Anal. Chem. **2005,** *77,* 417−423

Latex-Coated Polymeric Monolithic Ion-Exchange Stationary Phases. 2. Micro-Ion Chromatography

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Latex-coated monolithic polymeric stationary phases are used for micro-ion chromatography (μ-IC) of inorganic anions. Monolithic columns were prepared by the in situ polymerization of butyl methacrylate, ethylene dimethacrylate, and 2-acrylamido-2-methyl-1-propanesulfonic acid within fused-silica capillaries of varying internal diameters. Introduction of ion-exchange sites was achieved by coating the anionic polymeric monolith with either Dionex AS10 or Dionex AS18 quaternary ammonium functionalized latex particles to give total ion-exchange capacities in the range 9−24 nequiv for a 30-cm column. The resultant μ-IC columns were used for the separation of anionic analytes using chloride or acetate as the eluent-competing ion and direct UV spectrophotometric detection at 195 nm or using hydroxide as the eluent-competing ion and suppressed or nonsuppressed contactless conductivity detection. Separation efficiencies of 13 000 plates/m were observed (for iodate), and separation efficiency was maintained for large increases in flow rate (up to 42 μL/min, corresponding to a linear flow velocity of 18.5 mm/s), enabling highly reproducible, rapid separations to be achieved (seven analyte anions in less than 2 min). Use of a hollow fiber micromembrane suppressor enabled effective suppression of hydroxide eluents over the range 0.5−5.0 mM, thereby permitting suppressed conductivity detection to be performed. However, the relatively large size of the suppressor resulted in reduced separation efficiencies (e.g., 5400 plates/m for iodate). Detection limits obtained with suppressed conductivity detection were in the range 0.4−1.2 μM.

Ion chromatography (IC) has developed into a mature and widely used technique for the separation and analysis of a considerable variety of analytes.[1] Traditionally, IC columns have been prepared using spherical ion-exchange (IE) particles, which are packed into columns of varying dimensions and held in place with the use of frits. The IE functional groups on these particles are either bound covalently to the surface of the particle or are affixed electrostatically in the form of nanometer-sized functionalized latex particles, which are bound to the surface of the particle. The latter approach yields the highest chromatographic separation efficiency because of the favorable mass-transfer characteristics of the latex agglomerate stationary phases. Nevertheless, there is still a trend to further improve efficiency by reducing the diameter of the stationary-phase particles. High-speed separations are also a major goal in modern IC, but current stationary phases have limited applicability for this purpose, especially when high eluent flow rates are used. High flow rates lead to loss of separation efficiency due to deteriorating mass transfer and also back-pressure limitations.

The recent introduction of monolithic stationary phases has, to some extent, overcome the flow limitations of using small-diameter packing materials.[2,3] Monolithic stationary phases consist of a single piece of stationary-phase material (generally silica or organic polymers) having interconnecting flow-through pores. Higher flow rates are achievable with these columns since their porosities are ∼15% greater than particle-packed columns.[4] The use of monolithic columns for liquid chromatographic separations has increased dramatically in the past few years, though almost all of this work has focused on high-performance liquid chromatography separations.[2,3,5−11] Monolithic columns are now available commercially and have been used extensively for reversed-phase separations.[2] IE separations have also been reported for proteins and oligonucleotides[12−15] and small inorganic anions and cations.[4,16] In the case of inorganic cations (alkaline earth cations), separations were facilitated by derivatizing a commercial silica monolith

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10.1021/ac048747l CCC: $30.25
Published on Web 12/07/2004

with iminodiacetic acid to produce a chelating IE column.[16] For the separation of inorganic anions, a C18 silica monolith was coated permanently with the double-chained surfactant didodecyldimethylammonium bromide[4] to introduce IE sites and to enable the rapid separation (under 30 s) of a series of inorganic anions using high eluent flow rates (up to 10 mL/min).

A major advantage of monolithic columns is their relatively straightforward preparation within a variety of housings, including capillaries and microchips. They are therefore well-suited for use in miniaturized separation systems such as capillary electrochromatography and liquid microchromatography (μ-LC). In a manner similar to the larger scale monoliths, the majority of the work using monolithic microcapillary systems has been directed toward reversed-phase separations in both capillary electrochromatography (CEC)[17−20] and μ-LC.[21−24] However, IE-based CEC separations have also been performed[25−27] in which the IE sites were introduced onto the monolith by including a charged monomer in the polymerization mixture,[25,26] coating an ionic polymer onto the monolithic surface,[27] or using an adsorbed layer of functionalized latex particles.[28]

In this work. we have investigated the production of μ-IC columns by using a charged (in this case, anionic) monolithic polymeric network as a substrate for adsorption of quaternary ammonium functionalized latex particles to produce an anion-exchange monolithic stationary phase. Similar stationary phases were recently used for ion-exchange CEC separations and in-line preconcentration for capillary electrophoresis.[28] The major justification for developing latex-coated monolithic stationary phases for μ-IC is that stationary-phase synthesis is simplified since the same monolithic template can be used to support any desired latex of appropriate charge. Moreover, monolithic μ-IC media should be suitable for high-speed separations without the need for unduly high eluent flow rates and can be directly interfaced with mass spectrometric detection.

EXPERIMENTAL SECTION

Materials, Butyl methacrylate (BMA, 99%), ethylene dimethacrylate (EDMA, 98%), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS, 99%), 1-propanol (99.5+%), and 1,4-butanediol (99%) were obtained from Aldrich (Milwaukee, WI). (γ-Methacryloxypropyl)-trimethoxysilane (γ-MAPS, 98%) and basic alumina (activity grade I) were obtained from Sigma (St. Louis, MO). Azodiisobutyronitrile (AIBN, 99%) was obtained from Du Pont (Bayswater, Victoria, Australia). BMA and EDMA were filtered through basic alumina (activity grade I) to remove any inhibitors and were stored in the freezer until required. All other chemicals were used as supplied. Eluents and standard solutions were prepared in MilliQ water (Millipore, Bedford, MA) from the appropriate sodium or potassium salt. Eluents were further filtered through 0.45-μm Nylon filters (Millipore) prior to use.

Preparation of Polymer Monoliths. To stabilize the monolithic bed under applied pressure, the fused-silica capillaries used to house the monoliths were derivatized with γ-MAPS to improve attachment of the monolith to the capillary wall.[29] In summary, this involved rinsing the capillary consecutively with acetone, water, 1.0 M sodium hydroxide, 1.0 M hydrochloric acid, and ethanol. γ-MAPS (20% w/w) in ethanol (adjusted to pH 5 with acetic acid) was then flushed through the capillary and left overnight. Finally, the γ-MAPS solution was rinsed out of the capillary with ethanol, and the capillary was dried with nitrogen.

Polymer monoliths were prepared using the method introduced by Svec and co-workers.[30] The polymerization mixture consisted of 40% monomers (40:60, EDMA/BMA with 1.5% AMPS) dissolved in 60% porogenic solvent. The porogenic solvent consisted of 55% propanol, 35% butanediol, and 10% water. a 1% solution (with respect to the monomers) of AIBN was added as a free-radical initiator. The polymerization mixture was then placed in an ultrasonic bath and purged with nitrogen prior to introduction into a derivatized capillary (~1 m in length) by syringe. Both ends of the capillary were then sealed with rubber septa, and the capillary was submerged in a water bath at 60 °C for 24 h. The resultant monolith was flushed for 2 h, first with methanol and then water at a pressure of ~13 MPa.

Latex particles (65-nm diameter) with quaternary ammonium functionalities were obtained from Dionex (Sunnyvale, CA) as an 11% (w/v) suspension. The latex was dialyzed through a cellulose membrane for 48 h with water changes every 4−6 h and then filtered through a 0.45-μm nylon syringe filter (Activon, Thornleigh, Australia). The latex suspension was further diluted by a factor of 10 with water and flushed through the monolithic columns at a pressure of ~6.9 MPa for at least 2 h in order to coat the surface of the monolith.

The polymerization mixture remaining after the capillary was filled was sealed in a screw top sample vial and placed in the water bath along with the capillary. Following polymerization, the glass vial was carefully broken to retrieve the bulk monolith which was then Soxhlet extracted for ~8 h and dried under vacuum at 60 °C. This monolith was used for pore volume and size distribution measurements using a Poresizer 9310 mercury intrusion porosimeter (Micromeritics, Norcross, GA).

Chromatography. A Dionex IP25 isocratic pump was connected to an M-472 microsplitter valve (Upchurch Scientific, Oak Harbor, WA) to convert milliliter per minute flow rates to microliter per minute, with the majority of the flow being recycled back to the eluent reservoir. Injection was performed with an

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M-435 microinjection valve (Upchurch Scientific). UV detection was accomplished by connecting a 75 μm × 13 cm open-tubular section of capillary to the end of the monolithic column and feeding this into an Agilent ^{3D}CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. All separations were performed at room temperature.

For both nonsuppressed and suppressed conductivity detection an in-house-prepared contactless conductivity detector was used.[31] This was positioned on an open-tubular section of fused-silica capillary connected either directly to the outlet of the column (nonsuppressed detection) or to the outlet of the microsuppressor (suppressed detection). The microsuppressor consisted of a ~20 cm length of 100-μm-i.d. cation-exchange (Nafion) capillary tubing. This tubing was passed through the middle of a standard 25-cm PEEK column through which regenerant (5 mM sulfuric acid) was passed in a countercurrent direction. Each end of the ion-exchange tubing was connected to 365-μm-o.d. PEEK capillary, which could be directly connected to the separation column and the detection capillary using zero dead volume connectors (Upchurch Scientific).

RESULTS AND DISCUSSION

Synthesis and Characterization of the Stationary Phase. The characteristics of the latex-coated monolithic columns depend chiefly on the structure of the monolithic backbone and the nature of the latex particles used. Previous work in this laboratory on stationary phases for capillary electrochromatography and in-line preconcentration in capillary electrophoresis has focused on the use of the quaternary ammonium functionalized AS5A and AS18 latexes.[28,32] In the present study, the quaternary ammonium AS10 latex was used when direct UV spectrophotometric detection was employed. It was also chosen because the majority of the analytes investigated had moderately low ion-exchange selectivity coefficients. However, AS18 latex, which shows strong selectivity toward hydroxide, was used when suppressed conductivity detection was employed. The ability to use the same template monolithic polymer and to coat it with different latexes to achieve a desired selectivity is one of the prime advantages of the approach described here.

One advantage of monolithic columns in comparison to particle-packed columns is their superior mass-transfer characteristics at higher flow rates. Because one of the aims of this study was to obtain rapid separations, desirable monoliths would exhibit pore sizes large enough to sustain reasonably high flow rates, yet small enough to minimize mass-transfer effects. Pore size can be controlled relatively easily in the methacrylate-based monoliths used in this study by varying the reaction conditions.[30] A porogen mixture comprising 55% 1-propanol, 35% 1,4-butanediol, and 10% water gave rise to monoliths exhibiting pore sizes in the region of 5 μm (as determined by mercury intrusion porosimetry). Table 1 shows some of the other physical characteristics of the monolith determined by mercury intrusion porosimetry. Of particular note is the high porosity of the material, which should enable it to be used at relatively high flow rates.

Table 1. Monolith Properties Determined by Mercury Intrusion Porosimetry

total intrusion volume	1.695 cm^3/g
total pore area	0.7712 m^2/g
median pore diameter	5.054 μm
total density	0.351 g/cm^3
skeletal density	0.867 g/cm^3
porosity	59.49%

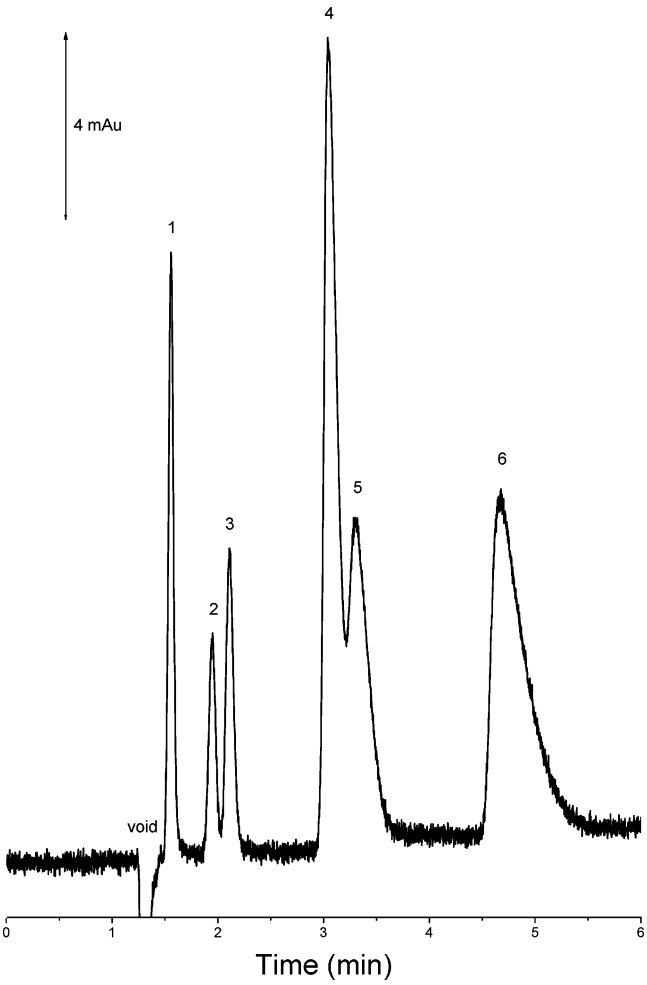

Figure 1. Micro-IC separation of anions using a 250 μm i.d. × 30 cm monolithic column coated with the quaternary ammonium latex AS10 (Dionex). Conditions: column: 250 μm × 30 cm; eluent, 20 mM Tris/Cl^-, pH 8.1; sample loop, 50 μm × 5.0 cm; analytes, 0.5 mM; detection, 195 nm; flow rate, ~8.0μL/min. Peaks: 1, iodate; 2, bromate; 3, nitrite; 4, bromide; 5, nitrate; 6, benzenesulfonate.

The ion-exchange capacity of the AS10 and AS18 latex-coated monolithic columns was determined by an adsorption/elution method where the column was first loaded with the UV-absorbing anion bromide, which was then eluted using 100 mM perchlorate eluent. The bromide was quantified by comparison with a calibration curve. The capacity determined for a 250-μm monolithic column was ~300 pequiv/cm of column, corresponding to a total capacity of 9.0 nequiv for a 30-cm column. Based on capacity per unit volume, the monolithic column has only ~4% of the ion-exchange capacity of an equivalent column packed with latex-agglomerate ion-exchange particles. The loading of the latex was therefore low, as observed in our previous study of latex-coated monoliths for capillary electrochromatography.[28] The main reasons

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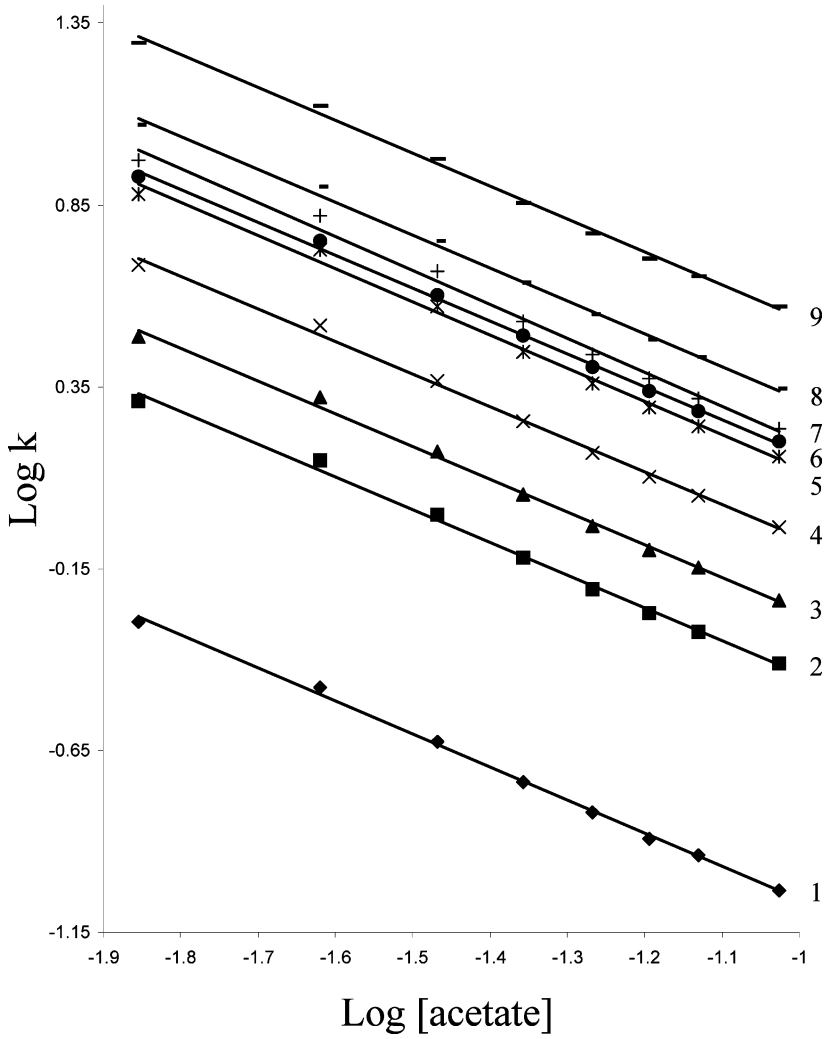

Figure 2. Log k' versus log [acetate] for the separation of anions using a 250 μm i.d. × 30 cm monolithic column coated with the quaternary ammonium latex AS10 (Dionex). Peaks: 1, iodate; 2, bromate; 3, nitrite; 4, benzoate; 5, bromide; 6, toluate; 7, nitrate; 8, benzenesulfonate; 9, toluenesulfonate. Other conditions as in Figure 1.

for this outcome were that the prepared monoliths had relatively large pores, decreasing the surface-to-volume ratio, and the degree of negative charge on the polymer was low due to the low concentration of AMPS used in the polymerization.

Separation of Anions on an AS10 Latex-Coated Monolith Using UV Detection. Initial investigation involved preparation of the latex-coated monoliths within small-diameter (50−100 μm) fused-silica capillaries, but the resultant columns were prone to blockages and exhibited low flow rates (>0.5 μL/min) and relatively long separations, although peak shapes were generally good. Faster runs could be obtained by reducing the column length from 30 to 10 cm, but this resulted in a corresponding loss in separation efficiency. Increasing the capillary diameter to 250 μm dramatically improved the flow reproducibility and allowed for higher flow rates due to reduced back pressure. Figure 1 shows the separation of six anions using a 250-μm monolith coated with AS10 latex particles. It can be seen that the speed of separation is high (the flow rate was ~8 μL/min, corresponding to a linear flow velocity of ~2.3 mm/s) and the observed efficiency was ~13 000 theoretical plates/m for iodate.

Despite the relatively low ion-exchange capacity of the monolithic column, the separation of a series of inorganic anions and organic acids could be accomplished as long as relatively weak eluents were used, such as Tris/chloride (used in Figure 1) or Tris/acetate. The role of anion exchange in the separation was examined by varying the concentration of acetate in a Tris/acetate eluent and plotting log k versus log [acetate]. These plots are shown in Figure 2, and the slopes were all in the range −0.90 to −0.94, which are in approximate agreement with the theoretical slope of −1.0 for anion-exchange elution of a singly charged analyte anion with a singly charged eluent anion. As with conventional IC systems, the selectivity of the separations performed on the monolithic columns could be varied by changing the competing ion used in the eluent. For example, use of acetate, chloride, and perchlorate as competing ions caused changes in elution order, especially for bromide and nitrate.

The porous nature of the prepared monoliths potentially allowed for very rapid separations. Figure 3 shows a Van Deemter plot for the latex-coated monolithic stationary phase and illustrates the efficiency versus flow rate for the separation of iodate. It can be seen that although there is an initial decrease in efficiency as the flow rate increases, this quickly levels off; as the flow rate is increased above 20 μL/min there is no subsequent loss in efficiency. Figure 4 shows chromatograms obtained at higher flow

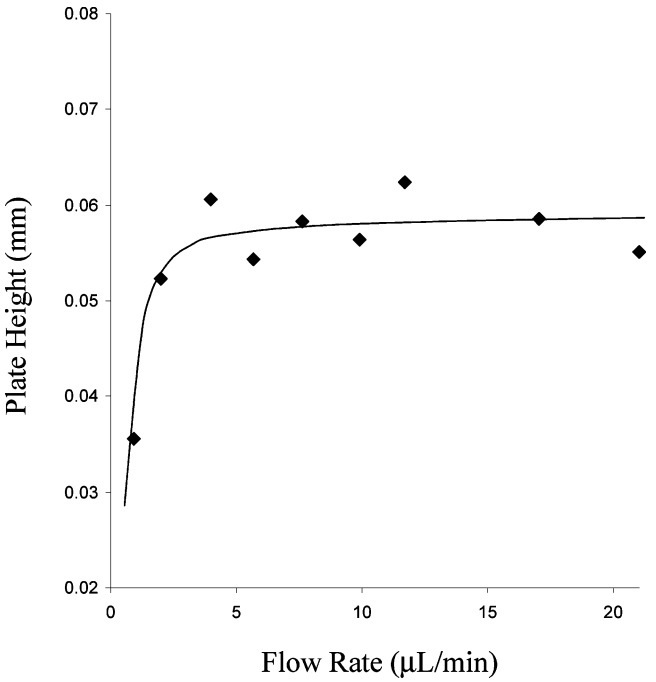

Figure 3. Van Deemter plots for the separation of iodate using a monolithic column coated with the quaternary ammonium latex AS10 (Dionex). Column, 250 μm i.d. × 30 cm; eluent, 20 mM Tris/20 mM acetate, pH 8.0.

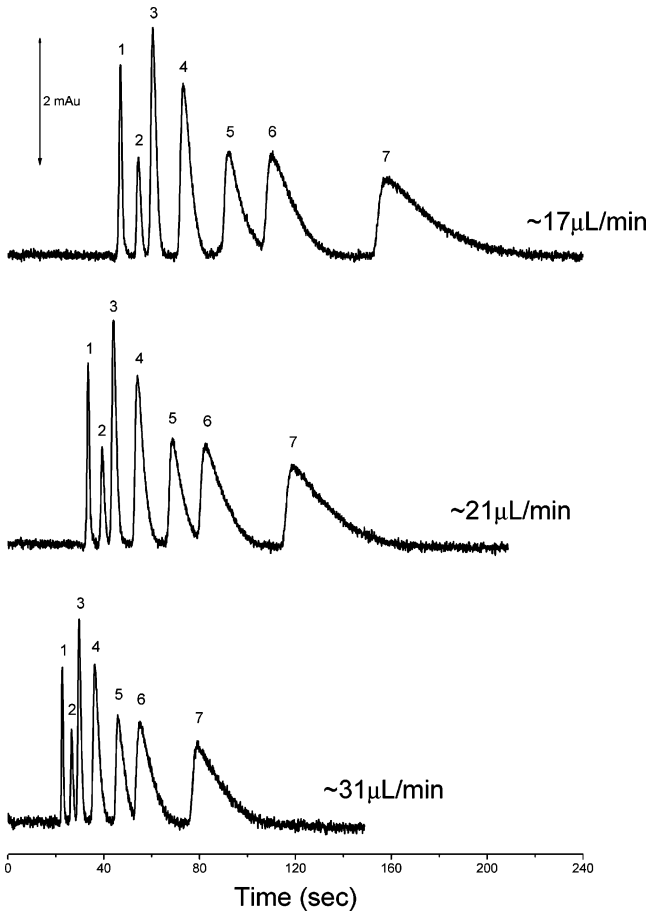

Figure 4. Effect of higher flow rates on the separation of inorganic anions and organic acids using a monolithic column coated with the quaternary ammonium latex AS10 (Dionex). Conditions: column, 250 μm i.d. × 30 cm; eluent, 40 mM NaF; sample loop, 50 μm × 5.0 cm; analytes, 0.5 mM; detection, 195 nm; Peaks: 1, iodate; 2, bromate; 3, nitrite; 4, benzoate; 5, nitrate; 6, benzenesulfonate; 7, toluenesulfonate.

rates. With a flow rate of 31 μL/min (corresponding to a linear flow velocity of 13.5 mm/s), baseline separation of all seven analyte anions in the test mixture could be achieved in under 2 min. A further increase in flow rate to 42 μL/min (corresponding to a linear flow velocity of 18.5 mm/s) permitted the baseline separation of iodate, bromate, and nitrite in 20 s after injection, with the three analytes being eluted within a total time period of 4.5 s; data not shown.

The high-speed separations discussed above could be performed reproducibly, both as replicate analyses performed sequentially and performed after storage of the column for up to 50 days (see Table 2). The RSDs in retention time, peak height, and peak area over 10 consecutive injections were 0.39, 2.7, and 2.9%, respectively. The good reproducibility of the separation performed after storage of the column (which was stored without the ends being sealed) indicated that the electrostatic binding of the latex particles to the monolithic surface was very stable.

Separation of Anions on an AS18 Latex-Coated Monolith Using Conductivity Detection. When conductivity detection is to be used in IC, the choice of the eluent-competing ion becomes critical. The types of analytes to be separated and whether chemical suppression of the background conductance of the eluent is to be used must be carefully considered. Numerous eluent competing ions are suitable for either nonsuppressed or suppressed conductivity detection, but perhaps the most versatile of these is the hydroxide ion. When used with a suitable stationary phase, hydroxide acts as an effective competing ion for a wide range of analyte anions, hydroxide eluents can be generated electrolytically from water, and hydroxide is very amenable to chemical suppression. For these reasons, the present studies on μ-IC with conductivity detection focused on hydroxide eluents. This required the use of a hydroxide-selective functionalized latex to coat the monolithic support. A Dionex AS18 quaternary ammonium latex was selected for this purpose. and the latex-coated monolithic stationary phase was prepared in the same manner as described above for the AS10 latex. The same monolithic substrate was used for the AS10 and AS18 coatings. The capacity determined for a 250-μm monolithic column was ~800 pequiv/cm of column, corresponding to a total capacity of 24 nequiv for a 30-cm column. The higher ion-exchange capacity of the AS18 column compared to the AS10 column can be attributed partly to the higher capacity of the AS18 latex itself.

Figure 5 shows the separation of five inorganic anions using an AS18 latex-coated methacrylate monolith and 1.0 mM potassium hydroxide as eluent, with detection by indirect conductivity. The analytes appear as negative peaks because their limiting equivalent conductances are less than that of the eluent ion (hydroxide). The sensitivity of indirect conductivity is only moderate, chiefly because of the high background conductivity of the eluent. Suppressed conductivity detection offers the possibility of very sensitive detection but requires a microsuppressor that is compatible with the capillary separation column.

The development of small-scale suppressors suitable for use with the low flow rates encountered when using capillary columns

Table 2. Average Retention Times and Relative Standard Deviation (n=10) Using Latex-Coated Monolithic Columns for the Micro-IC Separation of Inorganic and Small Organic Anions

	iodate	bromate	nitrite	benzoate	nitrate	benzene-sulfonate	toluene-sulfonate
			10 consecutive runs				
average retention time (min)	0.524	0.608	0.676	0.827	1.027	1.220	1.746
relative standard deviation (%)	0.386	0.391	0.364	0.461	0.657	0.768	0.854
			50 days later				
average retention time (min)	0.529	0.606	0.675	0.816	1.024	1.203	1.702

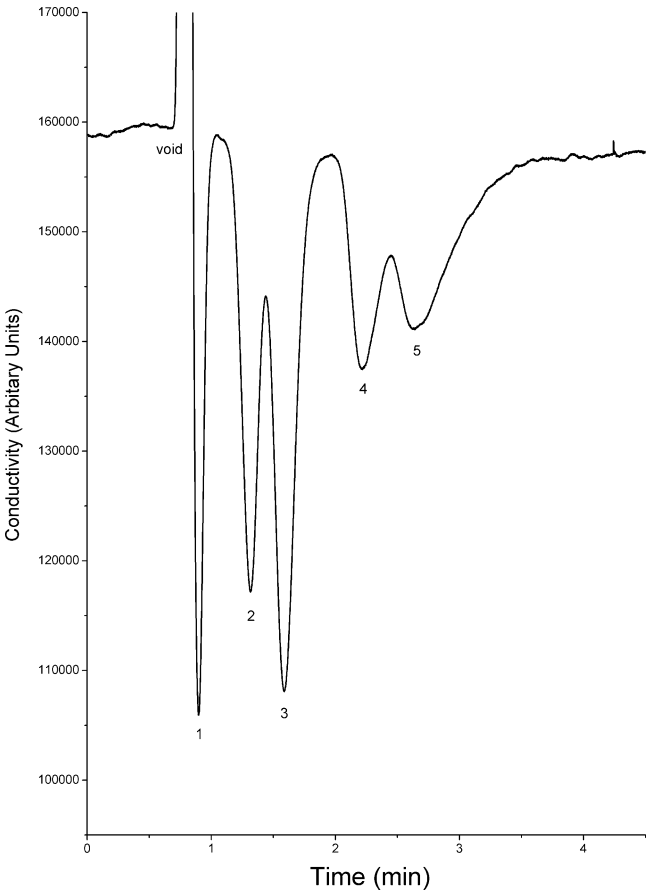

Figure 5. Separation and nonsuppressed conductivity detection of inorganic anions using a methacrylate-based monolithic column coated with the quaternary ammonium latex AS18 (Dionex). Conditions: eluent, 1.0 mM KOH; column, 250 μm i.d. × 35 cm, 3.5−μm pore size coated with AS18; flow rate, ~18μL/min; sample loop, 50 μm × 5.0 cm; analytes, 0.5 mM. Peaks: 1, iodate; 2, bromate; 3, nitrite; 4, bromide; 5, nitrate.

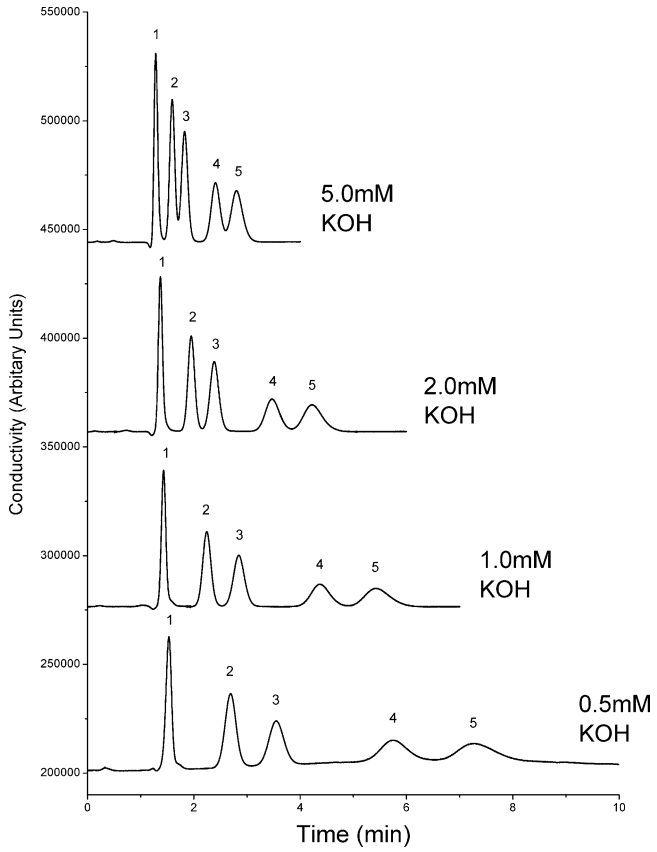

Figure 6. Effect of increasing concentration of hydroxide in the eluent on the observed separation using suppressed conductivity detection. Conditions: eluent, as marked; column; 250 μm i.d. × 30 cm, 3.5−μm pore size coated with AS18; flow rate, ~12.5μL/min; sample loop, 50 μm × 5.0 cm; regenerant, 5 mM H_2SO_4 flowing at 0.5 mL/min; analytes, 50 μM. Peaks: 1, iodate; 2, bromate; 3, nitrite; 4, bromide; 5, nitrate.

(typically <1−100 μL/min) has been limited. Rokushika et al.[33] used a 200-μm Nafion tube suspended in a solution of the regenerant (dodecylbenzenesulfonic acid) for suppression of carbonate/bicarbonate eluents. Subsequent versions of such hollow fiber microsuppressors have been reported by Pyo and Kim[34] and Sjogren et al.,[35] with the latter work also demonstrating the use of an electrodialytic generator for the generation of hydroxide gradients. In the present study, a hollow fiber microsuppressor similar to that described by Sjogren et al. was used. This suppressor consisted of a hollow section of 100-μm-i.d. cation-exchange tubing contained within a standard 25-cm PEEK column. The two ends of the ion-exchange tubing were connected to PEEK capillary, which in turn could be connected directly to the outlet of the columns and to an open-tubular section of fused-silica capillary to which the contactless conductivity detector was attached. Suppression of the hydroxide eluent could be achieved by pumping H_2SO_4 through the 25-cm PEEK column and using the ion-exchange tubing to transport H^+ and Na^+ between the eluent and regenerant. Figure 6 shows the separation and suppressed conductivity detection of five inorganic anions using a range of potassium hydroxide eluents. The observed separation efficiencies were relatively low (~5400 plates/m for iodate) compared to those obtained with direct UV spectrophotometric detection. This can be attributed to the relatively large suppressor used (~28 cm long), which resulted in the suppressor being

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(34) Pyo, D.; Kim, H. *Anal. Sci. Technol.* **1999**, *12*, 89−93.
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approximately the same length as the microcolumn and therefore contributing significantly to band broadening. Despite this, suppressed conductivity detection was ~70 times more sensitive than direct UV detection for UV-absorbing ions such as iodate. Detection limits (calculated at three times baseline noise) for the five analyte anions in Figure 6 at 2.0 mM KOH (where baseline separation was still maintained) were in the range 0.4−1.2 μM. The performance of the suppressor remained essentially constant for eluent hydroxide concentrations in the range 0.5−5.0 mM, suggesting that even higher eluent concentrations or hydroxide gradients could be used successfully.

CONCLUSIONS

Micro-IC using latex-coated monolithic polymeric structures as stationary phases has been demonstrated. Separations could be performed on these microcolumns using eluent flow rates in the microliter per minute range with direct UV, suppressed conductivity, and nonsuppressed conductivity detection. The separation efficiencies achieved are moderate but are retained at relatively high eluent flow rates, which enables rapid separations to be achieved. The hydrolytic stability of the organic polymer-based monolithic stationary phase allowed alkali metal hydroxide eluents to be used in conjunction with suppressed contactless conductivity detection. Silica-based monolithic stationary phases show hydrolytic degradation at alkaline pH and would be unsuitable when used with such eluents. Further studies are in progress to improve the separation efficiency of the stationary phase, to increase the ion-exchange capacity of the stationary phases, to miniaturize the suppressor, and to perform separations in the gradient elution mode with suppressed conductivity detection.

ACKNOWLEDGMENT

Financial support from the Australian Research Council is gratefully acknowledged.

Received for review August 23, 2004. Accepted October 13, 2004.

AC048747L