2.2)^+_{aq} \quad (1)$$

This similarity arises from the fact that the hydrophobic tail on D2.2.2 has very little effect on the thermodynamics of its metal cation binding, as it attaches to an aliphatic portion of the parent molecule. Hence the equilibrium constants for its reaction with cations are similar to those of 2.2.2[1]. On the other hand, if the second sorption mechanism predominates the reaction for K^+ uptake ought to be more similar to the extraction equation.

$$K^+_{aq} + A^-_{aq} + 2.2.2_{org} \rightleftharpoons (K2.2.2)^+A^-_{org} \quad (2)$$

where "aq" represents species in the aqueous phase and "org" species in the organic phase.

We may test which of the two above descriptions best conforms to the results in Fig. 9. The equilibrium constant K for reaction (1) in water is 2.0×10^5 (ref. 1) and may be expressed as

$$K\,[K^+]_{aq} = \frac{[K2.2.2]^+_{aq}}{[2.2.2]_{aq}} \tag{3}$$

If the cryptand is predominantly in the water phase, then at the lowest concentration of potassium hydroxide (0.10 mM), simple calculation shows that 95% of the cryptand sites should be occupied by K^+ ions. The fact that very little retention or separation of anions is observed at 0.10 mM potassium hydroxide implies that the concentration of $K2.2.2^+$ complex sites cannot be that high. Indeed, eqn. 3 states that if 99% of the cryptand sites are occupied by K^+ at $[K^+] = 0.75$ mM (the position of the maximum from Fig. 9), the value of the thermodynamic binding constant must be closer to 10^4 than to $2.0 \cdot 10^5$.

Although we do not yet have a measured value for the extraction constant, K_e, for eqn. 2, we can deduce a reasonable value. We have measured the extraction constants of alkali and alkaline earth metal salts with simple inorganic anions between water and toluene or phenylhexane containing lipophilic crown ethers[27]. These K_e values are typically 1–2 orders of magnitude smaller than the corresponding aqueous equilibrium constants with the hydrophobic parent macrocycles. Therefore, K_e for eqn. 2 is likely between $2.0 \cdot 10^3$ and $2.0 \cdot 10^4$, a range that does accommodate the data in Fig. 9. We conclude that the cryptand sites are not primarily present in the aqueous mobile phase environment, but that the eluent potassium salt is extracted into the hydrophobic stationary phase environment. Given the amphiphilic nature of the resulting complex, it is reasonable to speculate that the ionic head of the complex molecule resides at the surface of the C_{18} coating.

Variation of ion-exchange sites by changing eluent cation

A novel characteristic of the anion chromatographic system described above is that the ion-exchange sites on the stationary phase may be altered simply by changing the eluent cation. Further, the percentage of adsorbed D2.2.2 molecules that contain cations may be varied by using cations with various thermodynamic affinities for the ligand, or by changing the eluent cation concentration.

Fig. 10 shows the chromatograms of six anions, Cl^-, Br^-, NO_3^-, I^-, HPO_4^{2-} and SO_4^{2-}, all as K^+ salts, using eluents that differ only in the eluent cation. The eluent in each instance was 0.1 mM $MHCO_3$ (M = Na^+, K^+ or Cs^+)–6.05 mM H_3BO_3 at a flow-rate of 0.6 ml/min. It was attemped to keep the eluent pH values and component concentrations a comparable as possible. The pH values were Cs^+ 7.55, K^+ 7.86 and Na^+ 7.65, all below the level that causes column deterioration according to the manufacturer's specifications. Conductivity detection was used following chemical suppression. All peaks were positively identified by standard addition experiments. Table I compares the capacity factors for the six anions with each of the three solvent cations. These k' values (averages of three determinations with standard deviations)

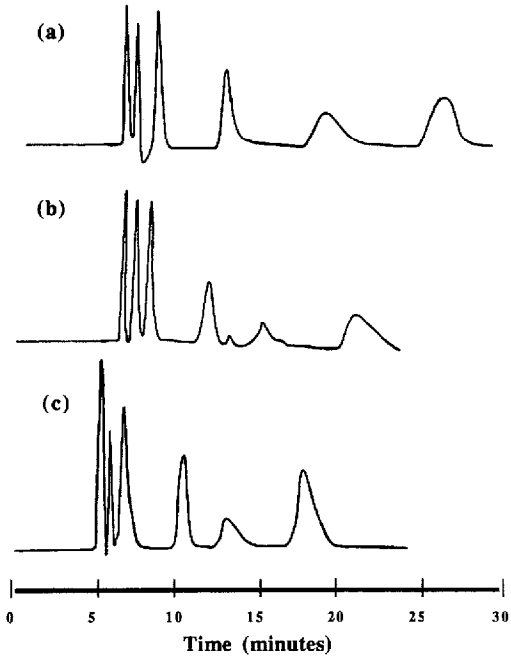


Fig. 10. Chromatogram of six anions (K^+ salts) using three eluents of the type 1.0 m*M* $MHCO_3$–6.05 m*M* H_3BO_3 at 0.6 ml/min. (a) M = K^+; (b) M = Cs^+; (c) M = Na^+. Column, ODS (5 μm) loaded with D2.2.2. Anions elute in the order Cl^-, 19 μM; Br^-, 33 μM; NO_3^-, 45 μM; I^-, 35 μM; HPO_4^{2-}, 42 μM; SO_4^{2-}, 34 μM in all three chromatograms.

consistently increase in the eluent cation series $Na^+ < Cs^+ < K^+$. Small variations in eluent pH may play some role in this trend. The K^+ eluent provides the greatest anion retention. However, the Na^+ eluent provides excellent resolution in a considerably shorter time. The detector response for the anions was the same within experimental error for all three eluent cations.

Fig. 11 demonstrates the effect of three eluent alkali metal cations on the separation of a mixture of F^-, Cl^-, NO_3^-, NO_2^-, HPO_3^{2-}, I^- and SO_4^{2-} using the ACT column. In all instances the eluent consisted of 10 m*M* MOH (M = Li, Na, or K) in

TABLE I

CAPACITY FACTORS (k') FOR SIX ANALYTE ANIONS USNG THREE ELUENTS WHICH DIFFER ONLY IN THE ELUENT CATION

Eluent[a]	*Sample anion*					
	Cl^-	Br^-	NO_3^-	I^-	HPO_4^{2-}	SO_4^{2-}
Na^+	0.026 ± 0.034	0.099 ± 0.035	0.22 ± 0.04	0.74 ± 0.05	1.09 ± 0.06	1.75 ± 0.08
Cs^+	0.10 ± 0.03	0.21 ± 0.03	0.35 ± 0.03	0.93 ± 0.04	1.49 ± 0.06	2.36 ± 0.04
K^+	0.12 ± 0.01	0.24 ± 0.01	0.454 ± 0.008	1.22 ± 0.05	2.29 ± 0.05	3.44 ± 0.02

[a] See text for experimental details.

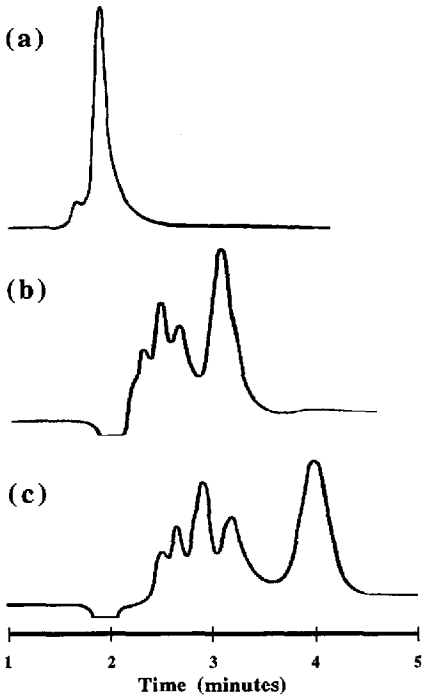


Fig. 11. Chromatograms of seven anions (F^-, Cl^-, NO_3^-, NO_2^-, HPO_4^{2-}, I^- and SO_4^{2-}, concentrations similar to those in Fig. 10; all as K^+ salts) using three eluents of the type 10 m*M* MOH in methanol–water (5:95, v/v) at 1.0 ml/min. (a) M = Li^+; (b) M = Na^+; (c) M = K^+. Column, ACT loaded with D2.2.2. The first four peaks in (c) correspond to F^-, Cl^-, NO_2^- and NO_3^-, with all other species eluting together in the last peak.

methanol–water (5:95, v/v) at a flow-rate of 1.0 ml/min. Hardly any separation is observed with the Li^+ eluent, consistent with the very weak binding of Li^+ to D2.2.2[1]. The small differences between the chromatograms obtained with Na^+ and K^+ and K^+ eluents cannot be due to differences in the relative percentages of bound D2.2.2, as virtually all of the cryptand molecules would be expected to be occupied under these circumstances with both cations. The relative complex size may be important: the Na^+ complex should be more compact. These observations are the subject of further investigations.

TABLE II

CAPACITY AND RESPONSE FACTORS FOR NO_3^- ELUTION USING K^+ ELUENT WITH VARIOUS SAMPLE CATIONS

Sample cation	*k'*	*Response factor*
Mg^{2+}	0.51 ± 0.04	1.6 ± 0.1
Rb^+	0.52 ± 0.04	1.5 ± 0.2
Na^+	0.50 ± 0.04	1.5 ± 0.1
Cs^+	0.49 ± 0.08	1.3 ± 0.4
K^+	0.54 ± 0.02	1.53 ± 0.07

Effect of sample cation

An experiment was performed to determine whether the sample cation affects the analysis of anions in these systems, as it does in other macrocycle-based systems where pure water is used as the eluent. An eluent containing 1.0 m*M* $KHCO_3$–6.05 m*M* H_3BO_3 was used with the ODS column at a flow-rate of 0.6 ml/min. Cations were chosen among those which do not have a greater affinity for D2.2.2 than does K^+. The results for NO_3^- analysis are shown in Table II. Replicate chromatograms were obtained in series to eliminate possible systematic variations due to changes in the chromatographic system conditions with time. The NO_3^- capacity factors are independent of the sample cation within experimental error, as is the detector response. This result is not unexpected, as sample cations with a D2.2.2 affinity less than that of K^+ should pass through the column with the void volume, leaving retained sample anions behind. This feature makes the present system much more amenable to application than aqueous eluent systems where cation–anion pairs elute in all their permutations.

It is possible that sample cations that have greater affinity for D2.2.2 than the eluent cation will cause variations in k'. Such cations will definitely compete with the eluent cation for D2.2.2 sites. However, the overwhelming concentration effect of the eluent cation may be employed to advantage. Further, the eluent cation may best be chosen from among those cations which bind the macrocycle most strongly. This topic is undergoing further investigation.

Column capacity

Because of its high affinity for the cryptand, Sr^{2+} is an appropriate species with which to measure the capacity of the D2.2.2-loaded column. The macrocycle-coated ACT column was equilibrated with Sr^{2+} by pumping 10 m*M* $Sr(OH)_2$ eluent for 2 h. The column was rinsed for 30 min with water, then stripped of residual Sr^{2+} with a 30-min rinse of 0.1 *M* hydrochloric acid, all at 1.0 ml/min. Inductively coupled plasma spectroscopic analysis of the acid stripping solution showed that there were 79 $\pm$ 4 μmol Sr^{2+} on the column, giving an overall capacity of approximately 30 μmol/ml of cryptand, given a total stationary phase volume of 2.5 ml. This value represents a lower limit, in that a small amount of Sr^{2+} may be lost in the rinsing step. The assumption that the loss on rinsing is small is based on kinetic data for the rate of the $(Sr2.2.2)^{2+}$ dissociation reaction[1]. When the same eperiment was performed with K^+, which forms a much more labile complex, 37 $\pm$ 7 μmol of K^+ were recovered. The capacity determined by the Sr^{2+} experiment compares favorably with that obtained by Cassidy and Elchuk[23] using dynamically coated ion exchangers.

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