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Universal stationary phase for the separation of anions on suppressor-based and single-column ion chromatographic systems

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ABSTRACT

A stationary phase composed of a hydroxyethyl methacrylate-based macroporous copolymer with quaternary amine functional groups for the separation of anions was evaluated. The material is rigid, stable and compatible with a wide variety of eluents including *p*-hydroxybenzoic acid, phthalic acid, borate–gluconate, and carbonate–hydrogencarbonate. The column packed with this anion exchanger may be used with single-column ion chromatography in addition to suppressor-based ion chromatography. The hydrophilic nature of the methacrylate copolymer provides improved peak shapes for polarizable anions and eliminates system peaks typically encountered with poly(styrene–divinylbenzene) ion exchangers. The column performance with a wide variety of eluents is demonstrated with applications using both single-column and suppressor-based ion chromatography systems.

INTRODUCTION

Ion chromatography (IC) has become one of the most widely used techniques for the determination of inorganic anions. At present, two types of IC are in practical use. The first, the suppressor-based system, was developed by Small *et al.* [1]. In this system, a high-conductivity eluent is attenuated in the suppressor column prior to entering the detector by converting the highly conductive eluent to a low-conductivity form, and at the same time the anions are converted to highly conducting acids. The second type is single-column ion chromatography (SCIC), which was introduced by Gjerde *et al.* [2]. In this system, an eluent with low equivalent conductance is chosen so that the separator column can be coupled directly to the detector.

Since the introduction of IC, several stationary phases for the separation of anions have been developed. Some are microporous agglomerated pellicular anion exchangers [3] which were developed for suppressor-based IC, macroporous poly(styrene–divinylbenzene) (PS–DVB) copolymer anion exchangers [4–6] developed for SCIC and silica-based anion exchangers [7,8], also developed for SCIC. The ultility of these anion exchangers is limited to a few specific eluents. For example, silica-based ion exchangers can only be used in the pH range 3–7. High-pH eluents such as hydroxide and carbonate–hydrogencarbonate will dissolve the silica-based packing.

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PS–DVB ion exchangers are pH stable, but work in our laboratory has demonstrated that they are not suitable for separating anions using carbonate–hydrogencarbonate or borate–gluconate eluents.

Recently, a new PS–DVB-based anion exchanger that can be used with both types of IC was developed [9]. This material may be used with carbonate–hydrogencarbonate eluent to separate the seven common inorganic anions by suppressor-based IC, and it may also be used with sodium hydroxide eluent for the analysis of weakly acidic anions by SCIC.

The stationary phase described here is compatible with both types of IC. It is demonstrated that this macroporous hydroxyethyl methacrylate-based (HEMA) anion exchanger is rigid, chemically stable and shows good ion-exchange kinetics with a wide variety of eluents including *p*-hydroxybenzoic acid, phthalic acid, borate–gluconate, hydroxide–benzoate and carbonate–hydrogencarbonate. The advantages of this HEMA anion exchanger over PS–DVB- and silica-based anion exchangers is discussed in terms of its universal characteristics, system peaks and peak shapes.

EXPERIMENTAL

Instrumentation

The system used was a Wescan ion chromatography system (Alltech, Deerfield, IL, U.S.A.). It consists of an Alltech Model 325 high-performance liquid chromatographic (HPLC) pump and a Wescan ICM-300 ion chromatography module equipped with a Model 315 conductivity detector, an active temperature control (ATC) module and a Rheodyne Model 9125 metal-free injection valve (100-μl sample loop). The temperature of the ICM-300 was maintained at 30°C. All data were recorded on a Spectra-Physics (Santa Clara, CA, U.S.A.) SP 4400 Chromjet integrator.

For suppressor-based IC, the above system was used with the addition of a suppressor built in-house using a 2 m × 0.61 mm I.D. × 0.84 mm O.D. Nafion 811X perfluorosulfonate tubular cation-exchange membrane (Perma Pure Products, Toms River, NJ, U.S.A.) as described by Dasgupta [10]. Sulfuric acid (12 m*M*) was used as a regenerant.

Columns

The universal stationary phase, a hydroxyethyl methacrylate-based anion exchanger with quaternary amine functional groups, was obtained from Tessek (Aarhus, Denmark). This material has a particle size of 10 μm and an ion-exchange capacity of 0.1 mmol/g. The material was slurry packed into 50 mm × 4.6 mm I.D., 100 mm × 4.6 mm I.D. and 150 mm × 4.6 mm I.D. columns (Alltech Universal Anion columns). A Wescan Anion/R (250 mm × 4.1 mm I.D.) column (Alltech) was used for comparison purposes.

Reagents

All eluents were prepared from EZ-LUTE buffer concentrate (Alltech), with the exception of borate–gluconate, for which analytical-reagent grade chemicals (Aldrich, Milwaukee, WI, U.S.A.) were used. The standards were prepared from Alltech certified IC standards. HPLC-grade water (Alltech) was used to prepare all eluents and standards solutions.

Eluents

Five eluents were tested with the HEMA column: *p*-hydroxybenzoic acid, phthalic acid, borate–gluconate, hydroxide–benzoate and carbonate-hydrogencarbonate. *p*-Hydroxybenzoic acid was 5 m*M*, and the pH was adjusted to 7.9 with lithium hydroxide. Phthalic acid was 4 m*M*, adjusted to pH 4.5 with lithium hydroxide. Two compositions of borate–gluconate eluents were tested. One (borate–gluconate 1) was prepared by diluting 40 ml of a concentrate containing 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w), 7.2 g/l lithium hydroxide monohydrate, and 94 ml/l glycerol with 120 ml of acetonitrile and 840 ml of water. The other (borate–gluconate 2) was prepared by diluting 40 ml of a concentrate containing 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w) and 7.2 g/l lithium hydroxide monohydrate with 960 ml of water; neither acetonitrile nor glycerol was used in borate–gluconate 2 eluent. Hydroxide–benzoate eluent was 2.5 m*M* in lithium hydroxide and 0.005 m*M* in sodium benzoate. Carbonate–hydrogencarbonate eluent contained 2.8 m*M* sodium hydrogencarbonate and 2.2 m*M* sodium carbonate. Table I summarizes the concentrations of the eluents used.

RESULTS AND DISCUSION

Chemical and physical properties

HEMA is a macroporous copolymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate. It is extensively cross-linked to produce a matrix with high chemical and physical stability. The maximum operating pressure is 2500 p.s.i. Fig. 1A shows the structure of HEMA. The tertiary α-carbonyl structure of pivalic acid is one of the most stable and least hydrolyzable esters known [11], which allows the HEMA stationary phase to be used with a variety of eluents in the pH range 2–12. The excess hydroxyl groups on the HEMA matrix also increase the hydrophilicity of this material, which will be shown later to result in improved peak shapes for polarizable anions. The strong-base anion exchanger of HEMA shown in Fig. 1B is prepared by treating the HEMA precursor with an aqueous solution of trimethylamine [12]. The preparation procedures and the influence of different functional groups on sorbent selectivity were discussed by Vlacil and Vins [12,13]. The sorbent contains *ca.* 0.1 mmol/g of quaternary amine ion-exchange functional groups. The particle size is 10 μm.

TABLE I

ELUENTS USED FOR EVALUATING THE UNIVERSAL ANION COLUMN

Eluent	Concentration and pH
p-Hydroxybenzoic acid	5 m*M*, adjusted to pH 7.9 with lithium hydroxide
Phthalic acid	4 m*M*, adjusted to pH 4.5 with lithium hydroxide
Borate–gluconate 1	40 ml of concentrate, 120 ml of acetonitrile and 840 ml of water. Concentrate contained 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w), 7.2 g/l lithium hydroxide monohydrate and 94 ml/l glycerol
Borate–gluconate 2	40 ml of concentrate and 960 ml of water. Concentrate contained 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w) and 7.2 g/l lithium hydrocide monohydrate
Hydroxide–benzoate	2.5 m*M* lithium hydroxide–0.05 m*M* sodium benzoate
Carbonate–hydrogencarbonate	2.2 m*M* sodium carbonate–2.8 m*M* sodium hydrogencarbonate

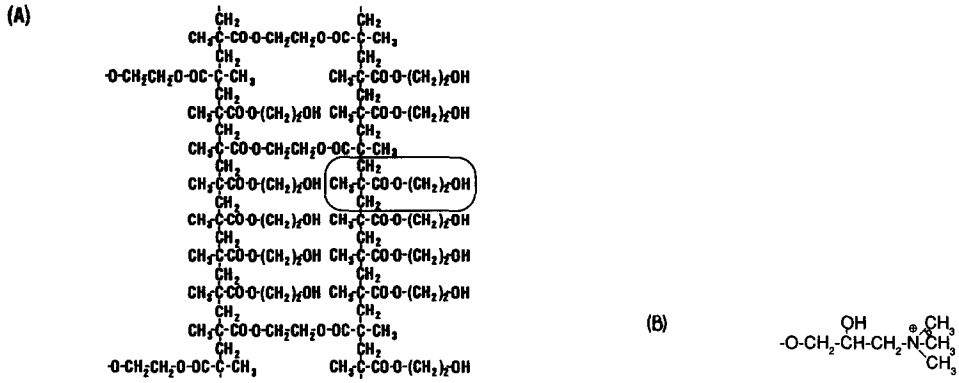

Fig. 1. Structures of (A) HEMA and (B) strong-base anion exchanger of HEMA.

Column performance with various eluents

The column performance with a wide variety of eluents was evaluated by injecting a standard mixture containing 10 ppm fluoride, 20 ppm each of chloride, nitrite, bromide, and nitrate and 30 ppm each of phosphate and sulfate. *p*-Hydroxybenzoic acid, phthalic acid, borate–gluconate and hydroxide–benzoate eluents were used in the SCIC mode and carbonate–hydrogencarbonate eluent was used with suppressor-based IC. The concentrations and pH of the eluents were optimized to give the optimum separation of the anions tested.

p-Hydroxybenzoic acid. *p*-Hydroxybenzoic acid (pHBA) is one of the most useful eluents in SCIC [14,15]. This large aromatic acid exhibits the characteristics desired for SCIC eluents. It has a lower equivalent conductance than most inorganic anions, and is capable of eluting both monovalent and divalent anions in one run. pHBA is used most commonly with PS–DVB-based anion exchangers in the pH range 8.0–8.6. The separation of fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulfate on the HEMA column with pHBA is shown in Fig. 2. The seven anions were effectively separated within 20 min. The peak shapes for polarizable anions such as nitrate and bromide were sharper than those obtained with a PS–DVB ion-exchange column.

Phthalic acid. Phthalic acid is another useful eluent in SCIC [14]. It is useful in the pH range 4.0–7.0 and is used most commonly with silica-based ion-exchange columns. The chromatogram shown in Fig. 3 was obtained using 4 m*M* phthalic acid, adjusted to pH 4.5 with lithium hydroxide (LiHP). Under these conditions, a good separation of fluoride, chloride, nitrite, bromide, nitrate and sulfate was achieved. The phosphate peak was eluted in the column void volume with this eluent.

Borate–gluconate. A mixture of boric acid, sodium tetraborate and gluconic acid was introduced in 1986 as an eluent system for SCIC [16]. This eluent is popular because of its low background conductance and relatively strong eluting power. Borate–gluconate 1, which contained acetonitrile and glycerol, is the standard eluent composition used with most polyacrylate-based columns [16,17]. As can be seen in Fig. 4A, the efficiency of this eluent with HEMA column was poor. Borate–gluconate 2 was then prepared and tested with this column. As shown in Fig. 4B, a better

Fig. 2. Separation of the standard anion mixture using *p*-hydroxybenzoic acid eluent. Column, 150 mm × 4.6 mm I.D.; packing, Alltech Universal Anion; eluent, 5 m*M* *p*-hydroxybenzoic acid, pH 7.9 adjusted with lithium hydroxide; flow-rate, 1.0 ml/min; detection, conductivity. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = phosphate (30 ppm); 7 = sulfate (30 ppm).

Fig. 3. Separation of the standard anion mixture using phthalic acid eluent. Eluent, 4 m*M* phthalic acid, pH 4.5 adjusted with lithium hydroxide; other conditions as in Fig. 2. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = sulfate (30 ppm).

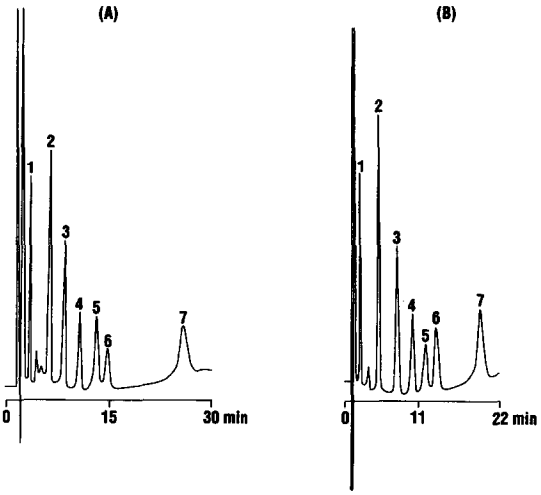

Fig. 4. Separation of the standard anion mixture using borate–gluconate eluent. (A) Eluent, borate–gluconate 1, prepared by diluting 40 ml of a concentrate containing 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w), 7.2 g/l lithium hydroxide monohydrate and 94 ml/l glycerol with 120 ml of acetonitrile and 840 ml of water; flow-rate, 1.5 ml/min; other conditions as in Fig. 2. (B) Eluent, borate–gluconate 2, prepared by diluting 40 ml of a concentrate containing 25.5 g/l boric acid, 13.2 ml/l gluconic acid (50%, w/w) and 7.2 g/l lithium hydroxide monohydrate with 960 ml of water; flow-rate, 1.5 ml/min; other conditions as in Fig. 2. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = phosphate (30 ppm); 7 = sulfate (30 ppm).

separation was obtained with this eluent. Some workers have suggested that the purpose of acetonitrile in a borate–gluconate eluent is to facilitate phase transfer and produce sharper peaks and shorter retention times [16]; however, the use of these additives with the HEMA column resulted in excessive retention of sulfate.

Lithium hydroxide–sodium benzoate. Hydroxide–benzoate eluent is used in SCIC for the analysis of weak acid anions such as borate, silicate, cyanide and sulfide [14]. As hydroxide has a higher equivalent conductance than most other anions, the peaks for the sample anions appear as negative peaks (decrease in conductance). Hydroxide ion is weakly held by the strong-base anion exchangers and it is not a good choice for separating the common inorganic anions. Fig. 5 shows a chromatogram of the standard anions using 2.5 m*M* lithium hydroxide–0.05 m*M* sodium benzoate eluent. This eluent is too weak to elute phosphate and sulfate.

Carbonate–hydrogencarbonate. This eluent is the most common eluent used in suppressor-based IC. As its equivalent conductance is very close to that of the common inorganic anions, it cannot be used in the SCIC mode. To evaluate the performance of the HEMA column with this eluent, a suppressor column was installed between the separator column and the conductivity detector. The chromatogram shown in Fig. 6A was obtained using 2.8 m*M* sodium hydrogencarbonate–2.2 m*M* sodium carbonate eluent on a 150 mm × 4.6 mm I.D. column. The resolution between the anions was good, but the retention times were too long. As shown in Fig. 6B, by modifiying the eluent concentration and using a shorter column, the retention times were reduced.

Selectivity and efficiency

The selectivity of the HEMA-based column with all the eluents tested is presented in Table II. The results indicate that this material has a "universal" character.

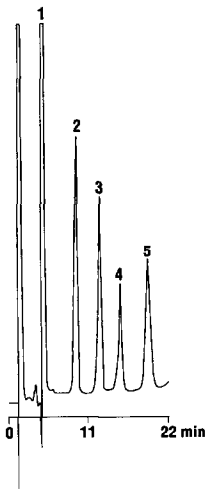

Fig. 5. Separation of the anion mixture using hydroxide eluent. Eluent, 2.5 m*M* lithium hydroxide–0.05 m*M* sodium benzoate; flow-rate, 1.5 ml/min; other conditions as in Fig. 2. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm).

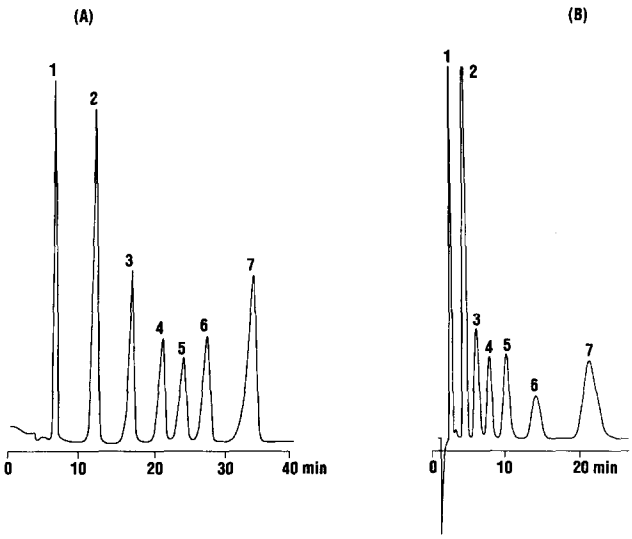

Fig. 6. Separation of the anion mixture using carbonate–hydrogencarbonate eluent. Packing, Alltech Universal Anion; suppressor, membrane, 12 m*M* sulfuric acid regenerant; detection, conductivity. Other conditions: (A) column, 150 mm × 4.6 mm I.D.; eluent, 2.2 m*M* Na_2CO_3–2.8 m*M* $NaHCO_3$; flow-rate, 0.7 ml/min; (B) column, 50 mm × 4.6 mm I.D.; eluent, 0.3 m*M* Na_2CO_3–5.7 m*M* $NaHCO_3$; flow-rate, 0.8 ml/min. Peaks: 1 = fluoride (10 ppm); 2 = chloride (20 ppm); 3 = nitrite (20 ppm); 4 = bromide (20 ppm); 5 = nitrate (20 ppm); 6 = phosphate (30 ppm); 7 = sulfate (30 ppm).

It gives good results with a wide variety of eluents and shows compatibility with both SCIC and suppressor-based IC.

The efficiency expressed as number of theoretical plates per meter with various eluents is shown in Table III. The results show good ion-exchange kinetics with a wide variety of eluents, and are comparable to those given by other commercially available columns [18].

TABLE II

ANION EXCHANGE CAPACITY FACTORS, k' ON THE UNIVERSAL ANION COLUMN

Anion	Eluent[a]				
	pHBA (pH 7.9)	LiHP (pH 4.5)	Borate–Gluconate 2	LiOH–benzoate	Na_2CO_3–$NaHCO_3$
Fluoride	1.5	1.7	1.4	1.7	0.6
Chloride	2.9	3.4	4.2	4.0	2.0
Nitrite	4.0	4.6	6.6	5.8	3.3
Bromide	4.9	5.9	8.6	7.2	4.3
Nitrate	6.2	7.6	10.5	9.3	5.0
Phosphate	8.0	–	11.9	–	5.8
Sulfate	12.6	12.5	18.1	–	7.4

[a] Concentrations of the eluents are given in Table I.

TABLE III

COLUMN EFFICIENCY WITH VARIOUS ELUENTS

Eluent	Efficiency[a] (plates/m)
5 m*M* *p*-hydroxybenzoic acid, pH 7.9 (lithium hydroxide)	21 573
4 m*M* phthalic acid, pH 4.5 (lithium hydroxide)	18 920
Borate–gluconate 2[b]	20 460
2.5 m*M* lithium hydroxide–0.05 m*M* sodium benzoate	25 813
2.2 m*M* sodium carbonate–2.8 m*M* sodium hydrogencarbonate	21 087

[a] Calculated at half-height using nitrate peak.

[b] The concentration of borate–gluconate 2 eluent is given in Table I.

Comparison between HEMA-, PS–DVB- and silica-based columns

The hydrophilic character of the HEMA-based anion exchanger offers several advantages over PS–DVB-based materials. System peaks typically encountered with PS-DVB anion exchangers when used with pHBA eluent [14] are not observed with the HEMA-based media. System peaks result from a change in equilibrium between the molecular form of the eluent and the resin matrix [14, 19, 20]. As HEMA is less hydrophobic than PS–DVB, the adsorbtion of the molecular form of pHBA by the resin is reduced. This may explain why the system peak is not observed. More research is required to determine the exact mechanism which causes system peaks.

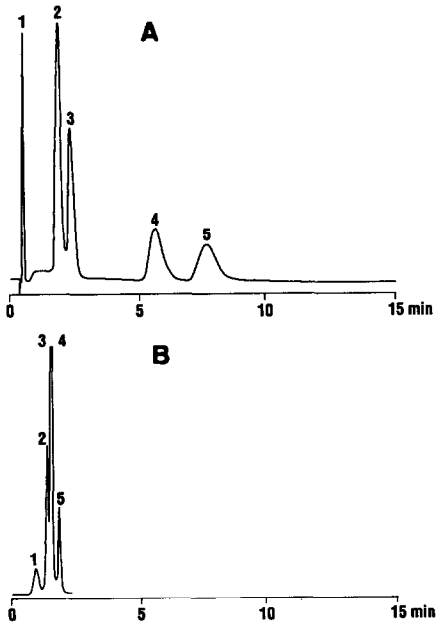

Fig. 7. Reversed-phase separation of five organic components on PS–DVB-based and HEMA-based columns. Column, 100 mm × 4.6 mm I.D.; eluent, acetonitrile–water (65:35); flow-rate, 1.0 ml/min; detection, UV at 254 nm. Packing: (A) PS–DVB (Wescan Anion/R); (B) Alltech Universal Anion. Peaks: 1 = uracil; 2 = phenol; 3 = benzaldehyde; 4 = N,N-diethyl-*m*-toluamide; 5 = toluene.

The peak shape for polarizable anions such as nitrate and bromide is improved with HEMA. PS–DVB-based anion exchangers have some hydrophobic or reversed-phase characteristics [6] and tend to cause peak tailing for polarizable anions. A Wescan Anion/R column (PS–DVB based) was used for a comparison study. The chromatograms in Fig. 7 show the hydrophilicity of HEMA as compared with PS–DVB. These chromatograms were obtained using reversed-phase conditions [acetonitrile–water (65:35) as the eluent with UV detection at 254 nm]. The five components were well resolved on the PS–DVB column (Fig. 7A) and were unretained on the HEMA column (Fig. 7B). Fig. 8 shows the peak shape of nitrate on both HEMA and PS–DVB columns. It is likely that the hydrophobic characteristics of PS–DVB cause peak tailing for nitrate owing to the retention of this polarizable anion on the packing by a "reversed-phase" mechanism.

Silica-based material provides excellent rigidity of the packing, but creates problems with eluent pH limitations and adsorption of fluoride ion, making it difficult to determine this ion. This HEMA-based sorbent may be used with phthalic acid eluent as a replacement for silica-based columns, with the additional advantage of allowing the determination of fluoride.

Applications

The physical, chemical and ion-exchange properties of HEMA allow a wide variety of separations. Fig. 9A shows the separation of chloride, chlorate and sulfate on a 100 mm × 4.6 mm I.D. HEMA column with pHBA eluent. The total analysis

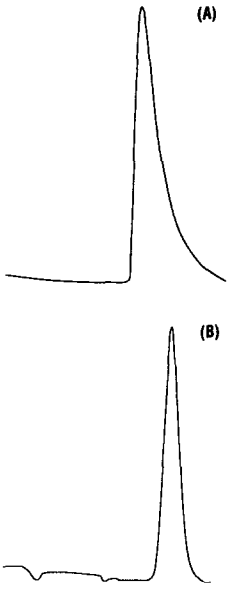

Fig. 8. Comparison of peak shape for nitrate ion on PS–DVB-based and HEMA-based columns. (A) Column, 250 mm × 4.1 mm I.D.; packing, PS–DVB (Wescan Anion/R); eluent, 5 m*M* pHBA, pH 8.5 adjusted with lithium hydroxide; flow-rate, 1.0 ml/min; detection, conductivity; sample size, 20 μl. (B) Column, 150 mm × 4.6 mm I.D.; packing, Alltech Universal Anion; eluent, 5 m*M* pHBA, pH 7.9 adjusted with lithium hydroxide; other conditions as in (A).

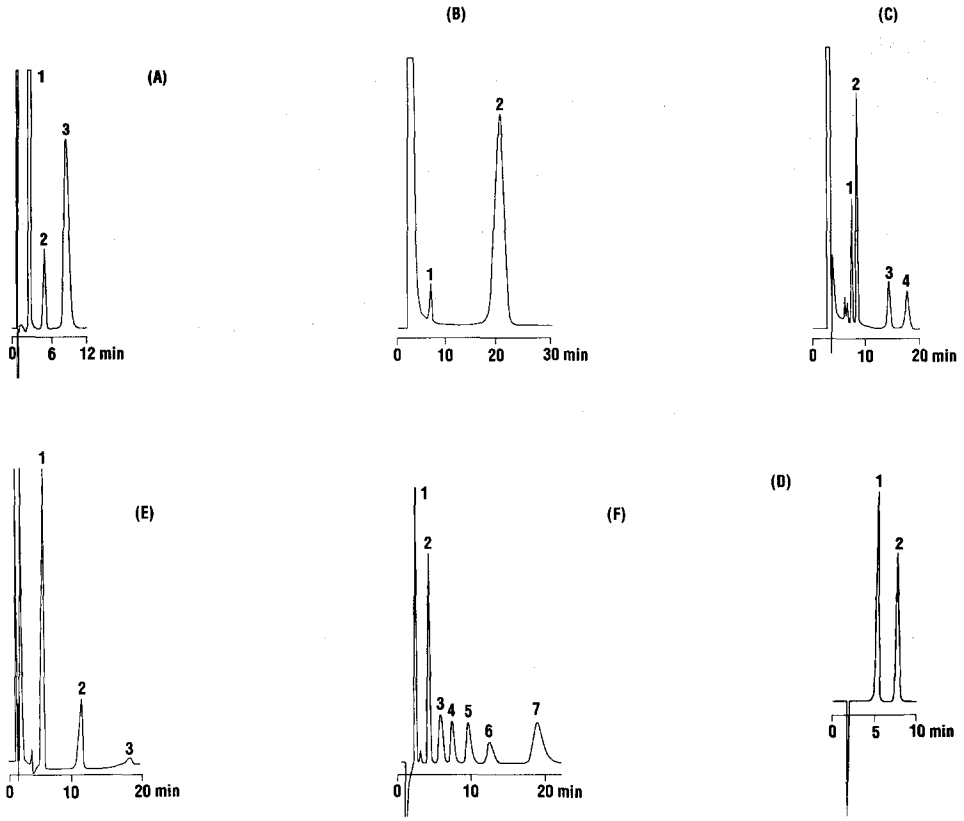

Fig. 9. Separation of various anions on HEMA (Alltech Universal Anion) column. (A) Separation of (1) chloride (100 ppm), (2) chlorate (40 ppm) and (3) sulfate (100 ppm): column, 100 mm × 4.6 mm I.D.; eluent, 4 m*M* pHBA, pH 7.9 adjusted with lithium hydroxide; flow-rate, 0.9 ml/min; detection, conductivity. (B) Separation of (1) chloride and (2) sulfate in surfactant: column, 150 mm × 4.6 mm I.D.; eluent, 4 m*M* LiHP in methanol–water (5:95), pH 4.5; flow-rate, 1.0 ml/min; detection, conductivity. (C) Separation of (1) chlorite, (2) chloride and (3) chlorate; peak 4 is a system peak: column, 300 mm × 4.6 mm I.D.; eluent, 10 m*M* sodium hydroxide; flow-rate, 1.0 ml/min; detection, conductivity. (D) Separation of (1) nitrite and (2) nitrate: column, 150 mm × 4.6 mm I.D.; eluent, 15 m*M* sodium hydroxide; flow-rate, 1.0 ml/min; detection, UV at 214 nm. (E) Separation of (1) chloride, (2) phosphate and (3) sulfate in plant food: column, 100 mm × 4.6 mm I.D.; eluent, borate–gluconate 2; flow-rate, 1.0 ml/min; detection, conductivity. (F) Separation of anions in antifreeze: column, 50 mm × 4.6 mm I.D.; eluent 0.3 m*M* Na_2CO_3–5.7 m*M* $NaHCO_3$; flow-rate, 0.8 ml/min; suppressor, membrane, 12 m*M* sulfuric acid regenerant; detection, conductivity; peaks: 1 = fluoride; 2 = chloride; 3 = nitrite; 4 = bromide; 5 = nitrate; 6 = phosphate; 7 = sulfate.

time is 13 min. The hydrophilic nature of HEMA allows direct injection of surfactant samples, as shown in Fig. 9B. Normally, the surfactant sample must be pretreated prior to injection, to remove any hydrophobic components in the sample that could irreversibly bind to the polystyrene-based packing. Fig. 9C shows the simultaneous separation of chlorite, chloride and chlorate using a 300 mm × 4.6 mm I.D. HEMA column with hydroxide eluent. A shorter column (150 mm × 4.6 mm I.D.) combined with a weaker eluent (2.8 m*M* sodium hydroxide) has also been successfully used for

this separation. Sodium hydroxide eluent may also be used for the determination of nitrite and nitrate in water with UV detection at 214 nm, as shown in Fig. 9D. Fig. 9E shows the separation of chloride, phosphate and sulfate in plant food with borate–gluconate eluent. Fig. 9F shows the separation of anions in antifreeze using carbonate–hydrogencarbonate eluent with suppressor-based IC.

The universal characteristics of HEMA allows a wide variety of separations. The methods that have been developed for other columns can be transferred directly to a HEMA column with minor modifications to the eluent concentration and pH. This column is compatible with most eluents commonly used in IC analysis of anions.

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