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Adjustable-capacity anion-exchange separator

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Abstract

A cryptand-based anion exchanger has been developed in which the capacity and to a lesser degree, selectivity are adjustable simply by the choice of the mobile phase. Although much work has been done in the past using cryptand-based anion exchangers, these stationary phases were based on adsorbed cryptands rather than covalently bound cryptands. These phases suffered from the usual problems associated with adsorbed systems. A novel styrene-based cryptand has been synthesized which can be covalently attached to a solid support. A brief review of cryptands and binding constants as well as comparisons of adsorbed phases versus covalently bound phases will be discussed. Some of the unique chromatographic properties of this prototype column will be illustrated as well.

Keywords: Capacity gradients; Cryptands; Stationary phases, LC; Anion-exchangers; Inorganic anions

1. Introduction

Anion-exchange chromatography with macrocylic ligands such as cryptands adsorbed onto a stationary phase were studied in great detail from 1989 to 1998 [1–6]. This ability to perform anion exchange was due to the complexation of metal cations within the macrocyle. Once a metal cation has been complexed, a positively charged functional group is generated which then provides the site for anion exchange.

Although crown ethers have found use in adjusting selectivity for cation separations [3,7], they have found only limited use as anion exchangers. Cryptands, due to their much stronger ability to bind certain cations, were found to be quite suitable in anion-exchange separations. Studies clearly showed the unique ability to adjust the capacity of the column simply by the choice of the eluent cation. Capacity was directly proportional to the binding constant of the complexed cation. Perhaps the most powerful aspect of these phases was the application of what was termed ‘‘gradient capacity ion chromatography’’ [2]. One could keep the mobile phase ionic concentration the same, but by changing the cation in the mobile phase, one could decrease the capacity of the column ‘‘during’’ the run. The eluent hydroxide concentration remained the same, only the cation was changed. Hence a run could be started with sodium hydroxide, a moderate capacity mode, with a binding constant of 3.9. Then either a step change or a gradient with lithium hydroxide, a very low capacity mode, with a binding constant of 1.0 can be done. This allows separation and elution of both early eluting anions as well as anions that would not normally elute due to either higher charge or hydrophobicity. This technique also allows the separations of polyvalent anions such as oligosaccharides with relatively low concentrations of mobile phases [6].

These anion-exchange phases were all based on *n*-decyl derivatives adsorbed onto either silica or polymer stationary phases (Fig. 1). Unfortunately,

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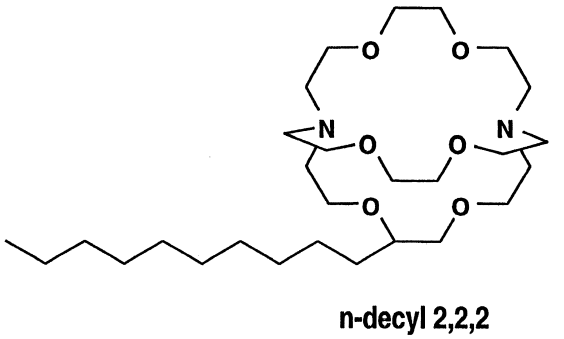

Fig. 1. Adsorbed *n*-decyl 2.2.2 cryptand onto silica C_{18} or polymer-based stationary phases. The numbers refer to the number of oxygen atoms in each bridge.

these adsorbed systems had several inherent drawbacks. The adsorbed macrocycles tended to bleed off the stationary phases with time, which resulted in capacity loss. The use of solvents is therefore very limited if not totally prohibited. This in turn decreases the ruggedness of the column since organic contaminants cannot be cleaned off by the use of solvents. Another drawback was that these phases exhibited low efficiency in most cases.

An alternative approach was needed to eliminate these drawbacks. An obvious solution would be to covalently attach the cryptand to the stationary phase. A novel styrenic 2.2.2 cryptand derivative was synthesized for the purpose of producing a covalently bound cryptand. This monomer cryptand was grafted onto various polymeric supports and tested as an anion-exchange separator (Fig. 2).

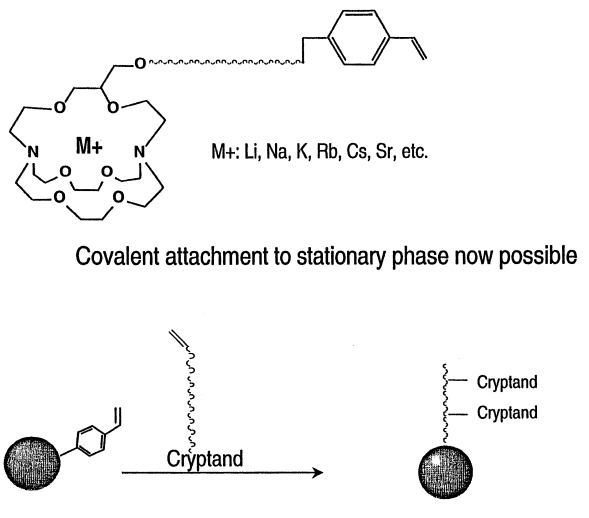

Fig. 2. Monomeric 2.2.2 cryptand used to graft to stationary phase.

This paper will investigate the chromatographic properties of these prototype phases, first with some common and relatively simple isocratic separations and then with what has been termed ''capacity gradients''. Some previously unreported separations involving common anions and polarizable anions will be shown, as well as how this new technology may solve some very difficult separation problems with complex matrices such as sulfuric acid.

2. Experimental

2.1. Apparatus

All experiments were carried out with a DX 500 ion chromatographic system (Dionex, Sunnyvale, CA, USA) consisting of a quaternary gradient pump (GP40) with automated membrane eluent degassing, a chromatographic oven (LC 30), and suppressed conductivity detection (CD 20 conductivity detector). Eluent flow rates for the 10-μm phases, unless otherwise stated, were 1.0 ml/min and for the 5-μm phases 0.5 ml/min. All instrument control, data collection and data processing were performed with the PeakNet Chromatography Workstation (Dionex). The output of the conductivity detector is automatically normalized so that a readout of 1 μS is equivalent to 1 μS/cm. The anion self-regenerating suppressors (ASRS-Ultra 4 mm and 2 mm) were used in the External Water Suppression mode. To remove carbonate from the eluents, an IonPac® ATC anion trap column was inserted in-line between the pump and the injection valve.

2.2. Stationary phases

The cryptand stationary phases studied consisted of conventional 55% cross-linked styrene–divinybenzene polymeric macroporous substrate beads with an average pore size of 150 Å and a surface area of 450 m^2/g. The monomeric 2.2.2 cryptand was then grafted onto the surface of the macroporous resin. The raw resins had average particle sizes of 10 μm tested in the 250 mm×4 mm I.D. format and 5

μm tested in the 150 mm×3 mm I.D. format. The quantities of cryptand bound to the solid support were approximately 330 μequiv./column (250 mm×4 mm) and 110 μequiv./column (150 mm×3 mm). It should be noted that these are the maximum dynamic capacities possible since the column's actual capacity during a run is related to the mobile phase being used.

2.3. Chemicals

Deionized water (18 MΩ cm resistivity at room temperature) from a water purification system (Continental Type I, Laboratory Reagent Grade Water System) was used for the preparation of the eluents and standards. Eluents were prepared from analytical reagent-grade 50% sodium hydroxide, 45% potassium hydroxide (Fisher) and lithium hydroxide monohydrate (Aldrich). Standards were prepared from analytical reagent-grade chemicals.

3. Results and discussion

3.1. Isocratic runs

Initially the first grafts were done on 10-μm resin and tested in a 250 mm×4 mm format. Since we were using a covalently bound cryptand instead of an adsorbed system, we first compared simple isocratic runs to verify expected results. Isocratic runs were made on the same column with three different mobile phases: potassium hydroxide, sodium hydroxide and lithium hydroxide. In each case a concentration of 70 m*M* is used. As can be seen in Fig. 3, results were as expected. Lithium hydroxide shows almost no retention for anions since lithium has a very low binding constant of less than 1.0. When sodium hydroxide was used as the mobile phase we see a higher capacity due to the higher binding constant for sodium (log K=3.9). As potassium has the highest relative binding constant (log K=5.4) run times increased appropriately.

Using 70 m*M* sodium hydroxide as the mobile phase and running at a higher flow rate of 2.0 ml/min excellent resolution of all seven common anions was accomplished in around 3 min. Fluoride was still well out of the water dip and fluoride and

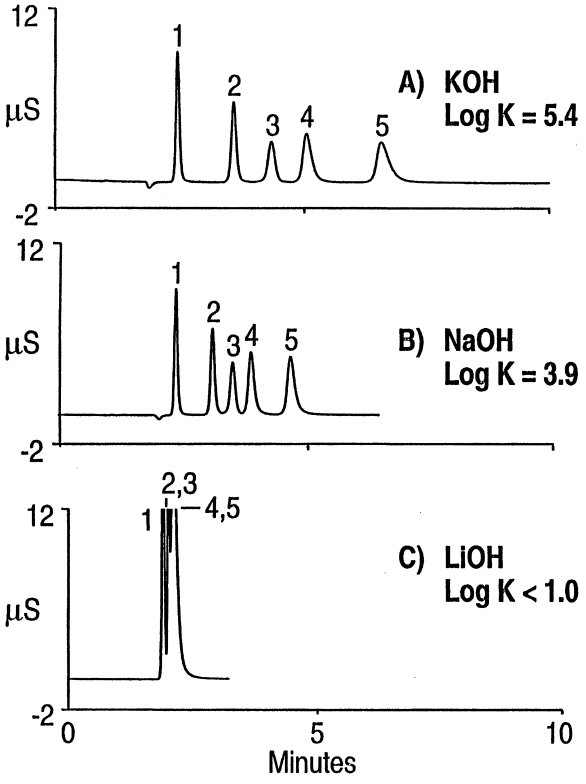

Fig. 3. Capacity versus cation form: hydroxide concentration is the same in each case, only the associated metal cation is different. Column: prototype 250 mm×4 mm I.D., 10 μm. Flow rate: 1.0 ml/min. Column temperature: 35 °C. Injection volume: 25 μl. Peaks: 1=fluoride (2 mg/l); 2=chloride (3 mg/l); 3=sulfate (5 mg/l); 4=nitrite (10 mg/l); 5=bromide (10 mg/l).

chloride were well resolved from acetate/formate. At this point it was clear that the covalent graft of the cryptand was successful and the column performed as expected based on results from the adsorbed systems previously studied. Although of academic interest, simply being able to accomplish separations of common anions was hardly of any commercial value. We needed to demonstrate that this column could accomplish separations that current column technology could not.

As the monomer was rather difficult to produce, expense was an issue. Two changes were made in the column format. First of all, grafts were done with 5-μm resins and secondly, these resins were packed into 150 mm×3 mm formats, which is approximately one-third the volume in the 250 mm×3 mm

column. Thus we were able to maintain efficiency as well as cut down on costs. All runs shown from this point on will be done with this new format.

3.2. Capacity gradients and mixed modes: adjusting capacity

One application that needed improvement was that of the common anions with the polarizable anions thiosulfate, iodide, thiocyanate and perchlorate. Conventional anion exchangers simply do not allow the elution of the hydrophobic anions even when using very steep gradients. The IonPac AS16 column was designed at Dionex specifically for this application and has met with great success. However resolution of bromide and nitrate peaks is not possible without resorting to rather long run times of around 25 min. Could the cryptand column perhaps show improved resolution with shorter run times?

As previously mentioned, the real power of this technology was demonstrated with the use of capacity gradients. A demonstration of the advantages of capacity gradients is shown in Fig. 4. Here we compare a 10 m*M* isocratic sodium hydroxide, run A, with a 10 m*M* capacity gradient run shown in run B. In the capacity gradient run, the column initially is running with 10 m*M* sodium hydroxide. At 0.1 min, a step change is made to 10 m*M* lithium hydroxide. The result is a dramatic improvement in run times, going from 15 min with poor peak shapes for the divalents to around 6 min with great peak shapes and still maintaining baseline resolution. The explanation for this is that initially the column is in the sodium form. When the step change to lithium occurs, the column capacity changes during the run to the lower capacity lithium form resulting in a much quicker run time. The column is then step changed back to 10 m*M* sodium hydroxide to convert the column back to the sodium form for the next injection. This conversion process takes just under 5 min as it does for most cases when going from lower binding constant complexes to higher binding complexes. Using this same approach with both common anions and the polarizable anions we see in run A of Fig. 5 baseline resolution of all peaks in around 10 min. This represents a significant improvement over existing separations.

Another interesting aspect to adjusting the capacity of the column involves what will be termed ''mixed modes''. Rather than starting totally in one form and then switching to another, one can also mix forms initially. This is shown in Fig. 5, runs B and C. Here we start the runs with the column initially in both sodium and lithium form. Again at 0.1 min, a step change to 10 m*M* lithium is made. In run B the mobile phase is an equal mix of 5 m*M* sodium hydroxide and 5 m*M* lithium hydroxide. Note that the hydroxide concentration is the same throughout the whole run. The column is now starting with a lower initial capacity, which in turn lowers the run times. In run C, the principle is extended even further by starting the run with a mobile phase of 2.5 m*M* sodium hydroxide and 7.5 m*M* lithium hydroxide. Again run times are shorter but we still

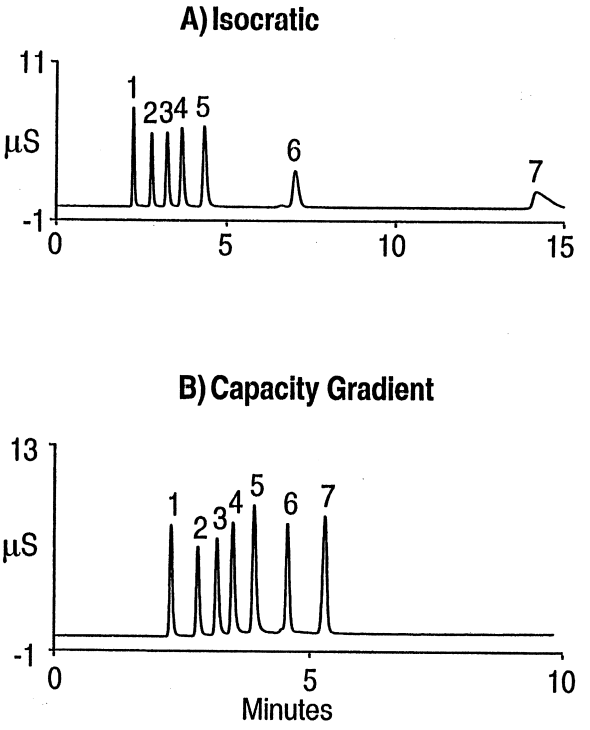

Fig. 4. Isocratic versus capacity gradient: by changing the complexed metal cation during the run, one can achieve much shorter run times and better peak shapes. Column: prototype 150 mm×3 mm I.D., 5 μm. Flow rate: 0.5 ml/min. Column temperature: 35 °C. Injection volume: 5 μl. Peaks: 1=fluoride (2 mg/l); 2=chloride (3 mg/l); 3=nitrite (5 mg/l); 4=bromide (10 mg/l); 5=nitrate (10 mg/l); 6=sulfate (5 mg/l); 7=phosphate (15 mg/l).

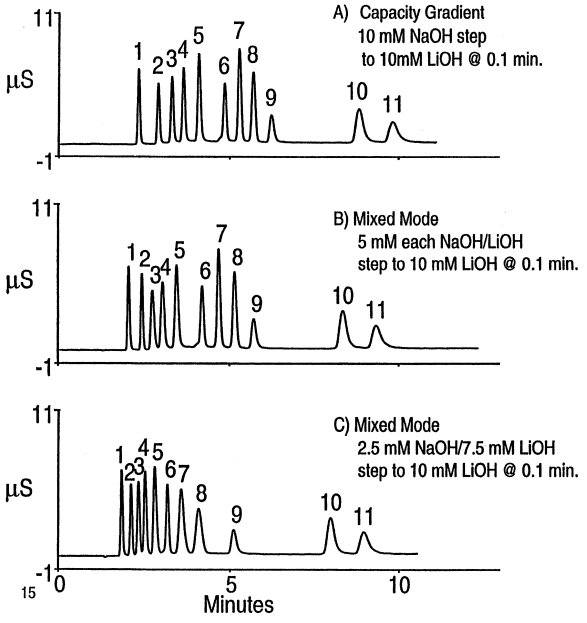

Fig. 5. (A) Simple capacity gradient starting with sodium and step changing to lithium. (B) Using mixed modes with a capacity gradient. The column is partially in the lithium form, partially in the sodium form. (C) The column is started with an even higher amount of lithium. In all three cases a step change to 10 m*M* lithium hydroxide takes place at 0.1 min. Column: prototype 150 mm×3 mm I.D., 5 μm. Flow rate: 0.5 ml/min. Column temperature: 35 °C. Injection volume: 5 μl. Peaks: 1=fluoride (2 mg/l); 2=chloride (3 mg/l); 3=nitrite (5 mg/l); 4=bromide (10 mg/l); 5=nitrate (10 mg/l); 6=sulfate (5 mg/l); 7=thiosulfate (10 mg/l); 8=phosphate (15 mg/l); 9=iodide (10 mg/l); 10=thiocyanate (10 mg/l); 11=perchlorate (15 mg/l).

maintain good resolution with fluoride well out of the water dip. The use of mixed modes along with the concept of a capacity gradient is clearly a new and powerful tool available to the chromatographer.

3.3. Ruggedness

Another area where this column should excel is in the ability to easily restore column performance when fouled by polyvalent contaminants. With conventional anion-exchange columns one has to resort to rather extreme measures to restore column capacity when it has been compromised from high-valency contaminants. As a test the cryptand column was injected multiple times with a 10% solution of polyphosphoric acid. After 10 injections we finally began to see a decrease in capacity and subsequent loss of resolution. Since we can easily convert the column to a near-zero-capacity state simply by running with lithium hydroxide, we should expect all the polyvalent foulants to no longer stick to the column since there would be no ion-exchange sites to hold them. Indeed we found this to be the case. Column capacity and resolution were fully restored after washing the column for 20 min with 30 m*M* lithium hydroxide.

3.4. Sample matrix cations

One area of concern had to do with cations in a sample matrix. If the column capacity was determined by the cation in the eluent, could the sample cations affect the column performance? What would happen if there was 1000 mg/l lithium chloride in a sample? Would there be a slight decrease in column capacity, which in turn might affect reproducibility? To answer this question four different sample matrices were made, each containing 1000 mg/l of various chloride salts. Sample anions added to each matrix were 1 mg/l each of quinate, glycolate, acetate, formate and 0.2 mg/l of fluoride. Therefore one sample matrix contained 1000 mg/l lithium chloride, another with 1000 mg/l sodium chloride, another with the same amount of potassium chloride and finally one with 1000 mg/l calcium chloride. Under normal isocratic conditions using 5 m*M* potassium hydroxide, all the sample anions would be baseline resolved. With the high chloride salt background, there will also be a large chloride peak eluting last. Each of the four sample matrices was run and the subsequent runs were overlaid to see if there was any difference in run times for the sample anions. No difference in run time was observed. This experiment was repeated with higher amounts of background cations. Amounts up to 2500 mg/l still did not adversely affect the run times of the sample anions. It was noted that when using potassium as the mobile phase, the complexed potassium is very strongly bound and the question arose as to whether a sodium-based eluent would be as stable since sodium has a weaker binding constant and may be more readily affected by matrix cations. The same series of experiments was repeated and again, no adverse effect was seen up to 2500 mg/l.

3.5. Complex matrices: sulfuric acid

The analysis of anions in concentrated acids is perhaps one of the most challenging and time-consuming matrices currently performed by ion chromatography. Typically multiple valves and columns are needed to obtain any kind of meaningful data. Analysis times can be as long as 1 h for each injection and high RSDs are typical, the main problem of course being the enormous concentration of the associated acid anion. What potential solution might the cryptand column offer? Could the column withstand injections of sulfuric acid at high enough concentrations that one could still quantitate the anions of interest? To answer this question a series of experiments was tried with a 2% solution of sulfuric acid. The eluent tried first was 10 m*M* sodium hydroxide. There simply was not enough capacity in the sodium form to prevent the column from being overloaded with the enormous amount of sulfate. A higher capacity was needed, therefore the column was then run using 10 m*M* potassium hydroxide. Now we could clearly see at least five anion peaks which are no longer affected by the sulfate peak. However the sulfate peak now takes more than 1 h to completely elute. This is due to the higher capacity of the column when run in the potassium mode. The solution was easy — simply combine both approaches (Fig. 6). Starting the run in the potassium mode (10 m*M* potassium hydroxide) gives the capacity needed to see the anions of interest. Then a step change is made at 10 min to the lower capacity sodium mode (50 m*M* sodium hydroxide). The step change accomplishes two goals: (1) to switch the column to a lower capacity mode to reduce the sulfate run time and (2) we have increased the amount of hydroxide to elute the sulfate off more quickly. As can be seen we now are ready to start a new analysis in under 20 min. One may ask ''why not step change to lithium hydroxide so as to elute off the sulfate even more quickly?'' The reason is that the difference in binding constants between potassium and lithium is so great that lithium simply is not able to switch the column to the almost-zero-capacity mode in a reasonable amount of time. It should be noted that we did not quantitate the sample anions. We are still at the prototype stage of development and simply wanted to demonstrate the potential this column might have. We will also be looking at anions in other acids such as phosphoric, perchloric and nitric acid. Any acid where the anions of interest elute ''before'' the associated acid anion should be good candidates for this approach.

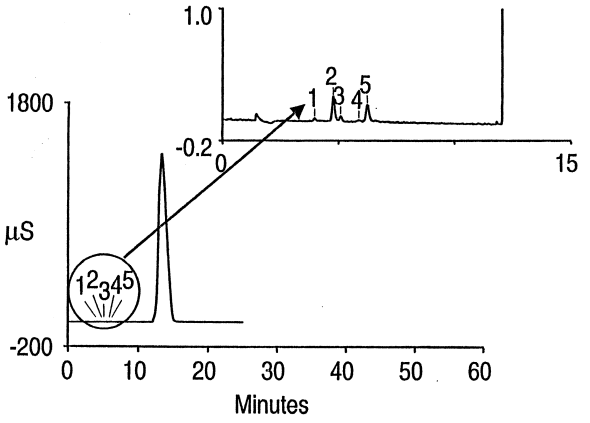

Fig. 6. Sulfuric acid (2%). Column is now run initially in high-capacity potassium mode and then with a step change to sodium to decrease column capacity. This combined with higher hydroxide concentration now elutes off the large sulfate peak in under 20 min. Column: prototype ×150 mm×3 mm I.D., 5 μm. Flow rate: 0.5 ml/min. Column temperature: 35 °C. Injection volume: 5 μl. Peaks: 1=fluoride; 2=acetate; 3=formate; 4=unknown; 5=chloride.

4. Conclusions

We have demonstrated the utility of a covalently bound 2.2.2 cryptand-based anion exchanger. The column easily separates common anions and polarizable anions with ease in under 10 min. The column is rugged, easily cleaned and is insensitive to wide variations in sample matrices. We have also demonstrated some very powerful potential analytical solutions to difficult matrices such as concentrated acids. Clearly much more work is in order before we have a commercial product, but the initial results are very encouraging indeed. Many other applications need to be investigated such as oligosaccharides, oligonucleotides, inositol phosphates, perhaps single- and double-stranded DNA as well. There are other members of the cryptand family that remain to be investigated as well.

Research into cryptand-based anion exchangers

has demonstrated that, just perhaps, the field of ion chromatography still has some exciting and new roads to travel.

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