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# Use of step gradients on different polymeric substrates in the separation of anions by macrocycle-based ion chromatography

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**Abstract**

Macrocycle-based ion-exchange columns have been used in our laboratory for the separation of anions. Column anion capacity is determined by the degree to which column macrocycles are bound with mobile phase cations. Capacity gradient separations have previously been performed by gradually changing the eluent cation from one with a high affinity for the macrocycle to one with a lower affinity over the course of the separation. In this work, we demonstrate that gradient separations can also be performed in step rather than linear fashion by switching eluent cations at the start of the separation and allowing the more strongly bound cation to slowly bleed from the column, reducing the column capacity during the separation. The column capacity is reduced at a rate determined by the rate of loss of the first cation from the column. Two different substrates were used as the basis for the macrocycle-based columns, Dionex MPIC and unsulfonated AS10 resins. The MPIC-based column showed retention characteristics similar to those that we have previously described with ramp gradients, while the AS10-based systems showed improved column efficiencies. Separations achieved with step gradients on these two substrates are comparable to linear gradients achieved with the same chemical systems, eliminating the need for pumps with gradient capabilities. Fourteen anions of widely varying character were separated on the AS10-based D222 column using a step gradient from NaOH to LiOH in just over 10 min.

## 1. Introduction

Until recently, gradient elution anion chromatography was considered incompatible with conductivity detection, which is the most common mode of detection in ion chromatography. The changes in eluent strength required to elute strongly retained anions caused severe changes in the baseline. With improvements in column and suppressor technology, gradient separations have become more feasible. Salts of weak acids have been used to perform gradient anion separations, and protonated cations of weak bases or amino acids have been used as eluents in cation gradients [1,2]. Gradients in unsuppressed ion chromatography have been performed using a concentration gradient between two isoconductive eluents [3]. Improvements in suppressor technology have allowed gradient separations with hydroxide eluents. All of the above types of gradients are based on an increase in eluent strength (gradient elution). This increase in eluent strength can cause baseline disturbances with conductivity detection.

In our laboratory we have performed gradient separations of anions by changing the column capacity rather than the eluent strength during the course of the separation (gradient capacity). These gradients employ macrocycle-based ion-exchange columns [4–7]. Macrocycle-based col-

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umns have been used to separate cations with purely aqueous mobile phases based on the ability of macrocycles to selectively complex cations. Cations that are more tightly bound by the macrocycle are retained longer on the column [8–10]. Anions have also been separated on macrocycle-based columns with water as eluent. In this case, anions that facilitate closer interaction between the cation and the macrocycle are retained longer than other anions [11–14].

In our laboratory we have separated anions by an ion-exchange rather than a ligand-exchange mechanism using an alkali metal hydroxide in the eluent. The metal ion undergoes complexation with the neutral macrocycle on the stationary phase, creating a positively charged anion-exchange site. The hydroxide ions serve to elute the anions from the column. The number of ion-exchange sites is dependent on the degree to which the macrocycle complexes the eluent cation. Thus, eluent cations that are more strongly bound by the macrocycle generate higher column capacities than cations that are less tightly bound.

Capacity gradient separations have been performed based on the effect of eluent cation on column anion-exchange capacity. This effect is accomplished in macrocycle-based systems by changing the eluent cation from one with a high affinity for the macrocycle to one of a lower affinity during the separation. Column capacity decreases as the number of exchange sites diminishes due to loss of the strongly bound cation from the column [10–12]. The eluent ionic strength remains more or less constant as only the identity, rather than the concentration, of the eluent cation is changed. Resulting gradient separations show the ability to elute a wide variety of anions with little or no change in baseline.

In the past, capacity gradients of the type described have been performed by gradually changing from one cation to another over a period of time in a linear mode, with the column capacity changing slowly. In this work we describe gradient separations performed in a step mode, which requires no gradient programming with its associated hardware. Excellent separations were achieved with these step gradients.

The type of column polymer substrate plays an important role in the type of separation achieved in the systems we have developed. All of our previous work has been done using Dionex MPIC columns, which contain a polystyrene–divinylbenzene polymeric resin. We recently began using columns based on the resin used in Dionex AS10 anion separator columns, but which has not yet been derivatized to produce the anion-exchange functionalities. Macrocycle columns made using this resin show better efficiency and faster separations than the MPIC resin-based columns used previously.

## 2. Experimental

### 2.1. Materials

Cryptand *n*-decyl-2.2.2 (D222), whose structure is shown in Fig. 1, was obtained from EM Science (Gibbstown, NJ, USA). All compounds used in making eluents and standards were reagent grade or better. Water used in making eluents was purified to 18 MΩ resistivity using a Milli-Q purification system and was degassed by sparging with helium. Eluent purity and degassing is crucial to prevent baseline disturbances in gradient separations. Underivatized AS10 type resin was obtained from Dionex. Both the MPIC and AS10 substrates are macroporous, ethylvinylbenzene–divinylbenzene copolymeric resins with 55% cross-linking. The AS10 resin differs from the MPIC resin in particle size (8.5 $\mu$m versus 10 $\mu$m) and that it has a much lower surface area (100 $m^2/g$ compared to 300 $m^2/g$) due to increased pore size.

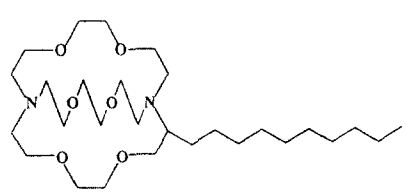

Fig. 1. Structure of the cryptand D222.

### 2.2. Apparatus

A Dionex 4000i series ion chromatograph was used in conjunction with Dionex anion micromembrane suppressors (AMMS) prior to conductivity detection with a Dionex CDM-2 conductivity detector. The suppressant was 12.5 m*M* $H_2SO_4$ flowing at 3–5 ml/min. Prepacked columns used were Dionex NS-1 MPIC columns.

### 2.3. Column preparation

Two different methods were employed to prepare macrocycle-based columns. Columns based on MPIC resin were prepared in the manner described previously [9] by circulation of a methanol–water (60:40) solution containing the appropriate amount of cryptand through the column for a period of 12 h.

Columns based on the underivatized AS10 resin were prepared by slurrying a methanol–water (60:40) solution containing the cryptand with the resin, also in a methanol–water mixture. The methanol was evaporated, and the resulting resin was packed into a 25 cm × 0.4 cm column.

## 3. Results and discussion

### 3.1. Macrocycle-based separation system

In macrocycle-based anion chromatography, the cation in the eluent has a great influence on the column capacity, and hence anion retention, as shown in Fig. 2. With the cryptand D222, lithium hydroxide eluent shows little retention of anions; all fourteen anions elute within 5 min. With a sodium hydroxide eluent at the same concentration, a high capacity results with only eight anions eluting in 60 min. This change in column capacity results from the much higher affinity of the $Na^+$ cation for the cryptand. The effect of the eluent cation on anion retention has been used to perform linear gradient separations by changing from an eluent that displays high capacity at the beginning of the separation, such as sodium, to an eluent cation that shows lower capacity, such as lithium, during the course of the separation [5,7].

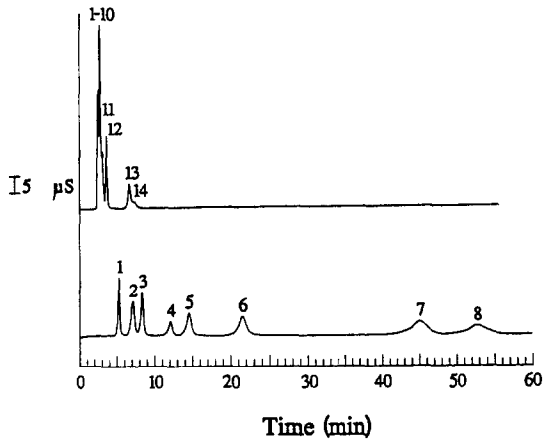


Fig. 2. Separation of 14 anion standard on D222-MPIC based column. Eluent: top chromatogram: 30 m*M* LiOH; bottom chromatogram: 30 m*M* NaOH; both at 1.0 ml/min. Peaks: 1 = $F^-$, 1.5 ppm (w/w); 2 = acetate, 10 ppm; 3 = $Cl^-$, 3 ppm; 4 = $NO_2^-$, 10 ppm; 5 = $Br^-$, 10 ppm; 6 = $NO_3^-$, 10 ppm; 7 = $SO_4^{2-}$, 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. From ref. 5.

### 3.2. Step gradients

Gradient separations of anions have been performed using linear gradients between two different eluents over a period of time to gradually increase eluent strength while lowering column capacity and provide separations of anions with widely varying affinities for the stationary phase. Such gradients require pumps that are capable of proportioning different eluent concentrations over the course of the separation. This need for high-pressure pumps with the capability to proportion eluents increases the cost and complexity of separations.

Step gradients have been reported for the separation of cations with widely different affinities for the stationary phases used in cation exchange chromatography, such as the separation of alkali metal and alkaline earth cations [15,16]. Anion selectivities do not show the same grouping of behavior as do the group I and II

cations. Rather, selectivities vary more smoothly between mono- and divalent anions. Hence, little has been reported on the use of step gradients in anion separations due to the need to more gently increase the eluent strength in order to provide optimum resolution of anions.

Capacity gradient separations with macrocycle-based ion chromatography are possible due to the bleeding from the column of the strongly bound cation employed at the beginning of the separation as the cation with lower affinity is introduced into the column. Since the strongly bound cation slowly bleeds from the column, step gradients in which the capacity of the column changes slowly are possible. Such a gradient is shown in Fig. 3, where a corresponding linear gradient is shown for comparison. The top chromatogram shows the separation of a fourteen-anion standard with a linear gradient between sodium and lithium hydroxides, each 30 m*M*, over the first 20 min of the separation. Good separation of the early eluting anions is achieved, with strongly retained anions eluting in a reasonable period of time. The lower chromatogram shows a step gradient between the same two eluents, but with the eluent switched from sodium to lithium at the time of injection. The resulting separation strongly resembles the linear gradient, with only minor differences in elution times, and some improvement in overall analysis time.

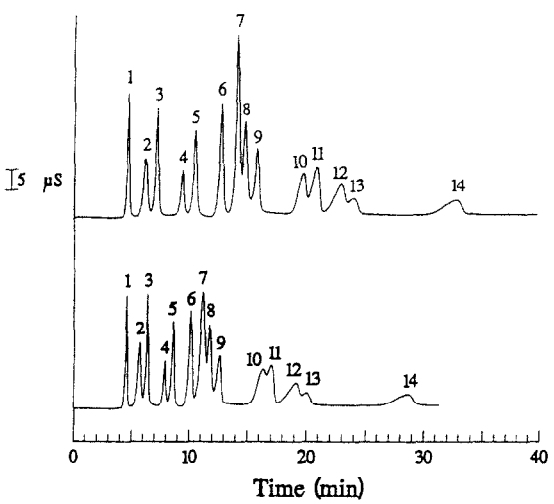


Fig. 3. Gradient separations of 14 anion standard on MPIC column loaded with D222. Gradient conditions: top chromatogram: linear gradient from 30 m*M* NaOH to 30 m*M* LiOH from 0 to 20 min; bottom chromatogram: step gradient from 30 m*M* NaOH to 30 m*M* LiOH at time of injection. Peaks as in Fig. 2.

### 3.3. *Effect of column substrate*

To date, we have reported cryptand stationary phases based on Dionex MPIC resin, a cross-linked, polystyrene–divinylbenzene substrate used for ion-pairing separations. In order to increase column efficiency, we have recently employed a new type of substrate for cryptand-based columns. This substrate is the underivatized resin used by Dionex as the basis for AS10 anion separator columns. This resin, while chemically similar to MPIC resin, differs in particle porosity and surface area. The resin is normally sulfonated and agglomerated with aminated latex to form AS10 anion separators. The underivatized resin, containing no ion-exchange sites, was combined in a slurry with the cryptand, and the resulting resin was packed into columns.

The effect of the column substrate on the separation of a seven-anion standard is shown in Fig. 4. The top chromatogram shows the seven-anion standard separated on the MPIC-based

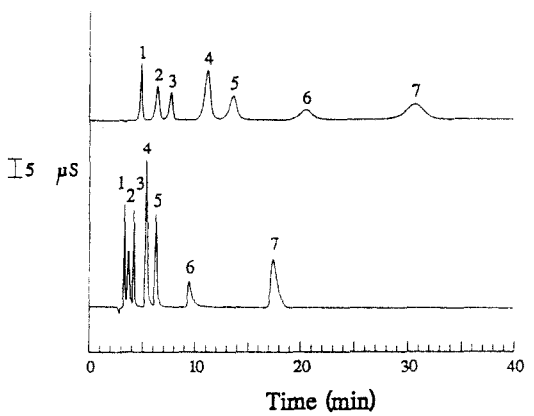


Fig. 4. Comparison of D222 on (top) MPIC resin and (bottom) AS10 resin. Eluent: 30 m*M* NaOH at 1.0 ml/min. Peaks: 1 = $F^-$, 1.5 ppm; 2 = acetate, 10 ppm; 3 = $Cl^-$, 3 ppm; 4 = $NO_2^-$, 10 ppm; 5 = $Br^-$, 10 ppm; 6 = $NO_3^-$, 10 ppm; 7 = $SO_4^{2-}$, 10 ppm.

D222 column used in the previously mentioned separations with a 30 m*M* NaOH eluent. The lower chromatogram shows the separation of the same standard on the AS10-based D222 column with the same 30 m*M* NaOH eluent. The AS10-based system shows higher efficiency and a shorter separation time (10 min as compared to more than 20 min for the MPIC-based resin).

### *3.4. Gradients on AS10 resin macrocycle columns*

Gradients, both linear and step mode, can be performed on the AS10 based column. Fig. 5 shows the separation of a fourteen-anion standard on the AS10–D222 column. The top chromatogram shows a linear gradient between 30 m*M* NaOH and 30 m*M* LiOH over the first 10 min of the separation, with good resolution of all fourteen anions in less than 20 min. The step gradient performed between these two eluents, with the eluents switched at the time of injection, is shown in the bottom chromatogram. All fourteen anions are resolved in just over 10 min, a factor of four times faster than similar separations on the MPIC based systems such as those shown in Fig. 3.

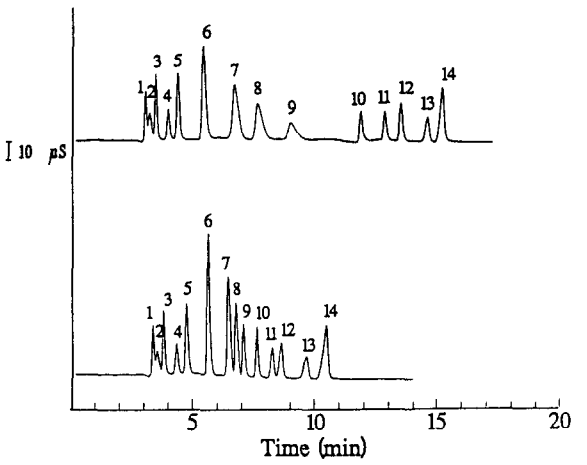


Fig. 5. Gradient separation of 14 anions on AS10 resin loaded with D222. Gradient conditions: top chromatogram: linear gradient from 30 m*M* NaOH to 30 m*M* LiOH from 0 to 10 min; bottom chromatogram: step gradient from 30 m*M* NaOH to 30 m*M* LiOH at time of injection. Peaks as in Fig. 2.

The difference between the two types of gradients can be explained by the rate at which the column capacity is changing. The rate of bleed of sodium from the column was measured by performing both linear and step gradients on the AS10 based column. The eluent was collected in 1-ml fractions before it entered the suppressor, where the eluent cations are removed from the eluent stream prior to detection. These samples were analyzed for the concentration of sodium present in the eluent as it left the column. The results are plotted in Fig. 6, with the concentration of sodium present in the eluent plotted against the time elapsed since the start of the gradient. Also included is the calculated gradient profile that should be observed if there were no macrocycle on the column. This plot shows that the sodium concentration in the eluent drops much more rapidly in the step gradient case than in the linear gradient case, resulting in a more rapid decrease in column capacity and faster elution of anions from the column. In both the linear and step gradient cases the concentration of sodium present in the column effluent is higher than predicted by the

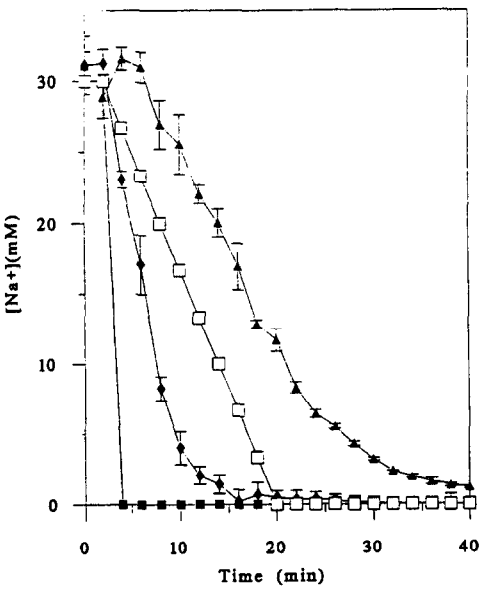


Fig. 6. Effect of gradient type on bleed of sodium from D222 column. Gradient conditions as in Fig. 3. ♦ = Step; ▲ = linear; ■ = theoretical step; □ = theoretical linear.

theoretical gradient profile due to the bleed of sodium bound to the macrocycle as the sodium in the eluent is replaced by lithium. The amount of sodium decreases rapidly in the early part of the gradient, but there is still some bleed of sodium from the column even after the sodium in the eluent has been completely replaced by lithium.

It should be noted that the baseline in both the linear and step gradient systems is unaffected by the gradient conditions; *i.e.* there is little change in the baseline during the course of the separation.

## 4. Conclusions

Gradient anion separations, in both linear and step mode, can be performed on macrocycle-based systems by changing only the identity of the eluent cation. Step gradients with these systems show resolving power similar to the linear gradients, with slightly reduced separation times. These step gradients simplify separations as compared to linear gradients, with less complex pumping equipment required. Such systems are highly dependent on the polymeric substrate. Specifically, the use of underivatized Dionex AS10 resin allows faster, more efficient separations than columns based on the chemically similar MPIC resin.

## 5. Acknowledgements

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