Research article

Preparation of ion exchange columns with longitudinal stationary phase gradients

Christopher A. Pohl *

Thermo Fisher Scientific, 1228 Titan Way, Sunnyvale, CA 94085, USA

ABSTRACT

Preparation of columns using electrostatic attachment of anion exchange latex particles with charge density gradients is demonstrated. When such columns are oriented with the highest charge density at the column outlet, the chromatographic performance at low linear velocity is enhanced. When multiple successive charge density gradients are prepared along the length of the column with the highest capacity oriented at the inlet end of the column, significant improvement in chromatographic performance is observed during gradient elution chromatography.

1. Introduction

Over my career spanning more than 47 years of chemistry R&D, I consider myself to be a very fortunate individual. Not only have I had a chance to make significant contributions to the field of ion chromatography, but I had the privilege of working with Hamish Small for almost 40 of those years. I knew Hamish as a mentor, a collaborator, a coinventor and a friend. He had a great sense of humor and I always looked forward to meeting with him to hear his latest jokes and stories. Arguably, even though he made many substantial contributions to analytical chemistry [1, 2, 3, 4, 5, 6, 7] it was Hamish's development of latex agglomerated stationary phases that was his greatest invention [8].

To understand the significance of this invention, it's important to put it into the context of what led Hamish to this invention and why it was critical to ion chromatography. Hamish began work at Dow Chemical in late 1955, doing research on the use of ion exchange resins. One of the earliest projects that he worked on was the problem of mixed bed resin agglomeration. In order to regenerate the resin after mixed bed resin has become expended, the particles are separated based on density differences between the anion-exchange resin which is less dense and the cation-exchange resin which is denser. By choosing an appropriate density slurry medium, particle should separate into two zones with the anion-exchange resin in the upper zone and the cation-exchange resin in the lower zone. Unfortunately, even though these particles were relatively large (roughly a millimeter in diameter) they tended to aggregate due to electrostatic attraction. This made it difficult to cleanly separate the anion-exchange resins from the cation-exchange resins which

compromised the water quality and resin capacity after regeneration. There was some evidence that the problem was worst with freshly prepared resins. As he studied the problem, he came to realize that if a mixture of anion-exchange and cation-exchange resins were vigorously shaken, the aggregation problem could be diminished or even eliminated if the shaking process was long enough. From this observation, he came up with the hypothesis that shaking the particles vigorously enough would result in polymer fragments from the oppositely charged resin being left on the surface as the particles were forced apart due to vigorous shaking. That gave Hamish the idea that he could accomplish the same process in a matter of seconds by simply exposing one of the two resins (either the anion-exchange or the cation-exchange resin) to an oppositely charged linear polymer. That idea led to Hamish's first patent, filed in 1956 during his first full year of employment at Dow Chemical Company [9].

In 1971 when Hamish began work on what we now refer to as Ion Chromatography, he had a problem to solve. Bill Bauman, who also worked at Dow Chemical, had suggested to Hamish the idea of using a "suppressor" column after the separation column to remove the eluent prior to detection with the conductivity detector. While Hamish understood the potential of the suggestion, he quickly realized that the concept wouldn't work unless the capacity of the separation resin was dramatically less than that of the suppressor column. He had previously developed a method for surface sulfonating styrene-divinylbenzene copolymer beads which allowed the preparation of low capacity cation exchange resin. Hamish used some of this resin to demonstrate the ion chromatography concept. Because the quaternization reaction of an amine with a

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chloromethylated styrene-divinylbenzene copolymer beads is not amenable to a surface reaction, he was stuck for a way to produce a similarly low capacity anion-exchange material. Then he remembered the work he had done to develop an anti-clumping reagent for mixed-bed ion-exchange resin. Since he knew he needed cross-linked anion-exchange material for the coating, he initially investigated this by grinding macroporous anion-exchange resin to an extremely fine powder in a mill. He then made a slurry of the finest fraction of the ground powder with surface sulfonated cation-exchange resin. Just as in the case of the anti-clumping reagent for mixed-bed ion-exchange resins, the electrostatic forces involved in binding these particles to the surface of the substrate particle proved strong enough to result in an essentially irreversible attachment of the anion-exchange particles on the surface of much larger surface-sulfonated cation-exchange substrate particles. While grinding resin was a reasonable starting point for preparing colloidal anion-exchange materials, at that time Dow Chemical was in the business of producing latex paint. He worked with some of the latex synthesis experts to produce an ion-exchange latex particle for use in second-generation anion-exchange materials for use in ion chromatography. It was this approach that was transferred to the polymer chemistry team in the early 70s at what was ultimately to become Dionex Corporation.

From that initial invention, electrostatically attached latex-based stationary phases were widely deployed in a variety of architectures including substantially smaller latex particles applied to smaller substrate particles in order to improve efficiency [10, 11], latex coated wide-pore substrates with pore sizes substantially larger than latex particle sizes in order to produce higher capacity [12], cation-exchange analogues (e.g. the Dionex IonPac CS3 column), dual layer anion and cation-exchange latex coated phases suitable for simultaneous retention via anion-exchange and cation-exchange (e.g. the Dionex IonPac CS5A column) and mixed-mode ion exchange phases combining ion-exchange with reversed phase (e.g. the Dionex OmniPac PAX-500 column) [13]. The wide use of this technology stems from a variety of useful attributes including the ease of preparing batch of stationary phase suitable for producing batches of stationary phase lasting years or even decades, the reproducibility of the stationary phase manufacturing process and the simplicity of the manufacturing process. Attaching the latex particle to the stationary phase is essentially instantaneous requiring only mixing a slurry of substrate particles with a slurry of latex particles while stirring. Adjusting the capacity of such latex-based stationary phases is equally straightforward. Capacity is directly proportional to latex diameter and inversely proportional to substrate diameter.

Given all the advantages of preparation of latex-based stationary phases it's easy to see why they have been widely deployed in so many different commercial products. One area that hasn't been explored, however, is the use of latex-based stationary phases for the preparation of columns with longitudinal gradients. A number of authors have investigated the fabrication and properties of columns with longitudinal gradients. Svec et al. [14] have investigated the use of photo polymerization to introduce capacity gradients on capillary monolithic columns for use in CEC. However, it is doubtful that such a technique could be used with larger diameter packed columns since most column hardware for larger diameter columns packed with small particle size media is opaque. Svec and Urbanova investigated using photo grafting to prepare hydrophobicity gradients for two-dimensional thin layer chromatography [15] but again such a fabrication technique would be impractical for larger diameter columns packed with small particle size media due to the opacity of suitable column hardware. Svec et al. also investigated the feasibility of producing longitudinal porosity gradients by varying the duration of Friedel-Crafts cross-linking reactions over the length of the column [16]. However, this method is only suitable for variation in column porosity and not suitable for introducing variable ion exchange capacity.

Fekete et al. published a theoretical study of the impact of a particle size gradient on chromatographic efficiency [17]. They concluded that under isocratic conditions a particle size gradient would provide no

performance benefits. They also predicted that a 15–20% gain in efficiency could be expected under gradient conditions.

Collinson et al. have studied columns with longitudinal selectivity and capacity gradients, including a paper where they destructively removed the stationary phase to a varying extent along the length of the column [18]. Although they observed differences in selectivity when they reversed the flow direction of the column, chromatographic performance was relatively poor. They also investigated the surface of a silica monolith in such a way as to produce a longitudinal amine capacity gradient with the goal of producing selectivity not achievable with a uniformly modified amine surface [19]. Again, they noticed small differences in selectivity based on the flow direction with relatively poor chromatographic performance. While these papers all indicate the potential of longitudinal gradients, they highlight the need to construct a gradient in such a way as to provide good chromatographic performance throughout the column bed.

In contrast to previous work, preparation of columns with longitudinal gradients using electrostatic attachment of latex is straightforward to execute without any compromises in chromatographic performance. The most common method for applying electrostatically attached latex stationary phases is to pack a column with substrate particles and then use an ion chromatography pump to drive the latex slurry through the column. While particulate is commonly problematic with pumps using mechanical check valves, latex particles are typically too small to affect the performance of check valves making ion chromatography pumps an ideal platform for application of such stationary phases. Furthermore, if two batches of latex particles, each with distinctly different properties, are delivered to the packed column in a suitable fashion, it should be possible to create a stationary phase gradient during the application process by using a standard gradient program commonly employed in gradient chromatography methods. A variety of different gradients are conceivable using latex-based stationary phases including capacity gradients, selectivity gradients, hydrophobicity gradients and charge density gradients. Capacity gradients can be accomplished using multiple techniques including a latex particle size gradient (capacity is directly proportional to latex diameter), a latex cross-link gradient (capacity is directly proportional to cross-link) and with a charge density gradient where the concentration of ion exchange sites in the latex particle is manipulated to adjust the capacity of batch of latex particles without altering the size of the particle.

Investigation of capacity gradients is of potential interest because an increasing capacity gradient should result in peak focusing. As with the particle size gradient study of Fekete et al. [17], capacity gradients are not expected to provide any efficiency benefit under isocratic conditions. Under such conditions the peak focusing effect of an increasing capacity gradient is offset by the relatively slow release of the band due to the high capacity at the outlet of the column. In contrast, when operating a column with a decreasing capacity gradient under isocratic conditions the band will be defocused as it passes over the column but the band will exit such a column relatively rapidly due to the low capacity at the outlet. Due to the symmetry of the two situations, the chromatographic performance is not expected to be different, in terms of efficiency, for a gradient in either direction under isocratic conditions.

However, there are several potential benefits beyond isocratic efficiency properties for a column with a capacity gradient. For example, loading capacity should be improved if the capacity is highest at the column inlet where sample zones are most concentrated. In addition, it would seem likely that a concentration gradient superimposed upon a capacity gradient would overcome the slow elution from a band eluting from the high capacity end of the column. If so, under gradient elution conditions it would seem likely that higher efficiency would be observed was a capacity gradient column. One might also expect that the van Deemter profile would be altered with the capacity gradient column, especially at the lowest flow rates with increasing capacity gradient focusing effects partially mitigating the consequences of diffusion in the mobile phase.

2. Materials and methods

2.1. Equipment

All chromatography was performed on a Thermo Fisher Scientific (Waltham, MA, USA) Dionex ICS-5000 + ion chromatography system comprised of a DP pump module, an EG eluent generator module, a DC chromatography module and an AS autosampler. The system used Chromeleon 7.2 software for data analysis. The EG eluent generator module contained a KOH EGC500 eluent generator cartridge, a CR-ATC eluent purifier cartridge, and a degasser cartridge. The DC chromatography module column compartment was operated at 30 °C, the conductivity cell operated at 35 °C, and a 4 mm AERS suppressor was used for all the chromatographic separations while in a 15 °C temperature control zone. Potassium hydroxide eluent produced by the eluent generator was used for all separations. The eluent flow rate in all cases was 1.0 mL/min unless otherwise noted. A Dionex GP-50 pump was used to deliver latex mixtures to the column. Latex particle size was measured on a Cilas Nano DS dynamic light scattering instrument.

2.2. Reagents

The vinylbenzylchloride (VBC) monomer (nominal isomer ratio, 60% meta and 40% para) was purchased from Dow. The Triton X-405, potassium persulfate, sodium metabisulfite, potassium bromide, divinylbenzene (DVB) (55%), N-methyldiethanolamine (MDEOHA) and N,N-Dimethylglycine ethyl ester (DMGEE) were all purchased from Sigma-Aldrich. HPLC grade acetonitrile was obtained from Fisher Scientific. The Dowex 50WX8 50–100 μm particle size hydrogen form resin was obtained from Bio-Rad.

2.3. Column packing

All columns were packed in 4 × 250 mm PEEK column hardware. Columns were packed with 6.55 μm particle size resin composed of surface sulfonated 55% divinylbenzene-45% ethylvinylbenzene copolymer beads with a 60% pore volume and a surface area of 20.8 m^2/g prior to the latex attachment step.

2.4. Latex synthesis

For the high charge density latex synthesis (Latex 1), aqueous solution was prepared in a 32 oz. narrow neck bottle with 800.04 g of water, 10.13 g of KBr, 51.01 g of a Triton X-405 surfactant, 2.72 g of potassium persulfate. Then 40.39 g of VBC and 1.83 g of 55% DVB was added to the aqueous solution followed by 2.51 g of sodium metabisulfite after which the mixture was stirred while purging with nitrogen for four minutes. The bottle was capped and tumbled in a water bath at 6.2 revolutions per minute and held at 32 °C for 11.5 h to complete the polymerization and form the latex particle suspension. The particle size of the resulting latex suspension was 37.5 nm. Once the polymerization was complete, 90.05 g of MDEOHA and 4.503 g of DMGEE were mixed with 180.56 g of water. The amine solution was poured slowly into 450.3 g of Latex 1 while stirring. The amine solution-latex particle suspension mixture was incubated in an oven at 65 °C for 4.5 days while stirring to form the anion-exchange particle suspension. At the end of the reaction the particle size of the anion-exchange latex suspension was 55.6 nm.

For the low charge density latex synthesis (Latex 2), an aqueous solution was prepared in a 16 oz. narrow neck bottle with 400.06 g of water, 5.16 g of KBr, 25.01 g of a Triton X-405 surfactant, and 1.36 g of potassium persulfate, 20.02 g of VBC, 0.92 g of 55% DVB, and 1.26 g of metabisulfite after which it was stirred and purged with nitrogen for 3 min. The bottle was capped and tumbled in a water bath at 6.2 revolutions per minute and held at 32 °C for 7 h to complete the polymerization and form the particle suspension. The particle size of the resulting latex suspension was 32.2 nm. Once the polymerization was complete, 90.04 g of MDEOHA and 26.965 g of DMGEE were mixed with 179.76 g of water. The mixture was poured slowly into 450.3 g of Latex 2 while stirring. The amine solution-latex particle suspension mixture was incubated in an oven at 65 °C for 21 h while stirring to form the anion-exchange particle suspension. The particle size of the resulting anion-exchange latex suspension was 59.2 nm.

2.5. Latex coating of columns

Latex agglomeration was performed at ambient temperature while the column was held in a vertical orientation at a 0.25 mL/min flow rate. Each latex was diluted 10:1 in deionized water. The latex was then passed through a column packed with 9 mL of Dowex 50W X8 resin to remove unreacted amine from 15-20 mL of latex suspension. Each latex was loaded in one of the eluent bottles of the GP-50 pump. The breakthrough volume for the 10:1 dilution of latex was found to be approximately 18.5 mL based on direct inspection of the column effluent for the high charge density latex and the low charge density latex which were approximately the same. Isocratic latex coatings were applied either individually or as a 50:50 blend using the proportioning valve and a static mixer to provide a homogeneous blend of both latex suspensions. For charge density gradient columns, the percentage of the low charge density latex was programmed to decrease from 100% to 0% in 75 min while the high charge density latex was programmed to increase from 0% to 100% at the same time. For the column prepared with five successive gradients, the composition of the two latex suspensions was programmed in a similar manner except that the duration of each gradient was reduced to 15 min and the gradient program was repeated five times for total duration of 75 min. In each case, after the latex deposition process was complete the columns were rinsed with deionized water for 10 min followed by a rinse of the column with 20% acetonitrile for an hour.

3. Results and discussion

### 3.1. Evaluation of columns prepared from each latex batch

Two separate columns were prepared. In one case, the column was prepared with 100% latex batch 1 (column 1) while in the other case the column was prepared with 100% latex batch 2 (column 2). Figure 1 shows the chromatographic performance and selectivity of column 1 prepared using the high charge density latex. Note that sulfate elutes after nitrate while carbonate elutes before bromide on this column. Column 2, prepared using the low charge density latex, is illustrated in Figure 2. There are two major differences between these two columns. If one compares the void volume adjusted retention times between the monovalent anions in Figure 1 with the void volume adjusted retention times of the monovalent ions in Figure 2, the low charge density latex substantially reduced the overall retention of monovalent species. For all monovalent species, retention times are roughly half that of the high charge density latex. However, in the case of divalent species, the retention time is reduced by an even greater factor. For column 2 in Figure 2, with the same eluent used in Figure 1 both carbonate and sulfate elute in between nitrite and bromide. This is clear evidence that latex batch 2 produced a latex with substantially lower charge density. The retention time of divalent species is substantially more sensitive to charge density than the retention time of monovalent species. This is due to the fact that a divalent ion interacts with two ionic sites simultaneously and is thus are more sensitive to the average distances between ion exchange sites. Table 1 lists efficiency information for both column 1 and column 2.

3.2. Evaluation of columns prepared with charge density gradients

Three different columns were prepared using a 50:50 ratio of two latex batches. The data for all three columns is shown in Table 1. One column (column 3), which serves as a control for comparison to columns
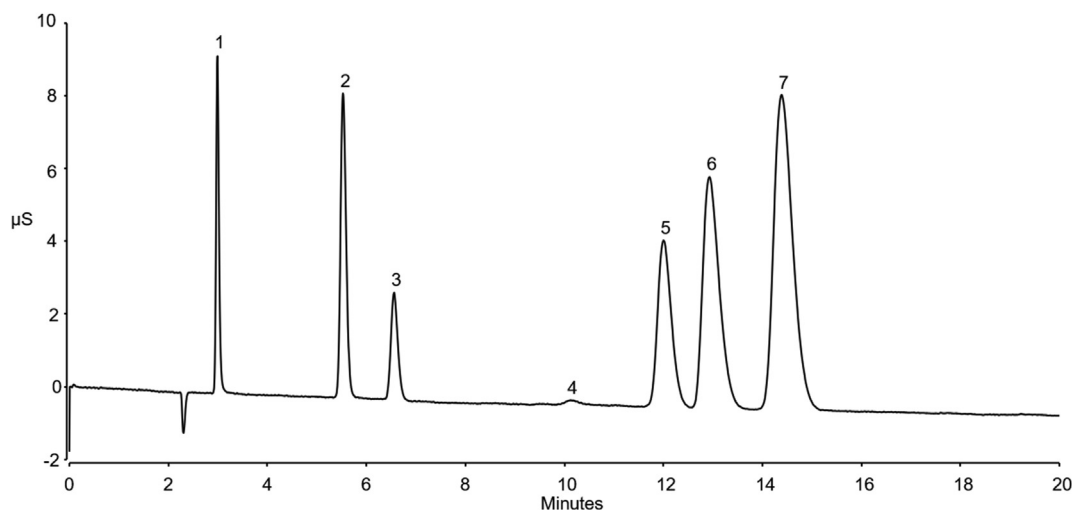
Figure 1. Isocratic separation of the common anions on a column prepared with the high charge density latex (latex batch 1). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, 30 mM KOH, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate.
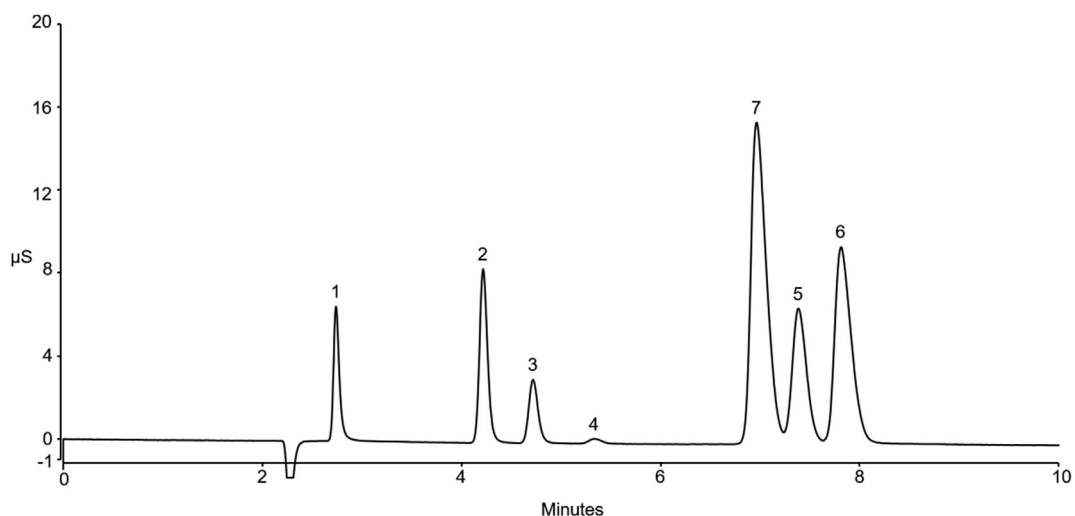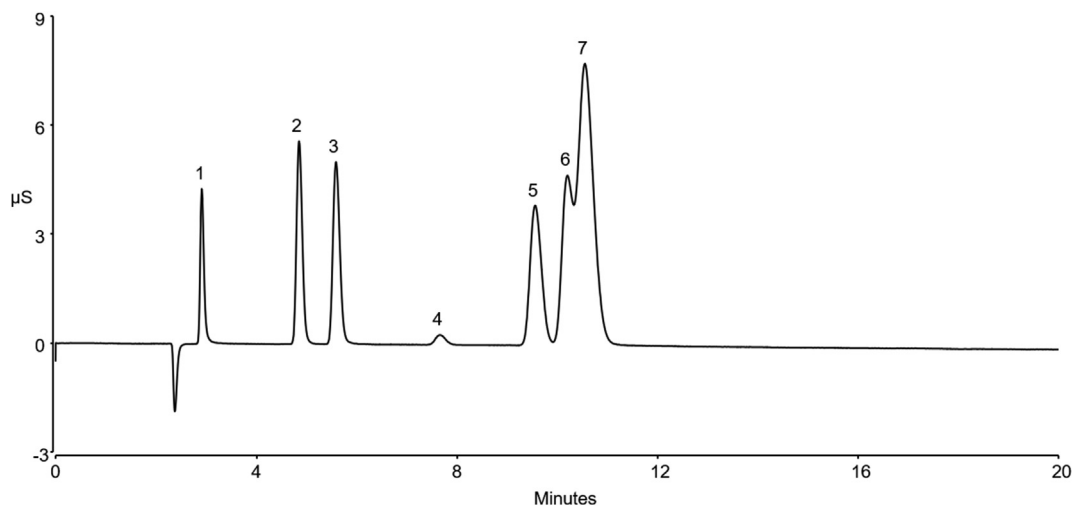

Figure 2. Isocratic separation of the common anions on a column prepared with the low charge density latex (latex batch 2). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, 30 mM KOH, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate.

Table 1. Characteristics of columns evaluated. Efficiency (plates per column). Eluent: 30 mM KOH, Temperature: 30 °C, Injection volume: 25 μL, Flow rate: 1 mL/min, Analytes: Fluoride (1 ppm) Chloride (3 ppm), Nitrite (5 ppm) and Bromide (10 ppm).

	Column 1	Column 2	Column 3	Column 4	Column 5
Latex	Batch 1	Batch 2	Batch 1&2	Batch 1&2	Batch 1&2
Deposition	Uniform	Uniform	Uniform	Single gradient	5 Gradients
Fluoride	12600	11500	7200	10000	10100
Chloride	11400	13400	8400	10100	11500
Nitrite	10500	12500	7900	8900	10500
Bromide	8700	12200	8100	7800	10400

with charge density gradients was prepared by delivering a 50:50 ratio of the low charge density latex and the high charge density latex using a 50% composition of each latex from the gradient pump during the latex coating process. The latex was applied with a continuous 50:50 ratio from the beginning to the end of the latex deposition process. Figure 3 shows a chromatogram from column 3. As can be seen from the chromatogram, the selectivity of column 3 is intermediate between that of column 1 and column 2. At the same time, it is evident that the selectivity

of the 50:50 latex blend is not precisely halfway between the selectivity of each individual latex. The selectivity is more similar to that of column 1, with sulfate eluting slightly after nitrate, only partially resolved from nitrate. If the selectivity had been exactly halfway between the two individual latex batches, sulfate would have eluted between bromide and nitrate. This is likely due to the higher swelling factor for the low charge density latex (84%) compared to the high charge density latex (48%). Particles that swell more upon functionalization cover a greater fraction

Figure 3. Isocratic separation of the common anions on a column prepared with a 50:50 blend of high charge density latex (latex batch 1) and low charge density latex (latex batch 2). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, 30 mM KOH, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate.
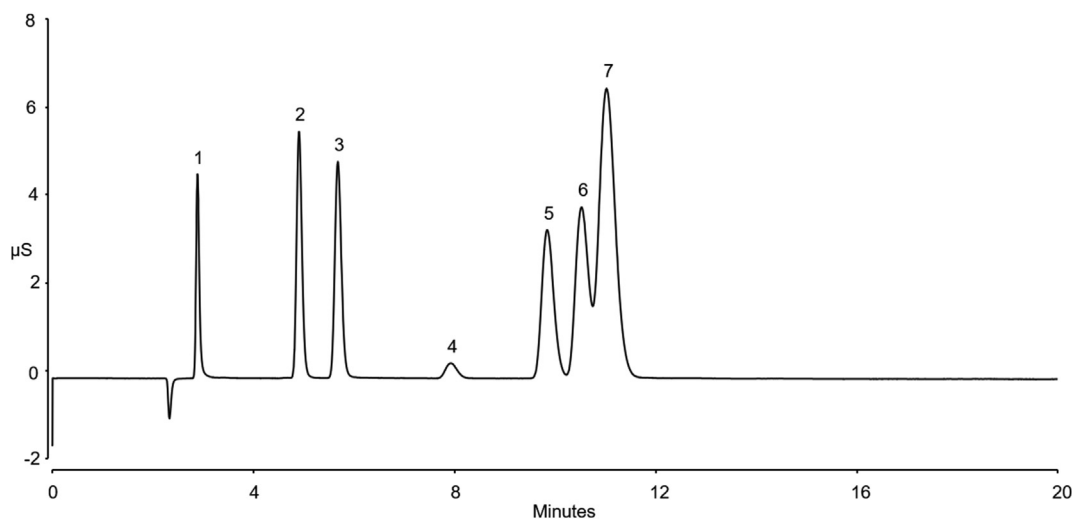
Figure 4. Isocratic separation of the common anions on a column prepared with a linear gradient from 100% high charge density latex (latex batch 1) to 100% low charge density latex (latex batch 2). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, 30 mM KOH, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate.
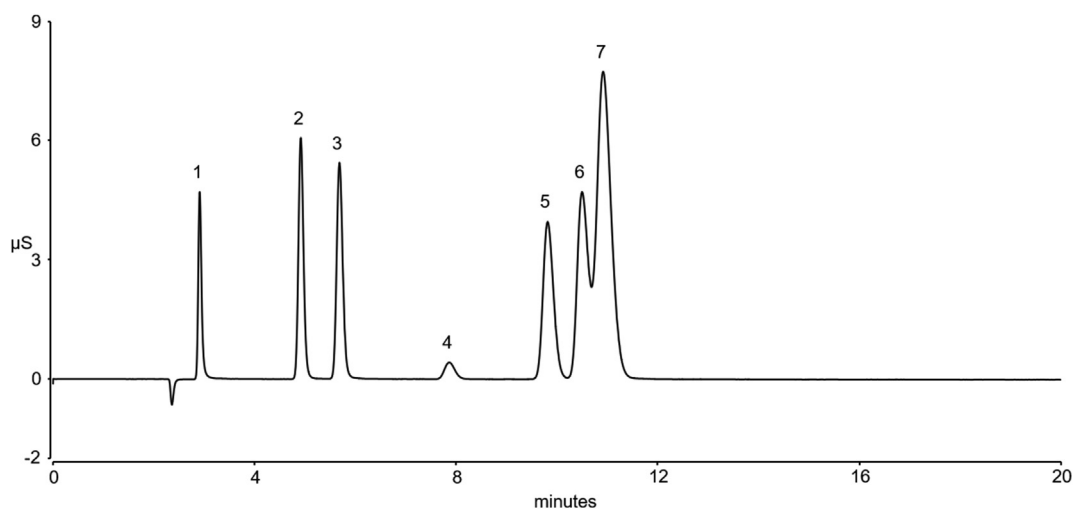
Figure 5. Isocratic separation of the common anions on a column prepared with 5 successive gradients from 100% high charge density latex (latex batch 1) to 100% low charge density latex (latex batch 2). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, 30 mM KOH, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate.

Table 2. Effect of charge density gradient on dynamic loading capacity.

Sulfate Conc.	50:50 latex blend		Single gradient		Five successive gradients	
	plates	% decrease	plates	% decrease	plates	% decrease
5 ppm	7200	0	5700	0	7800	0
7.5 ppm	7200	0	5600	2	7700	1
10 ppm	7100	1	5500	4	7600	3
20 ppm	6500	10	5100	11	6900	12
40 ppm	5600	22	4400	23	5900	24
60 ppm	4800	33	3700	35	4800	38
80 ppm	3900	46	3200	44	4000	49
100 ppm	3400	53	2800	51	3400	56

Table 3. Effect of linear velocity on chromatographic performance. Efficiency (plates per column) associated with column 5. Eluent: 30 mM KOH, Temperature: 30 °C, Injection volume: 25 μL, Analytes: Fluoride (1 ppm) Chloride (3 ppm), Nitrite (5 ppm) and Bromide (10 ppm). The flow rate was as specified below.

Analyte	High charge density at inlet			Low charge density at inlet		
	1 mL/min	0.5 mL/min	0.25 mL/min	1 mL/min	0.5 mL/min	0.25 mL/min
Fluoride	10100	11000	11500	9300	10900	11700
Chloride	11500	12300	12100	10800	12300	12400
Nitrite	10500	11300	11300	10000	11400	11500
Bromide	10400	11500	11700	10000	11500	11800

of surface area per particle. This reduces the overall contribution to capacity of highly swollen particles.

Another column (column 4) was prepared using the same two latex batches. In this case, the column was produced using a pump gradient program to delivering each latex batch to the column. A program was created that started with 100% of latex batch 2 and 0% of latex batch 1 with a 75-minute linear gradient of composition delivered to the column. At the end of the 75-minute gradient, the composition was 0% latex batch 2 and 100% latex batch 1. Afterward, column 4 was rinsed and cleaned as noted in section 2.5. The column resulting from this latex charge density gradient is shown in Figure 4. The average composition of the two latex batches is 50:50 since the gradient program was adjusted to match the volume required to fully coat the entire column. Not surprisingly, the retention time and selectivity of this column is quite similar to that of the column 3, produced using a 50:50 isocratic delivery process.

A third column (column 5) was prepared using the same set up described above but, in this case, the gradient program consisted of five consecutive gradients, each one 15 min in duration. The program was designed so that the composition reverted to initial conditions abruptly at the end of each gradient segment so that a sawtooth gradient was created with five consecutive shorter gradients instead of one long continuous gradient. Afterward, column 5 was rinsed and cleaned as noted in section 2.5. The column resulting from this sawtooth charge density gradient is shown in Figure 5. The selectivity of this column closely matches the selectivity of column 4, prepared with a single linear gradient shown in Figure 4.

3.3. Evaluation of the effect of charge density gradients on loading capacity

Columns 3, 4 and 5 were each tested under isocratic conditions. For this evaluation, sulfate was chosen as the test probe since divalent anions are more prone to overload when using hydroxide eluent. For each column, a range of concentrations from 5 to 100 ppm sulfate was injected using a 25 μL sample loop. In the case of gradient columns, the columns were oriented so that the inlet end of the column had the highest charge density. The results of this study are shown in Table 2. A small improvement is observed for column 4, composed of a single linear charge density gradient. But the effect is small and likely within the bounds of experimental error. A similar experiment was performed with

the flow direction reversed for all three columns (data not shown) but the results were similarly unimpressive.

3.4. Evaluation of the effect of flow rate on chromatographic performance

The column prepared with five successive gradients (column 5) was evaluated for the effect of flow rate on efficiency. The column was tested at three flow rates in both flow directions. The results are shown in Table 3. There are only rather modest differences in efficiency in either the decreasing charge density or increasing charge density flow directions. However, it is notable that higher efficiencies are observed at 1 mL per minute when the high charge density is at the inlet, while at the lowest flow rate the highest efficiency is observed for all analytes when the highest charge density is at the outlet. Presumably, at higher flow rates the highest loading capacity associated with the high charge density inlet provides the best efficiency. At lower flow rates, the focusing effects of the increasing charge density gradient tends to compensate for diffusion related band broadening, offsetting the disadvantage of low capacity at the column inlet. In fact, the efficiency for all four analytes is higher at the lowest flow rate suggesting that the van Deemter minimum is below 0.25 mL per minute when the column is in this orientation. Changes in efficiency versus flow rate when the flow direction was reversed were much more modest.

3.5. Evaluation of the effect of operation under gradient conditions on chromatographic performance

Columns 3, 4, and 5 were evaluated under gradient elution conditions. Given the fact that concentration gradients tend to focus analyte bands it is expected that the combination of an increasing concentration of eluent with a progressive decrease in charge density as the band travels toward the outlet will correct for the defocusing mechanism associated with the decreasing charge density as the band passes through the column and reduce bandwidth compared to a column with a homogeneous longitudinal charge density. For this purpose, a gradient was chosen that optimized the separation of anions under gradient conditions. For the gradient columns, the gradient columns were operated with the highest charge density at the inlet to the column. A representative chromatogram from column 5 is shown in Figure 6 All three columns were operated under identical gradient
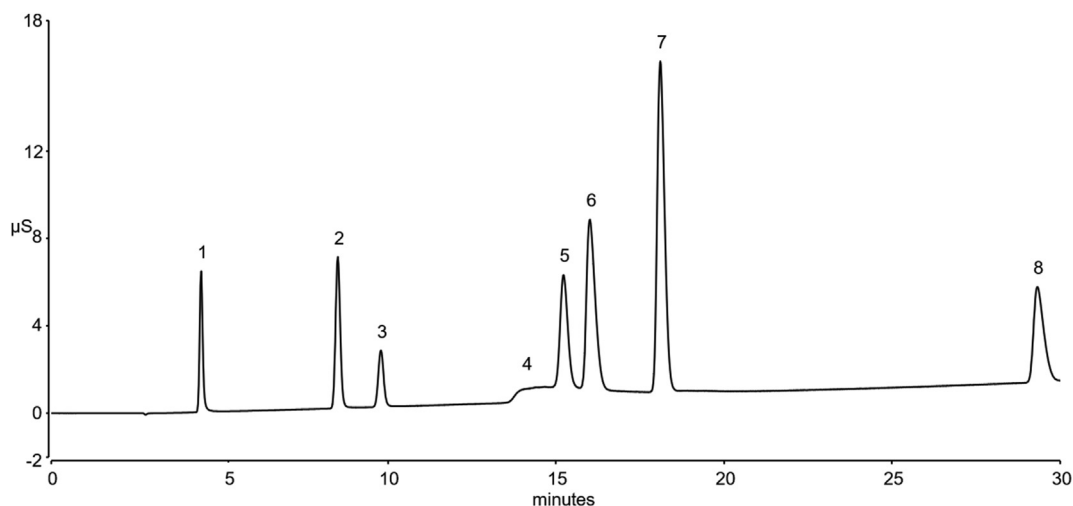
Figure 6. Gradient separation of the common anions on a column prepared with 5 successive gradients from 100% high charge density latex (latex batch 1) to 100% low charge density latex (latex batch 2). Column dimensions: 4 mm ID x 250 mm. Resin particle size: 6.55 μm. Flow rate: 1 mL/min, gradient from 10 mM KOH at time 0–50 mM KOH at 30 min, temperature: 30 °C, suppressed conductivity detection, 25 μL injection volume. Peaks: 1. 1 ppm fluoride; 2. 3 ppm chloride; 3. 5 ppm nitrite; 4. carbonate; 5. 10 ppm bromide; 6. 10 ppm nitrate; 7. 15 ppm sulfate; 8. 15 ppm phosphate.

Table 4. Effect of gradient elution on peak width. Peak width at half height (pw@hh) and efficiency (N, plates per column). Eluent: 10–50 mM KOH in 30 min, Temperature: 30 °C, Injection volume: 25 μL, Flow rate: 1 mL/min, Analytes: Fluoride (1 ppm) Chloride (3 ppm), Nitrite (5 ppm) and Bromide (10 ppm).

	Column 3		Column 4		Column 5	
	pw@hh	N	pw@hh	N	pw@hh	N
Fluoride	0.112	9000	0.127	10800	0.097	11800
Chloride	0.170	13900	0.172	14000	0.152	17300
Nitrite	0.190	14600	0.197	14100	0.173	17800
Bromide	0.270	17400	0.296	15100	0.246	21200
Nitrate	0.298	15700	0.337	12900	0.282	17900
Sulfate	0.246	28700	0.283	22300	0.241	31300
Phosphate	0.323	42700	0.345	29800	0.329	44000

elution conditions. The results of these chromatographic tests are included in Table 4. Chromatographic performance for each analyte is listed both as peak width at half height and in terms of plates per column. While the plates per column parameter isn't, strictly speaking, valid for gradient elution conditions, it provides a useful basis for comparison between the three columns since the gradient elution conditions were identical for all three columns. The plates per column measure is useful in order to compare different columns with slightly different analyte retention time which might otherwise not be apparent from the peak width at half height.

Reviewing the results shown in Table 4, there appears to be relatively little advantage to a single longitudinal charge density gradient associated with column 4 when compared to a column prepared with both latex batches homogeneously distributed across the length of the column in the case of column 3. In no case was the peak width at half height of any analyte associated with column 4 less than that of the corresponding data for column 3. Only in the case of fluoride and chloride efficiency was there even a small benefit associated with the linear gradient compared with the uniformly deposited latex coating of column 3.

In contrast, the performance of column 5 is substantially better than that of column 3 or column 4 both in terms of peak width at half height and chromatographic efficiency. It's not immediately clear why the column with five successive gradients perform so much better than the column with a single linear gradient. One possibility is that the concentration gradient refocusing effects are not well matched to the steepness of the charge density gradient of the column in the case of the linear charge density gradient column. In the case of the column with multiple successive gradients, the steepness of the concentration gradient and the

charge density gradient may be better matched. If so, an increaase in the steepness of the concentration gradient might provide performance advantages for the linear gradient although, if so, this would come at the expense of increased analysis time.

4. Conclusions

Electrostatically attached latex-based stationary phases represent an extremely flexible platform for the preparation of ion exchange stationary phases. The nature of the platform makes it ideally suited to the preparation of stationary phases with longitudinal gradients of chromatographic parameters such as capacity, charge density, and selectivity. Columns with longitudinal gradients in retention parameters can be easily fabricated using electrostatic attachment of latex particles. The effect of such gradients on loading capacity, efficiency at low flow rates and efficiency under gradient elution conditions suggest modest benefits are possible using this method preparing such phases. Multiple successive gradients appears to provide superior performance compared to columns with a single linear gradient. A potential disadvantage of capacity or charge density gradients is the low loading capacity associated with such columns when the low capacity or low charge density is positioned at the inlet of the column. A potential solution to this problem would be the creation of a column with a zone of high capacity or charge density at the inlet followed by an abrupt drop to a lower capacity or charge density which slowly increases along the length of the column. This would provide a benefit of higher loading capacity at the inlet with the focusing effect of a capacity or charge density gradient as the band travels toward the column outlet.

The stationary phase fabrication technique described in this work is covered in a pending patent application.

Declarations

Author contribution statement

Christopher A. Pohl: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] H. Small, T.S. Stevens, W.C. Bauman, Novel ion exchange chromatographic method using conductimetric detection, Anal. Chem. 47 (1975) 1801–1809.

[2] H. Small, Hydrodynamic chromatography. A technique for size analysis of colloidal particles, J. Colloid Interface Sci. 48 (1974) 147.

[3] T.S. Stevens, J.S. Davies, H. Small, Hollow fiber ion exchange suppressor for ion chromatography, Anal. Chem. 53 (1981) 1488.

[4] T.E. Miller, H. Small, Thermal pulse time-of-flight liquid flow meter, Anal. Chem. 54 (1982) 907.

[5] H. Small, T.E. Miller, Indirect photometric chromatography, Anal. Chem. 54 (1982) 462.

[6] H. Small, J. Riviello, Electrically polarized ion-exchange beds in ion chromatography: ion reflux, Anal. Chem. 70 (1998) 2205–2212.

[7] H. Small, Y. Liu, N. Avdalovic, Electrically polarized ion exchange beds in ion chromatography: eluent generation and recycling, Anal. Chem. 70 (1998) 3629–3635.

[8] Small H., Stevens T. S., High Performance Ion Exchange Composition, U. S. Patent 4,101,460, July 18, 1978.

[9] Small H., Method of Manufacturing Non-agglomerating Mixed Bed Ion Exchange Resins and Products Thereof, U. S. Patent 2,961,417, November 22, 1960.

[10] Stevens T. S., Langhorst M. A., High-Performance Analytical Column for Anion Determination, U. S. Patent 4,519,905, May 28, 1985.

[11] Pohl C. A., Coated Resin Ion Exchange Composition. U. S. Patent 4,376,047, March 8, 1983.

[12] Stevens T. S., High-Performance Ion-Exchange Composition, U. S. Patent 4,351,909, September 28, 1982.

[13] J.R. Stillian, C.A. Pohl, New latex-bonded pellicular anion exchangers with multi-phase selectivity for high-performance chromatographic separations, J. Chromatogr. 499 (1990) 249–266.

[14] V. Pucci, M.A. Raggi, F. Svec, J.M.J. Fréchet, Monolithic columns with a gradient of functionalities prepared via photoinitiated grafting for separations using capillary electrochromatography, J. Separ. Sci. 27 (2004) 37–44.

[15] I. Urbanova, F. Svec, Monolithic polymer layer with gradient of hydrophobicity for separation of peptides using two-dimensional thin layer chromatography and MALDI-TOF-MS detection, J. Separ. Sci. 34 (2011) 2345–2351.

[16] J. Urban, T. Hájek, F. Svec, Monolithic stationary phases with a longitudinal gradient of porosity, J. Separ. Sci. 40 (2017) 1703–1709.

[17] S. Codesido, S. Rudaz, J.L. Veuthey, D. Guillarme, G. Desmet, S. Fekete, Impact of particle size gradients on the apparent efficiency of chromatographic columns, J. Chromatogr. A 1603 (2019) 208–215.

[18] C.N. Cain, A.V. Forzano, S.C. Rutan, M.M. Collinson, Destructive stationary phase gradients for reversed-phase/hydrophilic interaction liquid chromatography, J. Chromatogr. A 1570 (2018) 82–90.

[19] V.C. Dewoolkar, L.N. Jeong, D.W. Cook, K.M. Ashraf, S.C. Rutan, M.M. Collinson, Amine gradient stationary phases on in-house built monolithic columns for liquid chromatography, Anal. Chem. 88 (2016) 5941–5949.