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Environmental applications of a cryptand adjustable-capacity anion-exchange separator

Andy Woodruff*, Christopher A. Pohl, Andrei Bordunov, Nebojsa Avdalovic
Dionex Corporation, 1228 Titan Way, Sunnyvale, CA 94085, USA

Abstract

A 2.2.2 cryptand-based anion exchanger was recently introduced as a commercial product. This new technology relies on a covalently bonded 2.2.2 cryptand, which allows one to selectively control the capacity of the column simply by the choice of eluent. This provides the analyst with more flexibility over conventional anion exchangers to suit the needs of the sample matrix. Since that time, a 2.2.1 version has also been developed and studied. In this paper we will compare the two types of columns and choose the best one for analyzing several environmental samples.

Keywords: Stationary phases, LC; Ion exchangers; Capacity gradients; Organic acids; Inorganic anions

1. Introduction

The history and development of a covalently bound 2.2.2 cryptand-based anion-exchange column recently introduced were previously discussed [1]. The cryptand macrocycle complexes cations such as lithium, sodium and potassium which in turn provide sites for anion exchange to take place. The associated hydroxide anion acts as a conventional pusher as in normal anion exchange. Cations with low binding constants such as lithium form few sites and hence almost zero capacity. Sodium has a higher binding constant and potassium is much higher. The higher the binding constant, the higher the number of sites which are formed. The higher the number of sights formed, the higher the capacity. The chromatographer now has two dimensions of control, both capacity and eluent strength. The ability to change the column capacity from high to low during a run is known as a capacity gradient. An example would be stepping from a sodium hydroxide eluent to lithium hydroxide. Using this approach one can easily elute highly retained hydrophobic anions along with common anions in much shorter times, yet maintain baseline separations. Samples containing polyvalent species such as polyphosphates are also easily eluted without having to resort to high eluent strengths. If high-resolution front end separations are needed, running with potassium hydroxide, which adjusts the column to the high-capacity mode, will provide good separations. A case in point would be with samples containing organic acids which elute in between fluoride and chloride. Then, if needed, one can step to the appropriate amount of sodium or lithium hydroxide to elute highly retained compounds.

Because of the high binding constant of potassium relative to sodium or lithium, one cannot accomplish the same degree of capacity gradient as with sodium to lithium step changes. A much less dramatic

*Corresponding author. Tel.: +1-408-481-4545; fax: +1-408-733-0645.
E-mail address: andy.woodruff@dionex.com (A. Woodruff).

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capacity gradient is possible by switching from potassium to sodium, but with much less utility than the sodium to lithium approach.

In the previously mentioned work [1], all of the research was done with only one type of macrocycle, the 2.2.2-based cryptand. In work done by Dr. Phil Drake at Proctor and Gamble (Fig. 1) he demonstrated a rather dramatic separation of lactic and acetic acid using an adsorbed *n*-decyl 2.2.1 cryptand column (unpublished research). This separation by conventional, fixed site, anion exchangers has been problematic for many years. The addition of solvent to the mobile phase is necessary to accomplish this separation. In all of the phases developed in our laboratories over the last 25 years, we have yet to develop a functional group that would accomplish this kind of separation with an aqueous mobile phase. The only difference between the 2.2.2 and the 2.2.1 macrocycles is one less oxygen and ethylene group in one of the bridges of the 2.2.1 as seen in Fig. 2. This structural difference was assumed to be the main reason a difference in selectivity was observed between the 2.2.2 and 2.2.1 phases. It was decided to pursue the development of a novel, styrene-based 2.2.1 cryptand which could be grafted onto stationary phases just as with the 2.2.2 version in the hope that new types of selectivity could be obtained.

Although more difficult to synthesize than the 2.2.2 monomer, a method for producing such a compound was developed. This compound was grafted onto a polymeric macroporous stationary phase. This new 2.2.1-based anion-exchange column had approximately the same total 85 μequiv./column capacity as the current commercial 2.2.2 column.

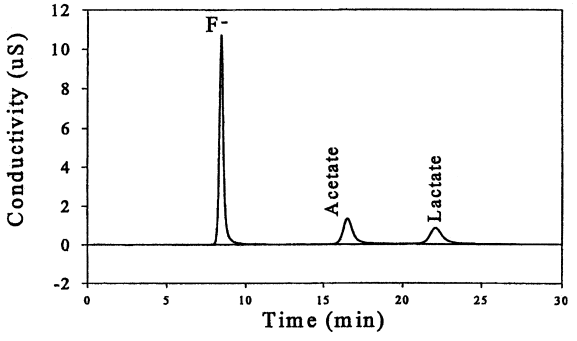

Fig. 1. Previous 2.2.1 work with adsorbed *n*-decyl 2.2.1: separation of fluoride, acetate, and lactate on a NS1 5 μm separator loaded with approximately 300 μequiv. of *n*-decyl 2.2.1. Selectivity and retention seemed fairly stable, but fronting became evident in a few hours. Eluent was approximately 40 m*M* NaOH, flow-rate was 1.25 ml/min.

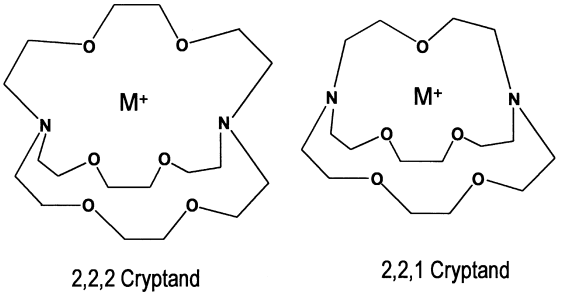

Fig. 2. Structures of 2.2.2 and 2.2.1 cryptand macrocycles.

When comparing relative binding constants, we see in Table 1 that the 2.2.1 has a slightly higher binding constant for lithium than the 2.2.2. Also note that the numbers for sodium and potassium are essentially flip-flopped. The highest binding capacity for the 2.2.1 is now sodium, with the intermediate capacity now being the potassium form. A capacity gradient using the 2.2.1-based column would now be a step change from potassium to lithium.

This paper compares the relative advantages and disadvantages of both the 2.2.2 and the 2.2.1 phases. Based upon this investigation, a phase will be selected to analyze various environmental and industrial samples.

Table 1
Comparison of cryptand binding constants ($\log K$), where $K = [\text{complex}]/[L][M^+]$, L = ligand, M^+ = cation

Cation	Log *K*	
	2.2.2	2.2.1
Li^+	~1	2.50
Na^+	3.9	5.40
K^+	5.4	3.95
Ag^+	9.6	10.6
Ca^{2+}	4.4	6.95
Ba^{2+}	9.5	6.30

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 or LC 25), and suppressed conductivity detection (CD 20 conductivity detector). Eluent flow-rates were 0.5 ml/min. Unless otherwise stated, temperature was maintained at 35 °C. All instrument control, data collection and data processing were performed with the PeakNet Chromatography Workstation (Dionex). An anion self-regenerating suppressor (ASRS-Ultra 2-mm) was 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. In some runs, the recently introduced Continuously Regenerated Anion Trap Column (CR-ATC) was used. This device electrolytically regenerates the resin continuously. Therefore, there is no need to manually remove the ATC trap column and regenerate it periodically. The efficiency of removal is also higher and backgrounds as low as 0.5 μS can be obtained.

2.2. Stationary phases

The cryptand stationary phases consisted of 55% cross-linked styrene–divinylbenzene polymeric macroporous substrate beads with an average pore size of 150 Å and surface area of 450 m^2/g which were grafted with monomeric 2.2.2 cryptand or 2.2.1 cryptand (Dionex). The raw resins had average particle sizes of 5 μm tested in the 150 mm×3 mm I.D. format. The quantity of cryptand bound to a solid support was approximately 85 μequiv./column for each phase. It should be noted that this is the maximum dynamic capacity possible, since the column's actual capacity 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 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 grade chemicals.

3. Results and discussion

3.1. Comparison of 2.2.2 and 2.2.1 stationary phases

3.1.1. Isocratic separation of lactic and acetic

The first comparison made between the two phases was simply to run an isocratic intermediate-capacity mode separation of lactic and acetic acid. It was already known that the 2.2.2 phase could not separate this pair of analytes. Unexpectedly, the 2.2.1 phase did not separate this pair either, even when running in the high capacity mode. In fact, when running under the optimal conditions for separation on each phase, the 2.2.2 seemed slightly better with partial resolution relative to the 2.2.1.

At this point, we only have a few speculations as to why these results were obtained. There are only two differences between our phases and those used by Dr. Drake. One is that, in our case, the macrocycle is covalently bound and his is an adsorbed macrocycle. The other being the linker arm between the macrocycle and the raw resin. The proprietary linker used for covalent attachment is hetero-atomic, giving a somewhat more hydrophilic linker relative to the very hydrophobic *n*-decyl linker Dr. Drake used. The raw resin used in both cases is essentially the same. Further grafts will have to be done to test existing hypotheses and the results will be reported in a future paper. Perhaps the most interesting hypothesis is that this efficient separation between acetic and lactic acids (for the adsorbed case) may have less to do with the functional group where the anion exchange takes place and more due to the micro-environment surrounding the functional group. If true, this may open the door for a new family of selectivities irrespective of functional group.

3.1.2. Capacity gradient comparison

The next comparison was a capacity gradient separating the seven common anions along with the hydrophobic anions thiosulfate, iodide, thiosulfate, and perchlorate. In this approach each phase was initially in the intermediate-capacity mode and at 0.10 min a step change was made to lithium hydroxide. A slightly less concentrated eluent system was used for the 2.2.1 (Fig. 3). Once again, very similar selectivities were observed.

The comparison in Fig. 4 shows something that has never been reported before in the literature for macrocyclic-based anion exchangers. A capacity gradient was done where the second eluent was simply deionized (DI) water. There is better resolution of thiosulfate from the phosphate/iodide peaks and perhaps a bit more resolution for the thiocyanate/perchlorate pair for the 2.2.1 column. Overall, however, not much difference is seen between the two phases when using DI water capacity gradients.

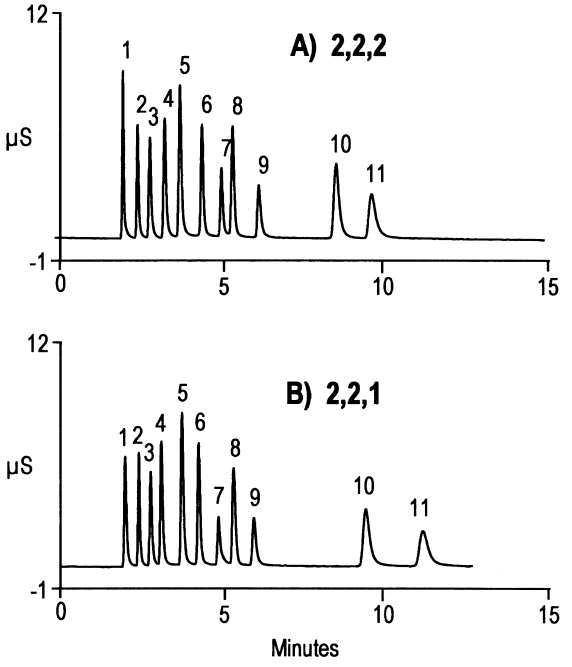

Fig. 3. Chromatographic comparisons of 2.2.2 vs. 2.2.1 with a standard capacity gradient. Run A begins with 10 m*M* sodium hydroxide and step changes to 10 m*M* lithium at 0.1 min for the 2.2.2 column. Run B is also run with a similar capacity gradient for the 2.2.1 column except eluent concentrations are 8 m*M* instead of 10 m*M* (potassium hydroxide to lithium hydroxide at 0.1 min). Columns, 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).

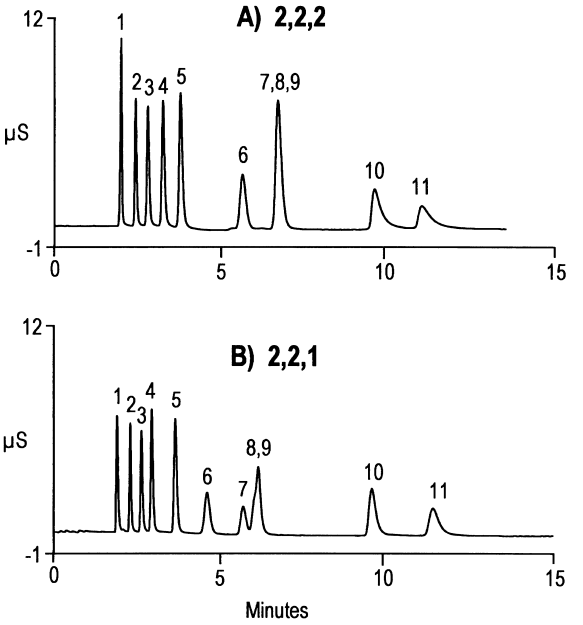

Fig. 4. Chromatographic comparison of 2.2.2 vs. 2.2.1 with a DI water capacity gradient. Run A begins with 10 m*M* sodium hydroxide and step changes to DI water at 0.1 min for the 2.2.2 column. Run B (2.2.1) begins with 8 m*M* potassium hydroxide and step changes to DI water at 0.1 min. Columns, 150 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).

Relative to using DI water capacity gradients, the question arises, how is this possible? Water alone should not be able to elute any anions, let alone very highly retained ones such as thiocyanate and perchlorate. The current understanding is that there are two mechanisms at work. Under these conditions where a step change is made to DI water at 0.10 min, perhaps the most predominant mechanism is related to the protonation constant (pK_a) of the nitrogens within the macrocycle. In the case of the 2.2.2

macrocycle, the literature [2] gives the first pK_a in aqueous solutions as 9.6 and the second as 7.3. When the DI water begins to flow through the column, the pH begins to decrease. At some point one of the nitrogens becomes protonated and ejects out the complexed metal cation due to charge–charge repulsion. This has the effect of temporarily eliminating any site for anion exchange. Momentarily, there is a period of zero capacity and anions are released. Although the pK_a values are different for the 2.2.1 phase, the same effect applies. Eventually the protonated nitrogens create a new set of anion-exchange sites. Because of this, DI water capacity gradients cannot be used for polyvalent species. They are simply too highly charged to avoid being retained by the protonated sites, which eventually arise from the change in pH. Indeed, this is exactly what is seen if a run is made with polyphosphates. None of the larger, oligomeric polyphosphates elute when using a DI capacity gradient.

A second mechanism has also been postulated based on observation, related to partition equilibrium. When the concentration of metal cation in the mobile phase is very low, the partition equilibrium seems to be shifted in favor of the mobile phase. At some critical low concentration, the metal cation no longer remains in the cryptand and partitions back into the mobile phase, thus eliminating sights for anion exchange. The same elution profile is observed (as in a DI water capacity gradient) when a step change is made to 0.1 m*M* sodium hydroxide. Under these conditions there is no protonation taking place, yet a near total loss of capacity is observed. The concentration at which this phenomenon occurs is dependent upon the binding constant of the metal cation in question. In general, the higher the binding constant, the lower the concentration must be before observing this effect.

3.1.3. Sample matrix cations

There was one last important comparison that needed to be made between the two phases. With the original work on the 2.2.2 phase [1], one concern was the effect of high concentrations of various cations in the sample matrix. For instance, if the stationary phase was being run with sodium hydroxide and a sample containing a large amount of potassium was injected onto the column, what effect would this have? After all, if the capacity of the column was determined by the cation present, might the presence of other sample cations negatively affect the chromatography? Fortunately, it was found that the column was not adversely effected even when samples contained over 1000 mg/ml of cations such as lithium, sodium, potassium or calcium. When the 2.2.1 phase was subjected to the same tests, results clearly indicated that it was not so resilient and, in fact, a loss of resolution for early eluting organic acid anions was observed. Based on these results, it was decided to use the 2.2.2 phase instead of the 2.2.1 for the environmental samples analyzed.

4. Samples

A very popular trend these days has been the use of water home treatment systems. One such system utilizes a removable cartridge, which fits into a two-compartment pitcher. The top compartment is where the tap water enters the system and slowly flows through the cartridge into the bottom compartment, from which the water is drawn for use. Usually the cartridge consists of an ion-exchange resin, porous carbon, and perhaps a microfiltration unit as well. The whole system is designed to remove toxic metals such as lead, other ions, organic contaminants, and bacteria. A before and after treatment of tap water is shown in Fig. 5. In this case the column was run isocratically in the high-capacity mode with 13 m*M* potassium hydroxide. The high-capacity mode was needed to separate the large amount of carbonate (several hundred mg/l) from the sulfate present. The untreated tap water shows the expected levels of chloride and sulfate as well as some nitrate. After being treated, the water showed increased chloride levels. No doubt it is due to the fact that the ion-exchange resin is initially in the chloride form prior to use. As the resin picks up other ions, chloride is displaced, which increases the chloride in the water after treatment. Most of the nitrate and carbonate were removed, however the sulfate concentration was essentially the same.

In Fig. 6, an alkaline wastewater from a light hydrocarbon plant is seen. Here is an example of increasing the hydroxide concentration while maintaining the column in the high-capacity mode. The

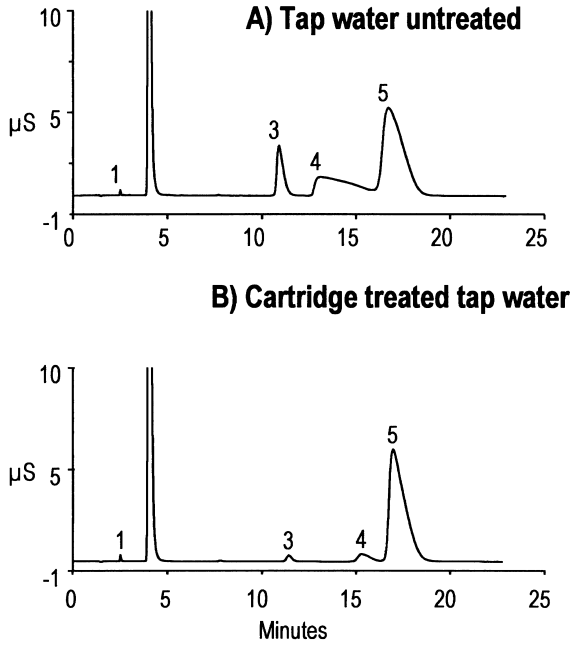

Fig. 5. Common tap water before and after cartridge treatment. Column, Cryptand A1 150 mm×3 mm I.D., 5 μm; eluent, 13 m*M* potassium hydroxide; flow-rate, 0.5 ml/min; column temperature, 35 °C; injection volume, 5 μl. Peaks: 1=fluoride, 2=chloride, 3=nitrate, 4=carbonate, 5=sulfate.

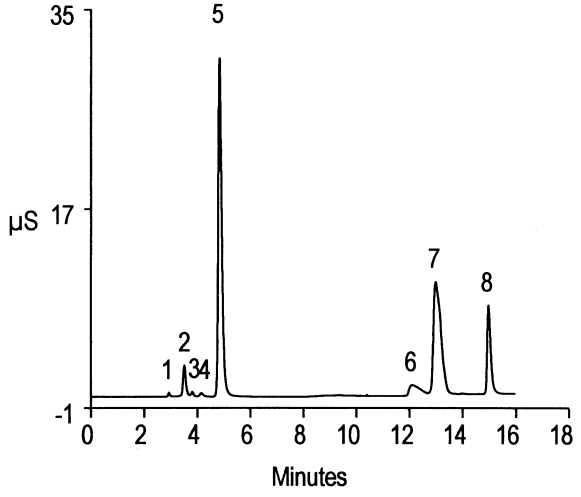

Fig. 6. Alkaline (pH 14) industrial wastewater from light hydrocarbon plant. Column, Cryptand A1 150 mm×3 mm I.D., 5 μm; eluent, 10 m*M* potassium hydroxide (continuous), at 5 min step to 22 m*M* lithium hydroxide until 10 min then step to 40 m*M* lithium hydroxide; flow-rate, 0.5 ml/min; column temperature, 35 °C; injection volume, 5 μl. Peaks: 1=fluoride, 2=acetate, 3=formate, 4=propionate, 5=chloride, 6=carbonate, 7=sulfate, 8=thiosulfate.

potassium hydroxide concentration was 10 m*M* throughout the entire run. The high-capacity mode is needed to separate the early eluting organic acids from fluoride and chloride. The hydroxide concentration was increased with a step change to lithium hydroxide to elute carbonate and sulfate. Finally, stepping to an even higher lithium hydroxide concentration eluted the highly retained thiosulfate.

An anticavity, fluoride treatment mouthwash sample was run with a conventional capacity gradient (Fig. 7). All expected peaks were found with the highly retained benzoate being eluted within 12 min.

Another advantage the cryptand column offers over conventional columns is in the method development phase. Often there may be highly retained unknowns contained in a sample. Often one can be fooled into thinking all the sample peaks have eluted off when beginning a method only to find long broad peaks eluting in subsequent injections from the original injection. By using a capacity gradient in the first run, one can easily see all the analytes in a sample. Knowing what the latest eluting peak is, a method can then be developed to ensure that all analytes are eluted from the column.

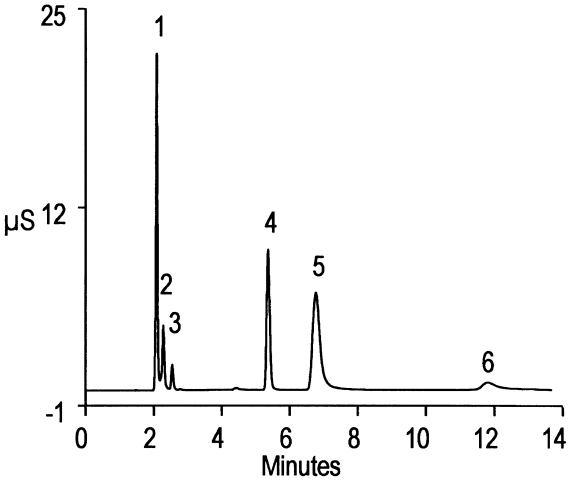

Fig. 7. Anticavity fluoride treatment mouthwash using a standard capacity gradient. Column: Cryptand A1 150 mm×3 mm I.D., 5 μm; eluent, 10 m*M* sodium hydroxide, step to 10 m*M* lithium hydroxide at 0.1 min; flow-rate, 0.5 ml/min; column temperature, 35 °C; injection volume, 5 μl. Peaks: 1=fluoride, 2=formate, 3=chloride, 4=propionate, 5=benzoate, 6=saccharin.

5. Conclusions

A new macrocyclic anion exchanger based on a 2.2.1 cryptand was compared with the commercially available 2.2.2 phase. The results obtained indicate similar selectivities for both stationary phases. The grafted 2.2.1 phase did not have the expected selectivity compared to previous work reported with adsorbed 2.2.1 phases. It was even slightly inferior compared to the 2.2.2 phase with respect to acetic/lactic acid separations. It was also determined that the 2.2.1 phase could be adversely effected if the sample matrix contained high concentrations of cations, whereas the 2.2.2 phase can be used for separations of such samples without losing resolution.

Capacity gradients were reported for the first time using DI water as the second eluent and two potential mechanisms were postulated to explain this behavior. Another unusual phenomenon discussed here was the near total loss of capacity when the eluent cation concentration was very low (while maintaining high pH).

Several environmental applications were shown using the 2.2.2-based stationary phase.

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