Anion separations on columns based on transition metal–macrocycle complex exchange sites

John D. Lamb*, Robert G. Smith, Randal C. Anderson, Max K. Mortensen

Department of Chemistry, Brigham Young University, Provo, UT 84602, USA

Abstract

Macrocycle-based ion chromatographic columns can provide fast and effective separations of inorganic anions. Chromatographic columns based on nitrogen-containing macrocycles such as cryptand decyl-2.2.2 (D222) and didecyl-diaza-18-crown-6 (DD22) have high affinities for transition metal cations. This work compares the chromatographic separation of anions using transition metal cation eluents in columns based on the macrocyclic ligands DD22 and D222 adsorbed on two different polystyrene resins, MPIC and AS10 (Dionex). When a transition metal ion, $e.g.$ Cu^{2+}, Ni^{2+}, or Co^{2+}, was present in the mobile phase flowing through an MPIC column loaded with didecyl-diaza-18-crown-6, anion separations of differing selectivities were obtained. Separations of anions were achieved with these columns even when only a cation that has low affinity for the macrocycles, such as Li^+, was included in the mobile phase. This is due to protonation of the nitrogen atoms in the macrocycles. Columns based on the AS10 type resin produced much higher efficiency and shorter retention times than did columns based on the MPIC resin.

1. Introduction

The unique selectivity of crown ethers and cryptands has been widely used in performing many types of separations including chromatographic separations [1–5]. Anions as well as cations can be separated on macrocycle-based columns. Because most macrocycles are uncharged, anion separator columns can be generated when macrocycle-cation complexes form on the column and serve as anion exchange sites. Anions must be associated with the positively charged complexes to maintain electrical neutrality on the column. Since macrocycles are generally hydrophobic and are associated with

the hydrophobic environment of the column, the more hydrophobic anions are retained most strongly [6–9].

In our laboratory, we have developed a macrocycle-based anion separation system that involves an ion-exchange mechanism (as opposed to a ligand-exchange mechanism, described below) by the inclusion of an alkali metal hydroxide in the eluent [10–13]. Eluent alkali metal cations form cationic complexes with the neutral column macrocycles. Gradient separations of anions have been performed by changing the eluent alkali metal cation from one with a high affinity for the column macrocycle to one of lower affinity. In such a gradient system, the column anion exchange capacity decreases during the course of the separation [11–13].

Transition metal cation–macrocycle complexes

---

* Corresponding author.

have the potential to function in this type of ion chromatographic system not only by an ion exchange mode, but also by a ligand-exchange mechanism. This latter mechanism involves the temporary association of sample anions with the inner coordination sphere of the bound transition metal. Since these interactions involve more specific coordination than simple charge–charge interactions, variations in selectivity from one transition metal cation to another might be expected. The ligands didecyl-diaza-18-crown-6 (DD22) and cryptand decyl-2.2.2 (D222) (shown in Fig. 1) have an affinity for transition metals and are sufficiently hydrophobic to remain adsorbed indefinitely on reversed-phase columns. In this research we have studied the use of DD22 and D222 for anion separations in this way, when loaded onto one of two different polystyrene-based reversed-phase columns, namely MPIC or AS10 (Dionex).

One goal of this research was to determine the extent to which macrocycle-based separations using transition metal ions involve a ligand-exchange mechanism. When this mechanism prevails, a change in selectivity is expected when compared to separations achieved with alkali metal cations, reported earlier [10–13]. The performance of such systems in separating anions was examined in three ways:
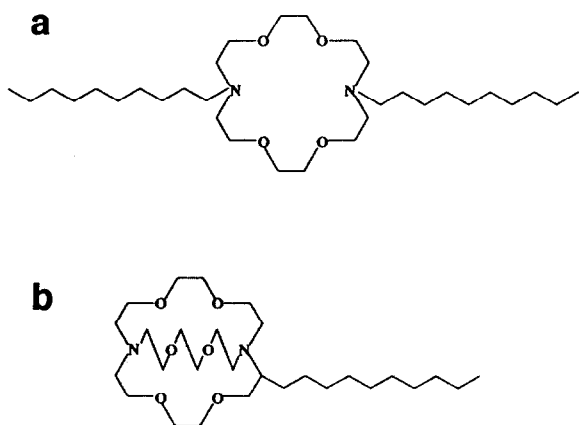
(1) changing the eluent cation, (2) changing the macrocycle adsorbed to the column, and (3) changing the type of reversed-phase resin. In the following sections we report anion separations with transition metal eluents on columns packed with MPIC and AS10 resin loaded with DD22 or with D222.

2. Experimental

2.1. Materials

DD22 was obtained from Parish Chemical (Provo, UT, USA). D222 was obtained from EM Science (Gibbstown, NJ, USA). All chemicals used for eluent preparation were reagent grade. The eluents were made with water purified to 18 MΩ resistivity with a Milli-Q water purification system (Millipore). Each eluent was degassed by sparging with helium.

2.2. Instrumentation

A Dionex 4000i series ion chromatograph was used in all chromatographic separations. A Shimadzu variable wavelength UV–vis detector was used for anion detection at 214 nm. The chromatograph was controlled and the data collected on a personal computer using the Dionex AI400 control software.

2.3. Column preparation

The MPIC column loaded with DD22 or D222 was prepared as previously described [10,11] by recirculating the ligand in a methanol–water (60:40 v/v) solution through the column for a period of 12 h. The AS10 resin loaded with DD22 or D222 was prepared by slurrying the appropriated macrocycle with the resin in a MeOH–water (60:40) solution and then evaporating off the methanol. The resin thus prepared was then packed into chromatographic columns.

Fig. 1. Structures of (a) didecyl-diaza-18-crown-6 (DD22) and (b) decyl-2.2.2. (D222).

3. Results and discussion

The purpose of this research was to explore the possibility of employing transition metal containing eluents to perform anion separations in macrocycle-based ion chromatography. Three different parameters were studied for their effect on the separation of anions: the type of macrocycle adsorbed onto the column; the identity of the eluent cation; and the type of reversed-phase resin substrate. Each of these parameters was evaluated on the basis of column capacity, selectivity, and efficiency.

3.1. Effect of macrocycle type

Macrocycles have been noted since their discovery for their ability to selectively complex metal ions. The selectivity of cation-macrocycle complexation is often determined by the ability of the metal ion to fit into the macrocyclic cavity. Cations that fit more closely are often bound more strongly than cations that do not fit as well.

The selectivity of cation binding by macrocycles is also a function of the types of heteroatoms in the macrocyclic ring. Oxygen-only crown ethers show affinity for alkali metal and alkaline earth cations, but little interaction with transition metal cations. Nitrogen-containing macrocycles show much higher affinities for transition metals than for alkali metal and alkaline earth cations. The binding of transition metal cations by 2.2.2 and dimethyl-diaza-18-crown-6 ($Me_2 2.2$), the water soluble parent macrocycles analogous to those employed in this study, but without the hydrophobic aliphatic substituent groups, is shown in Fig. 2, in which the log of the aqueous binding constant is plotted *versus* metal ion. These nitrogen containing crown ethers show very high affinity for copper ions among the transition metal ions studied.

The bicyclic cryptand 2.2.2 shows slightly higher affinity for the metal ions than the monocyclic crown ether derivative dimethyl-2.2 ($Me_2 2.2$). For this reason, columns incorporating cryptands have higher capacities than columns containing crown ethers due to the ability

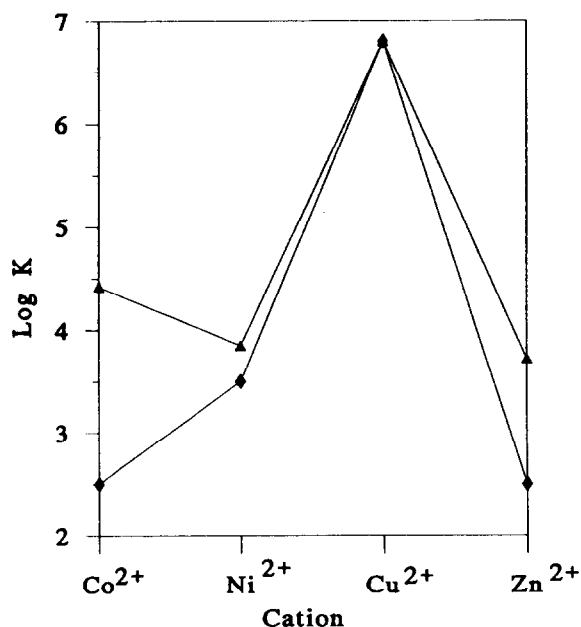Fig. 2. Aqueous binding constants of selected transition metal cations by $Me_2 2.2$ (▲) and 2.2.2 (◆) (from refs. 14 and 15).

of the cryptands to form more stable cation complexes [13].

The effect of macrocycle type on anion separations using $NiSO_4$ eluent is shown in Fig. 3. The same amount of each macrocycle was loaded onto separate MPIC columns. The separation of an 8 anion standard is shown using a 1 mM $NiSO_4$ eluent on each of these columns. The column loaded with D222 shows higher capacity than the column loaded with DD22. Specifically, with the D222 column the separation took 70 min; with the DD22 column, 45 min were required to elute all species. This effect can be attributed to the higher affinity of the cryptand column for eluent metal ions, yielding a column of higher capacity. Similar results were obtained with eluents containing copper, zinc, and cobalt — in all cases retention of anions on the D222 column was greater than on the DD22 column.

The selectivity among anions was observed to be a function of the column macrocycle type, as
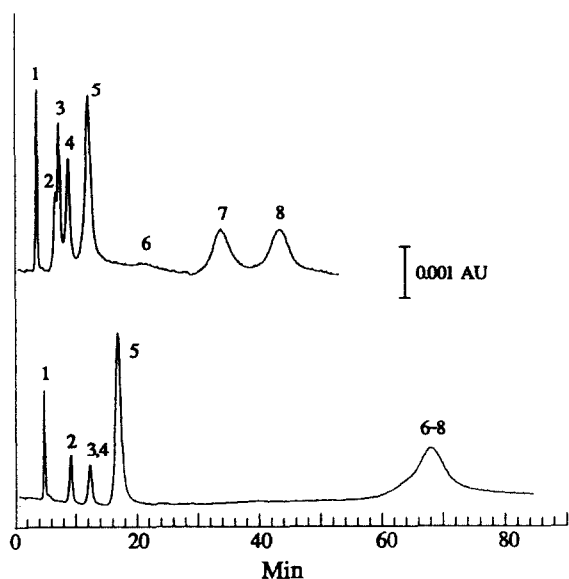
Fig. 3. Effect of macrocycle on anion separations with 1 mM NiSO$_4$ (pH = 7.8) eluent. Columns: MPIC columns loaded with DD22 (top chromatogram), D222 (bottom chromatogram). Peaks 1 = IO$_3^-$, 25 ppm (w/w); 2 = BrO$_3^-$, 50 ppm; 3 = NO$_2^-$, 5 ppm; 4 = Br$^-$, 25 ppm; 5 = NO$_3^-$, 10 ppm; 6 = Cr$_2$O$_7^{2-}$, 50 ppm; 7 = MoO$_4^{2-}$, 25 ppm; 8 = I$^-$, 10 ppm.

Table 1
Effect of macrocycle and column substrate on efficiency of anion separations using 1 mM NiSO$_4$ eluent

Anion	Column efficiency (Theoretical plates)		
	MPIC–DD22	MPIC–D222	AS10–DD22
IO$_3^-$	1380	2460	7360
NO$_2^-$	1150	2220	10 870
Br$^-$	1470	2220	11 110
NO$_3^-$	1100	1900	10 870

due to the structures of the macrocycles themselves. DD22 is tethered to the resin surface by two hydrophobic side chains on either side of the ring. Thus, it probably sits very close to the hydrophobic resin surface, so that the mass transfer of anions into and out of the stationary phase is hindered. D222, on the other hand, is tethered by only one side chain. Thus, it is probably not as closely bound to the resin surface, resulting in faster mass transfer and higher efficiency.

evidenced by Fig. 3. The DD22 column provided greater selectivity and resolution than the D222 column, with at least partial resolution of all 8 anions in the standard. In contrast, with the D222 column there was coelution of nitrite and bromide, and of dichromate, molybdate and iodide. The elution order was the same with both of these macrocycle-based columns. Improved resolution of anions was also observed in the DD22 column as compared with the D222 column when comparable cobalt or zinc eluents were used.

An important criterion in the evaluation of a separation is column efficiency. The efficiencies measured for several anions on both the DD22 and D222 columns using NiSO$_4$ eluent are listed in Table 1. The efficiency of the D222-based column is generally double that of the DD22 column. Specifically, an average efficiency of 2200 theoretical plates was obtained for the four anions listed using the D222 column as compared to 1300 for the DD22-based MPIC column. The higher efficiency of the D222 column is probably

3.2. Effect of eluent cation

As shown in Fig. 2, the nitrogen containing macrocycles DD22 and D222 have affinity for transition metal cations. Since the stationary phase macrocycles contain nitrogen in the ring, they also can be protonated under the proper pH conditions. This is an additional way positively charged anion exchange sites may be generated. The pK values for these nitrogens are 10.0 and 7.5 for 2.2.2 and 9.5 and 7.5 for Me$_2$2.2, the water soluble parent compounds of the macrocycles used in this study. Thus at the neutral pH employed with these eluents, the macrocycles can have a +1 or +2 charge due to the protonation of the nitrogens in the macrocyclic ring. The effect of macrocycle protonation is demonstrated by the observation that anions are separated even in the presence of a lithium sulfate eluent (the top chromatogram in Fig. 4). Li$^+$ ion has very little affinity for either of the ligands and hence little column anion capacity can result from the formation of metal ion-macrocycle
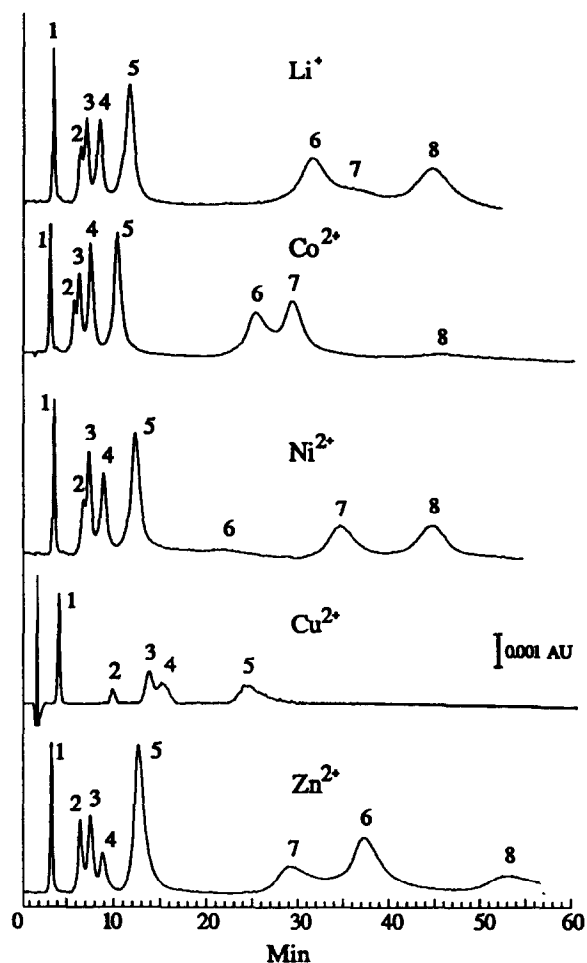
Fig. 4. Effect of eluent cation on anion separation on MPIC–DD22 column. Eluents (from top to bottom): Li_2SO_4 (pH 7.2), $CoSO_4$ (pH 6.9), $NiSO_4$ (pH 7.8), $CuSO_4$ (pH 7.8), $ZnSO_4$ (pH 7.5); each 1 mM. Peaks as in Fig. 3.

complexes. Yet with the Li^+ containing eluent, significant retention of anions was observed. This column capacity must be due to the protonation of the macrocycle nitrogens. Since the binding of metal ions by the macrocycle occurs in competition with the protonation reaction, we must consider the degree to which protonation occurs.

The reactions that can take place in the column to form anion exchange sites are (1) the protonation of each of the macrocyclic nitrogens and (2) the binding of a metal ion. These reactions can be written as:

$$H_{aq}^+ + L_s \rightleftarrows HL_s^+$$

$$H_{aq}^+ + HL_s^+ \rightleftarrows H_2L_s^{2+}$$

$$M_{aq}^{2+} + L_s \rightleftarrows ML_s^{2+}$$
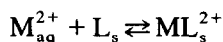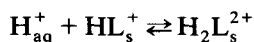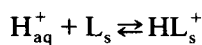
where L_s denotes the macrocyclic ligand adsorbed on the resin and M_{aq}^{2+} is the eluent cation. Each of these reactions has a corresponding expression for the equilibrium constant that can be written:

$$K_1 + \frac{[HL_s^+]}{[L_{f,s}][H_{aq}^+]}$$

$$K_2 = \frac{[H_2L_s^{2+}]}{[HL_s^+][H_{aq}^+]}$$

$$K_M = \frac{[ML_s^{2+}]}{[L_{f,s}][M_{aq}^{2+}]}$$

where K_1 and K_2 are the first and second protonation constants, K_M the equilibrium constant for the binding of the metal ion by the macrocycle, and $L_{f,s}$ the amount of free ligand on the stationary phase. The extent to which the macrocycles are bound to metal ions can be found by combining these three equilibrium expressions:

$$[ML_s^{2+}] = \frac{K_M[L_{T,s}][M_{aq}^{2+}]}{1 + K_1[H_{aq}^+] + K_1K_2[H_{aq}^+]^2 + K_M[M_{aq}^{2+}]}$$

where $L_{T,s}$ is the total amount of ligand adsorbed on the column. The protonation constants and the binding constants of a variety of metal ions in homogeneous aqueous solution with the parent compounds of these ligands have been tabulated [14,15]. Calculations using these values can be made as an indirect method to determine the relative population of complex and protonated species if the water soluble parent compounds were used. Although not directly applicable to our system, this exercise may be useful in explaining some of the trends in our results. Indeed, extraction equilbria often follow patterns similar to those observed in homogeneous solution data, although the absolute values of the association constants differ. For Me$_2$2.2, with a 1

mM $CuSO_4$ eluent at a pH of 7.8, the majority (approximately 98%) of the macrocycles would contain Cu^{2+} ions. 2.2.2, which also has high affinity for Cu^{2+}, would be bound to a similar degree by Cu^{2+} ions. The other transition metals have lower affinities for both $Me_2 2.2$ and 2.2.2, and would be bound to a much lower degree relative to protons. For example, with a 1 mM Ni^{2+} (pH 7.8) eluent, only 9% of the $Me_2 2.2$ and 3% of 2.2.2 macrocycles would contain a metal ion. Zinc and cobalt would be bound even less, with 2.4% and 1.3% of $Me_2 2.2$ macrocycles containing a metal ion for 1 mM Zn^{2+} (pH 7.5) and Co^{2+} (pH 6.9) eluents, respectively. For 2.2.2, the degree of binding of Zn^{2+} and Co^{2+} ought to be even lower, with 0.2% of 2.2.2 macrocycles containing metal ions for either of these ions at a concentration of 1mM. The degree of complexation could be increased by raising the eluent pH, but this approach is not feasible due to the precipitation of the metal ion hydroxide at pH values greater than 7. To the extent that these solution data can be used to predict species distributions in our multiphase system, one would expect Cu^{2+} to be the transition metal ion most likely to yield the highest number of stationary phase complexes.

When comparing eluents with different metal ions, two effects on retention were observed in our experiments. The first was the increase in retention of all the anions with eluents containing cations with higher affinity for the column macrocycle. This effect is shown in Fig. 4. Cu^{2+}, which has a much higher affinity for both DD22 and D222, showed the longest retention, with only 5 of the anions eluting within 1 h. The other transition metal cations exhibited only small differences in affinities for the macrocycles. Eluents containing Co^{2+}, Ni^{2+} and Zn^{2+} all showed similar capacities, as expected by their binding constants and by the likelihood that many of the column macrocycles contain protons rather than metal cations.

The second effect of transition metal-containing eluents involves differences in anion selectivity, due to complexation interactions between the metal ion and the analyte anion. This effect is illustrated by changes in the retention of MoO_4^{2-} and I^- with the Cu^{2+} eluent. Specifically, the

retention times of these two anions increased dramatically over the other anions. One possible explanation for this increased retention is inner sphere complex formation between MoO_4^{2-} or I^- and the Cu^{2+}–macrocycle complex. These ligands are known to form complexes with copper(II) [16]. With the Zn^{2+} eluent there was also a change in selectivity, with molybdate eluting before dichromate. For other anions the overall separations were very similar, with some slight variations. This is not unexpected since it is likely that many of the column macrocycles contain protons rather than metal ions with the Zn^{2+} eluent.

The effect of the eluent cation on the separation of anions can also be examined by comparison to separations achieved with alkali metal cations. DD22 has little affinity for alkali metal cations, and thus little retention was observed with these ions under alkaline conditions. On the other hand, D222 has a high affinity for alkali metal ions, and we have reported excellent separations of anions on D222 columns with alkali metal hydroxide eluents. The separation of anions on the D222 column using a NaOH eluent is similar to the separation observed with the LI_2SO_4 eluent in this work. We have concluded that the separation using Li_2SO_4 eluent resulted from retention of anions by the protonated cryptand, as shown in Fig. 5. With both the protonated macrocycle and the alkali metal cation–macrocycle complex, separation is postulated to occur by purely electrostatic interaction between the anion and the positively charged stationary phase complex. In contrast, with Ni^{2+} eluent, a noticeable effect on the selectivity of anion retention was observed, particularly with the retention of iodide and molybdate. Similar variations were seen with Co^{2+} and Zn^{2+} eluents. In addition, the copper eluent showed a dramatic increase in retention, with only five of the eight anions eluting within 90 min.

3.3. Effect of column substrate

Two different polymeric substrates were used in this research, both based on a polystyrene–divinylbenzene copolymer. The Dionex MPIC resin is designed to perform ion-pairing and
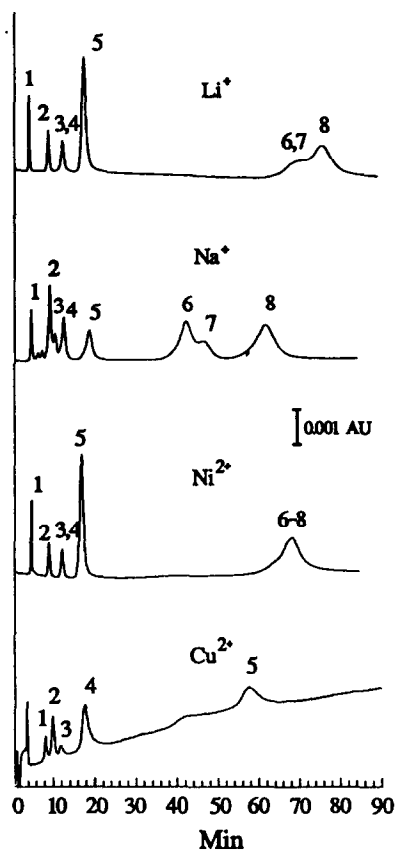
Fig. 5. Effect of eluent cation on separation of anions on MPIC–D222 column. Eluents (from top to bottom): Li₂SO₄ (pH 7.2), NaOH, NiSO₄ (pH 7.8), CuSO₄ (pH 7.8); each 1 mM except NaOH (20 mM). Peaks as in Fig. 3.

Fig. 6. Separation of anions on MPIC (top) and AS10 (bottom) columns, each loaded with DD22 and 1 mM NiSO₄ (pH 7.8) eluent. Peaks as in Fig. 3.

reversed-phase HPLC separations. The MPIC resin is a macroporous, high surface area resin with a particle size of 10 μm. The AS10 resin used in this study is the resin used to make for Dionex AS10 anion separator columns, but which has not yet been derivatized to introduce the ionic functionality. This resin is also a reversed-phase macroporous resin, but of a much lower surface area than the MPIC resin. The average particle size of the AS10 resin is 8.5 μm.

The effect of the column packing substrate is shown in Fig. 6. The same amount of DD22 was loaded onto each of the columns, and the separation was performed with a 1 mM NiSO₄ eluent in both cases. The AS10 based column provided much higher efficiency than th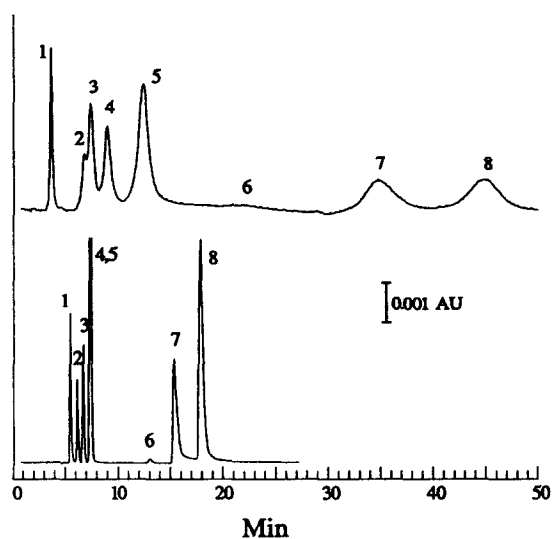e similar separation performed on the MPIC-based column. For example, the peak for nitrate on the MPIC resin has a measured efficiency of 1100 theoretical plates, while the same peak on the AS10 column shows a separation efficiency of 10 870 theoretical plates, an increase in efficiency by more than a factor of almost ten.

While the efficiency of the separation is greatly improved with the AS10 resin, the column capacity is also significantly decreased. This is evident from the fact that the retention times of all of the analytes are significantly lower on the AS10 column, with all 8 anions eluting in 20 min on the AS10 column, as compared to 45 min on the MPIC-based system. Similar results are seen with the D222-based system, with an increase in column efficiency and decrease in analysis time. This effect may be due to crowding since the same amount of ligand was loaded onto each column and the AS10 resin has a much lower surface area.

4. Conclusions

The use of transition metal containing eluents in conjunction with macrocycle-based columns can impart an extra selectivity on anion separations. The retention of MoO₄²⁻ and I⁻ is greatly

increased with Cu^{2+} eluents. However, in cases where the column may be largely protonated rather than bound with metal cations, no major changes in selectivity were observed, indicating that the ion-exchange mechanism is the predominant retention mechanism. The column substrate can play an important part in the efficiency of the separations, even among similar polystyrene-based resins. The use of AS10 resin, a reversed-phase resin intended for anion separator columns that has not yet been derivatized to introduce the ion exchange functionality, greatly improves the efficiency of separations of anions in macrocycle-based ion chromatography.

5. Acknowledgement

References

[1] K. Kimura, H. Harino, E. Hayata and T. Shono, *Anal. Chem.*, 58 (1986) 2233.
[2] M. Lauth and P. Gramain, *J. Chromatogr.*, 395 (1987) 153.
[3] E. Blasius and K.P. Janzen, *Top. Curr. Chem.*, 98 (1981) 163.
[4] M. Nakajima, K. Kimura, E. Hayata and T. Shono, *J. Liq. Chromatogr.*, 7 (1984) 2115.
[5] T. Iwachido, H. Naito, F. Samukawa and K. Ishimaru, *Bull. Chem. Soc. Jpn.*, 59 (1986) 1475.
[6] M. Nakajima, K. Kimura and T. Shono, *Bull. Chem. Soc. Jpn.*, 56 (1983) 3052.
[7] Y. Frere and P. Gramain, *Makromol. Chem.*, 183 (1982) 2163.
[8] E. Blasius, K.P. Janzen, W. Klein, H. Klotz, V.B. Nguyen, T. Nguyen-Tien, R. Pfeffer, G. Scholten, H. Simon, H. Stockemer and A. Toussaint, *J. Chromatogr.*, 201 (1980) 147.
[9] M. Takagi and H. Nakamura, *J. Coord. Chem.*, 15 (1986) 53.
[10] J.D. Lamb and P.A. Drake, *J. Chromatogr.*, 482 (1989) 367.
[11] J.D. Lamb, P.A. Drake and K. Woolley, in P. Jandik and R.M. Cassidy (Editors), *Advances in Ion Chromatography*, Vol. 2, Century International, Medfield, MA, 1990, p. 197.
[12] J.D. Lamb and R.G. Smith, *Talanta*, 39 (1992) 923.
[13] J.D. Lamb, R.G. Smith and J. Jagodzinski, *J. Chromatogr.*, 640 (1993) 33.
[14] R.M. Izatt, J.S. Bradshaw, S.A. Nielsen, J.D. Lamb and J.J. Christensen, *Chem. Rev.*, 85 (1985) 271.
[15] R.M. Izatt, K. Pawlak, J.S. Bradshaw and R.L. Bruening, *Chem. Rev.*, 91 (1991) 1721.
[16] E. Hogfeldt and A.E. Martell, *Stability Constants of Metal–Ion Complexes, Supplement No. 1.*, Burlington House, London, 1971, p. 75.