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Anion–cation separations on a mixed-bed ion-exchange column with indirect photometric detection

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ABSTRACT

Inorganic cations and anions are separated simultaneously on a mixed bed of anion and cation exchangers. Columns studied are mixtures of polymer based and silica based anion and cation exchangers and stationary phases that contain both chemically bonded anion and cation ionogenic groups. The major parameters that influence retention and resolution are the mobile phase counterions, their concentration, and the ion-exchange capacities. Because electrolyte solutions are required for elution, detection by conductivity is limited. Using an electrolyte that is composed of a chromophoric cation and anion not only satisfies elution requirements but also premits analyte cations and anions to be detected by an indirect photometric detection strategy.

INTRODUCTION

Columns exhibiting a mixed mode of interactions are becoming increasingly more important in liquid chromatographic (LC) separations. Most are based on polymer matrix type low capacity ion exchangers where the combination of either anion exchange or cation exchange with reversed-phase properties are present [1–4]. Ion exchange of charged analytes occurs at the chemically attached ionogenic group and retention of uncharged molecules occurs at the polymer matrix. For analytes that contain both charged and hydrophobic centers both sites are capable of participating in retention with the analyte. Because of these characteristics retention order depending on the analytes can differ sharply between an ion-exchange column, a reversed-phase column, and one that contains both sites.

Columns containing a mixed bed of ion exchangers can also provide a mixed mode of interaction [5]. Thus, a mixed bed of anion and cation exchangers or a matrix containing both anion and cation exchange sites (both hereafter are referred to as MBIE columns) can be used for an efficient, simultaneous separation of anions and cations [5–7]. In this application a single MBIE column, a single injection, and a single eluent are used for the separation. Since retention of the cation and anion analytes is by ion exchange, their succesful elution must involve an eluent counter-

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cation and counteranion of appropriate ion-exchange selectivity and concentration. If conductance is used for detection, as was done previously [6,7], post column suppression cannot be used to alter background conductance due to the eluent since this will remove either anions or cations depending on the type of suppressor used. Thus, to monitor anion and cation analytes simultaneously as they appear in the column effluent from the MBIE column, an eluent electrolyte must be chosen that provides a cation and anion ionic equivalent conductance which differs sharply from the cations and anions that are being separated and detected. In previous studies using MBIE columns aqueous lithium acetate eluents were successfully used [6,7]. However, the use of conductance for detection limits the type of eluent that can be used since eluent strength cannot easily be altered based on principles of ion-exchange selectivity and mass action of eluent electrolyte without having a major influence on conductivity.

This report focuses on using chromophoric counterions as the eluent electrolyte and indirect photometric detection (IPD) to detect separated analyte anions and cations. Using IPD, which has been shown to be a useful detection strategy in ion chromatography [8–11], allows eluent strength to be adjusted through the use of different counterions and/or via altering counterion concentration. The only requirement is that the counterion must have a favorable absorptivity, if IPD is used.

EXPERIMENTAL

Reagents

Benzyltrimethyl ammonium chloride and *p*-toluenesulfonic acid were obtained from Aldrich while $Ce(NO_3)_3$ was purchased from Fisher Scientific. LC water was obtained by passing distilled water through a Millipore Milli-Q Plus water purification unit. Dionex HPIC-CS5 and HPIC-AS7 columns were purchased from Dionex. Polystyrene divinylbenzene polymer based cation (PRP-SO_3^-) and anion (PRP-X100) exchangers were obtained from Hamilton and/or prepared by sulfonation [3] and quaternization [2,4], respectively, of PRP-1 (Hamilton). Bulk particles of Zorbax SCX and SAX were obtained from Fisher Scientific.

Instrumentation

A Varian M2010 pump, Waters U6K injector, Beckman M160 (254 nm) or a Spectra Physics 770 variable-wavelength detector, and a Hewlett Packard M3390A integrator were used. Columns used and their properties are listed in Table I.

Procedures

Benzyltrimethyl ammonium ($BTMA^+$) chloride was converted to the hydroxide by anion exchange. Aliquots of standardized solutions of BTMAOH and *p*-toluene sulfonic acid (PTSH) were combined in aqueous solution to give the BTMA–PTS eluent. The aqueous $Ce(NO_3)_3$ eluent was prepared by dilution of a known weight of $Ce(NO_3)_3$. Sulfonated and quaternized PRP-1 particles were prepared and their exchange capacities were determined as described elsewhere [2–4]. Polyion MBIE columns (see Table I) were prepared by mixing quantities of the two exchangers in 20 ml of a solution consisting of 30 g NaCl and 100 ml glycerol per liter of LC water. The slurry of exchangers was packed upward in a column using a high flow-rate/high pressure procedure. The Zorbax MBIE column was packed similarly except the glyce-

rol was omitted. Dionex columns were obtained prepacked. Columns were conditioned with the mobile phase of interest and column efficiencies were determined using NaCl as the analyte (see Table I).

Analyte solutions were prepared by dissolving known weights of inorganic salts in LC water. Sample aliquots of 1 to 25 μl were delivered by a 25-μl syringe. A peak area method was used to determine calibration curves. Inlet column pressure at 1 ml/min was about 1000 p.s.i. while column void volume was 0.4 and 1.5 ml for 70 and 250 mm columns, respectively. IPD was at 254 nm for the BTMA–PTS eluent and 240 nm for the $Ce(NO_3)_3$ eluent.

RESULTS

When a mixed bed of cation and anion exchangers or a stationary phase containing both cation and anion ionogenic groups are packed into a column and a charged analyte is passed through the column using an aqueous salt solution as the eluent, both cation and anion exchange will occur in the column [6,7]. The parameters influencing analyte cation and anion retention are those that affect cation and anion exchange. These include: (1) anion and cation exchange capacity, (2) ratio of anion to cation exchange capacity, (3) analyte ion concentration, (4) concentration of the eluent electrolyte, (5) ion exchange selectivity of the eluent countercation and counteranion, and (6) pH if the analyte or eluent counterions have weak acidic/basic properties. Optimization of each parameter, which is discussed elsewhere [5–7,12],

TABLE I

MIXED-BED ION-EXCHANGE (MBIE) COLUMNS

Column	Packing, mixed-bed ratio, dimensions	Cation-exchange capacity (equiv./g)	Anion-exchange capacity (equiv./g)	Particle size (μm)	Sodium efficiency (plates/m)	Chloride efficiency (plates/m)
Dionex HPIC-CS5	[a] 250 × 4.6 mm I.D.	160	70	10	10 000[c]	18 000[c]
Dionex HPIC-AS7	[b] 250 × 4.6 mm I.D.	43	250	10		
Polyion-1	$PRP\text{-}SO_3^-$/PRP-X100 (1:1) 70 × 4.1 mm I.D.	48	50	10	2 000[c]	1 000[c]
Polyion 2	$PRP\text{-}SO_3^-$/PRP-X100 (1:1) 70 × 4.1 mm I.D.	58	29	10		
Zorbax	Zorbax SCX/Zorbax SAX (1:1) 70 × 4.1 mm I.D.	32	245	6	8 000[c]	7 000[c]

[a] A polystyrene divinylbenzene core containing quaternary ammonium groups coated with sulfonated latex.

[b] A polystyrene divinylbenzene core containing sulfonated groups coated with quaternary ammonium latex.

[c] A 1.00 m*M*, 0.10 m*M* and 1,61 m*M* BMTA–PTS eluent, respectively.

must be done while recognizing that both anion and cation exchange are occurring simultaneously.

If the analyte cation and anion ion exchange selectivities differ, each analyte will appear in the column effluent at different elution times. By using an electrolyte in the eluent that is significantly different in ionic equivalent conductance from the analyte cation and anion, the separated analyte cation and anions can be detected using a conductance detector [6,7]. The conductance detector, however, limits the type of electrolyte that can be used in the eluent because of the electrolyte's contribution to the background conductance.

In ion chromatography it has been shown that cations separated on a cation exchanger [8,10] can be detected by IPD if a chromophoric counter cation is used in the eluent. Similarly, anions can be separated and detected by IPD [8,9] by using a chromophoric counteranion. Inorganic anions and metals as anionic EDTA complexes were separated on an anion exchange column using IPD and an EDTA eluent [13]. For a MBIE column IPD should be possible if the eluent electrolyte used in the mobile phase is composed of a chromophoric countercation and chromophoric counteranion. When this chromophoric eluent electrolyte passes through the column the ion exchange process between the chromophoric countercation and counteranion, AB, and the analyte cation and anion, MX, takes place as shown in eqns. 1 and 2, respectively,

$$R^-M^+ + B^+ \rightleftharpoons R^-B^+ + M^+ \tag{1}$$

$$R^+X^- + A^- \rightleftharpoons R^+A^- + X^- \tag{2}$$

where R is the anionic or cationic ionogenic group on the stationary phase. If the effluent is monitored where both A and B absorb and the mobile phase background absorbance due to AB is electronically offset by the detector, the chromatographic peaks for M and X will appear as negative peaks (absorbance decrease) when each passes through the detector. In general, background absorbance should be less than 0.8 providing the absorbance detector used has a favorable offset capability [8,11,12].

In order to obtain a favorable simultaneous detection of analyte cations and anions using IPD and a MBIE column, eluent counterions must meet several requirements: (1) they must be chromophoric and both must have an appreciable absorptivity, (2) they must provide appropriate eluting power, and (3) they must have a reasonable solubility and dissociation at the mobile phase pH required for the separation. Two mobile phase eluent electrolytes that meet these requirements and were chosen to be used in this study were cerium nitrate and BTMA–PTS. Both of these electrolytes also have the property whereby both the eluent countercation and counteranion will absorb at the same wavelength, thus permitting the use of a single wavelength type absorbance detector.

For the case where both the eluent countercation and counteranion absorb at the same wavelength the total absorbance (A_t) indicated by the detector when set at the wavelength of absorbance is given by eqn. 3,

$$A_t = a_m^+bc^+ + a_m^-bc^- \tag{3}$$

where a_m^+ and a_m^- are the molar absorptivities of the chromophoric countercation and counteranion, respectively, c^+ and c^- are the respective concentrations, and b is the detector cell path length. When an analyte cation and anion travel through the column the concentration of the chromophoric ion of the same charge decreases in the analyte peak as each emerges from the column (see eqns. 1 and 2). Because the analyte cation and anion displace an equivalent amount of chromophoric cation and anion in the mobile phase, respectively, negative peaks are obtained. For the case where an analyte cation displaces an equivalent amount of chromophoric cation in the mobile phase, the concentration of the chromophoric cation decreases while the concentration of the chromophoric anion and its contribution to absorbance is constant. For an analyte anion the concentration of the chromophoric anion would decrease causing an absorbance decrease while the concentration of the chromophoric cation and its contribution to absorbance is constant. In both cases, therefore, a linear relationship exists between the change in absorbance and the change in the concentration (of analyte cation and anion, respectively) according to Beer's law providing the ion-exchange processes are maintained well below a column overload condition. In all the experiments reported here the analyte ion sample sizes were always below 0.17 μequiv., which is well below the exchange capacity for each type of ion exchange site (see Table I), and in a region where capacity factor is independent of column loading.

Three kinds of MBIE columns were studied (see Table I). The Polyion and Zorbax columns are polymeric and silica based, respectively, and were packed as intimate mixtures of anion and cation exchangers. The polymer based exchangers have several unique features: (1) these exchangers can be prepared to vary widely in ion exchange capacity, and (2) if ion exchange capacity is low enough the low capacity exchanger can participate in reversed-phase interaction in addition to ion exchange at the polymeric matrix surface, thus providing an additional mode of interaction which is potentially useful when separating ionic and non-ionic samples and/or lipophilic type analytes [5]. The Polyion and Zorbax columns can be prepared by mixing anion and cation exchangers to obtain different anion–cation exchange ratios either by mixing different weights of the two exchangers or by mixing equal weights of the two exchangers that differ in exchange capacity. The Dionex MBIE columns are unique in that these stationary phases are not an intimate mixture of exchangers but rather are composed of a latex containing either sulfonated or quaternary ammonium groups that is deposited and held via electrostatic forces on a polystyrene–divinylbenzene core containing either quaternay ammonium or sulfonated groups, respectively. While the ionogenic group located on the latex layer will be the larger of the two ion exchange capacities (see Table I) both anion and cation exchange sites are available.

Fig. 1 shows that the retention of both analyte cations and anions on the two Dionex columns decreases as mobile phase BTMA–PTS concentration increases typical of mass action effects of eluent counterion on ion exchange. In all cases IPD at 254 nm, where both $BTMA^+$ and PTS^- absorb, was used to detect analyte cations and anions, respectively. In Fig. 1A where a Dionex HPIC-CS5 column was used, anions have lower retention than cations because the cation-exchange capacity is in excess (see Table I) and monovalent cations have lower retention than monovalent anions (see Fig. 1B). In these studies peak identification was confirmed by using different salts as analytes. For example, Na^+, K^+, Br^- and Cl^- peak assignments were based on the comparison of chromatograms obtained by using NaCl, NaBr,

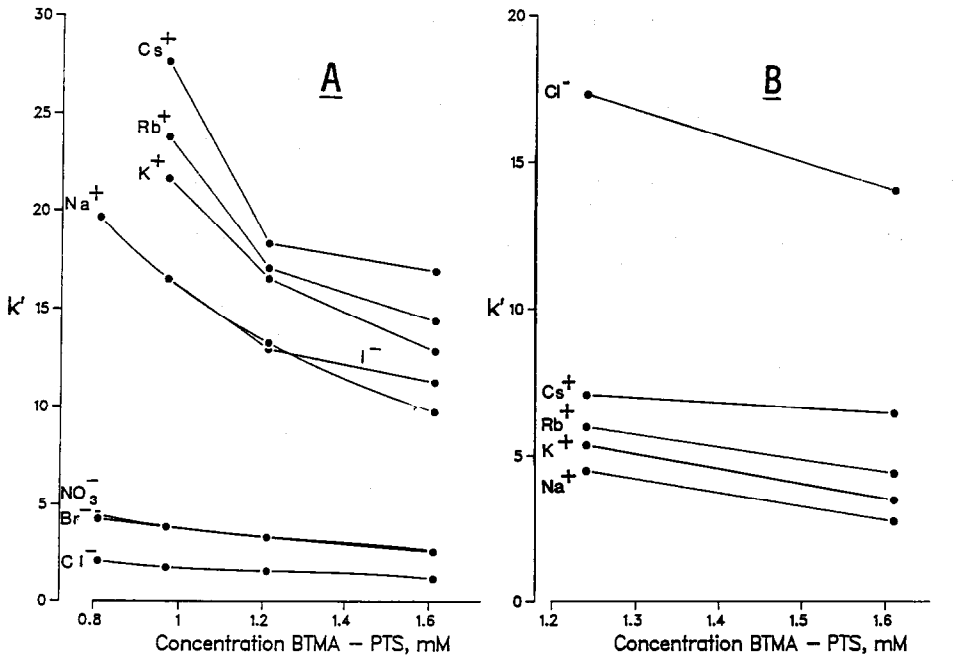

Fig. 1. Effect of BTMA-PTS concentration on the retention of analyte cations and anions on the Dionex columns. (A) Dionex HPIC-CS5 column and aqueous BTMA–PTS eluent at 1.0 ml/min with IPD at 254 nm, (B) Same as (A) except Dionex HPIC-AS7 column.

KCl and KBr as analytes. Other salts were also used to confirm peak assignment.

Fig. 2 shows a separation of anions and cations on the Dionex HPIC-CS5 column. BTMA–PTS mobile phase concentration can be reduced to improve anion resolution, however, if this is done elution time for cations is much longer. For a HPIC-AS7 column (see Fig. 1B) where anion exchange capacity is higher, analyte cations elute early and analyte anions are resolved at higher retention times depending on mobile phase BTMA–PTS concentration.

Calibration curves based on peak area were determined for both Na^+ and Cl^- on a Dionex HPIC-CS5 column using NaCl as an analyte. The range covered 29 to 3630 ng for Na^+ and 45 to 5620 ng for Cl^- in a 20-μl injection volume; no attempt was made to determine the upper limit of linearity. Detection limits for Na^+ and Cl^- using IPD and a Beckman M160 fixed-wavelength detector at 254 nm was about 31 and 3.0 ng, respectively, for a signal-to-noise ratio of 3:1. Correlation coefficients over the range studied exceeded 0.999 for both ions.

An eluent containing $Ce(NO_3)_3$ provides a countercation of high cation-exchange selectivity due to charge and a counteranion of modest anion-exchange selectivity. Both absorb at the same wavelength and IPD of separated analyte cations and anions is possible. Fig. 3 shows how mobile phase concentration of $Ce(NO_3)_3$ influences analyte cation and anion retention on a HPIC-CS5 column. Monovalent anions are more highly retained than the divalent cations because of the favorable cation-exchange selectivity exhibited by the trivalent cerium. Fig. 4 illustrates the separation

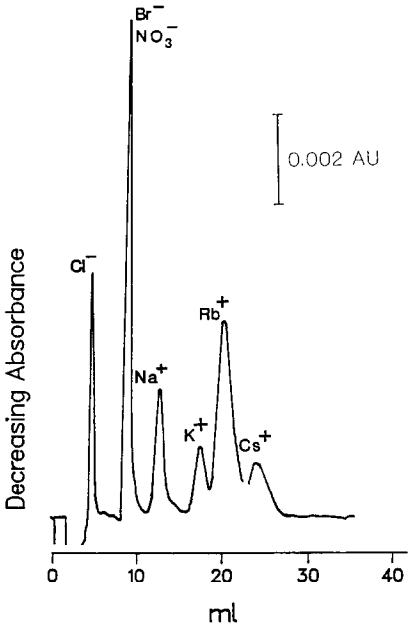

Fig. 2. Simultaneous separation and IPD of analyte cations and anions. Dionex HPIC-CS5 column and aqueous 1.31 m*M* BTMA–PTS eluent at 1.0 ml/min with IPD at 254 nm.

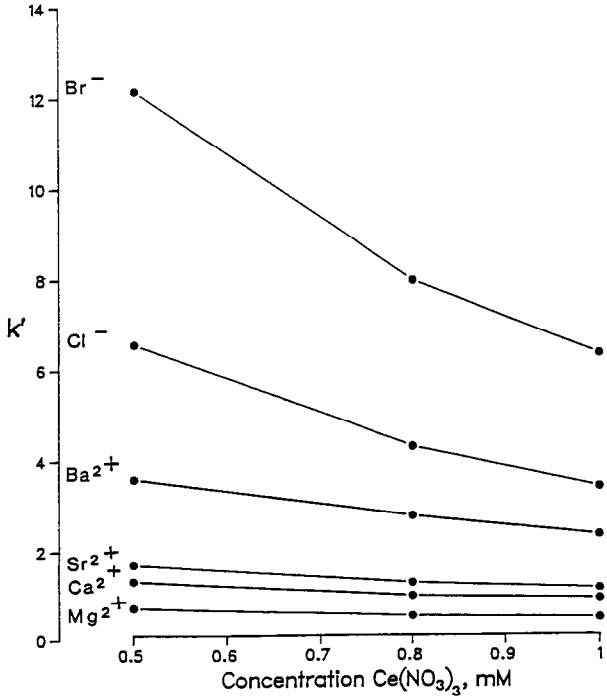

Fig. 3. Effect of $Ce(NO_3)_3$ on retention of analyte cations and anions on a Dionex column. Dionex HPIC-CS5 column and aqueous $Ce(NO_3)_3$ eluent at 1.0 ml/min with IPD at 240 nm.

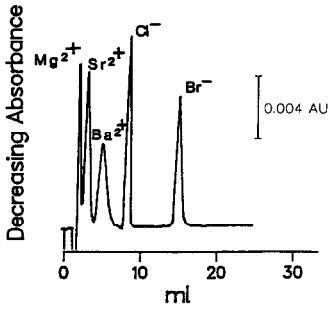

Fig. 4. Simultaneous separation of divalent cations and anions. Conditions are the same as in Fig. 3 except 0.50 m*M* $Ce(NO_3)_3$.

of alkaline earth and common halide ions on the HPIC-CS5 column using $Ce(NO_3)_3$ as the eluent and IPD at 240 nm.

When polymeric cation and anion exchangers (Polyion columns in Table I) and silica-based cation and anion exchangers (Zorbax column in Table I) were each mixed and packed into a column, analyte cations and anions could be separated on each of the columns. If cation-exchange capacity is in excess over anion-exchange capacity, retention of analyte cations is higher than analyte anions when compared to the reverse situation. In both columns BTMA–PTS and $Ce(NO_3)_3$ eluents could be used for the elution of cations and anions and for their indirect detection. Fig. 5, for example, shows how retention of analyte cations and anions decreases on a Polyion column where cation- and anion-exchange capacity are equal as mobile phase BTMA–PTS concentration increases. Since Polyion and Zorbax column efficiencies are lower than for the Dionex columns (see Table I) baseline separation of complex mixtures are more readily obtained on the Dionex columns.

Separations on the Polyion and Zorbax columns can also be improved by increasing column length [see Fig. 5 where capacity factors (k') for analyte cations and anions can be compared]. No attempt was made to optimize column length for the Polyion and Zorbax columns. The 7.0-cm length was chosen because of a limitation in the availability of the stationary phases and interest in studying the effects of exchange capacity on retention. This could only be easily done by preparing limited quantities of polymeric ion exchangers that differ in exchange capacities.

Fig. 6 illustrates two typical separations on the short 7.0-cm Polyion-2 (Fig. 6A) and Zorbax (Fig. 6B) column using a $Ce(NO_3)_3$ and a BTMA–PTS eluent. In Fig. 6A analyte cation retention is sharply reduced due to the $Ce(NO_3)_3$ eluent and analyte cations are eluted before analyte anions even though the cation-exchange capacity is twice the anion exchange capacity (see Table I). A Mg^{2+} and Ca^{2+} mixture would be separated similarly as Sr^{2+} and Ba^{2+} but at lower retention time. If all four cations are present only partial resolution is obtained because of the column efficiency and the short 7.0-cm column. For the Zorbax column (Fig. 6B) anion-exchange capacity is over seven times the cation-exchange capacity (see Table I) and analyte cations elute rapidly when the eluent BTMA–PTS concentration is increased in order to elute analyte anions at lower retention times. If BTMA–PTS mobile phase concentration is reduced resolution of the early eluted analytes is improved.

When the Polyion-2 column (see Table I) was used to prepare a peak area

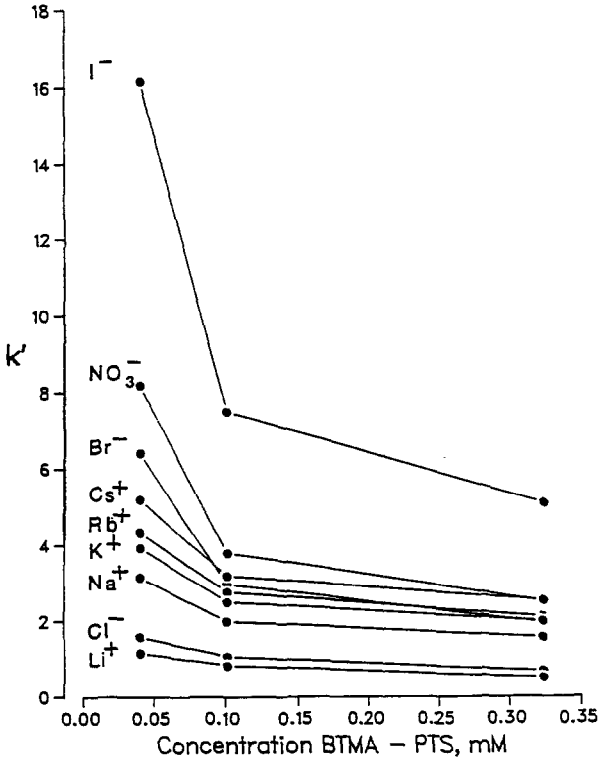

Fig. 5. Effect of BTMA–PTS concentration on the retention of analyte cations and anions on a Polyion column. Polyion-1 column and aqueous BTMA–PTS eluent at 1.0 ml/min with IPD at 254 nm.

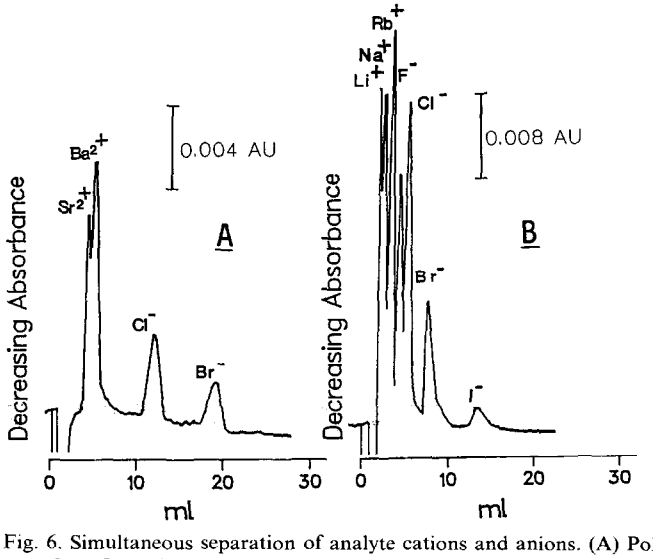

Fig. 6. Simultaneous separation of analyte cations and anions. (A) Polyion-2 column and aqueous 0.25 m*M* $Ce(NO_3)_3$ eluent at 1.0 ml/min with IPD at 240 nm. (B) Zorbax column and aqueous 1.61 m*M* BTMA–PTS eluent at 1.0 ml/min with IPD at 254 nm.

calibration curve for $CaCl_2$ as an analyte and aqueous 0.50 m*M* $Ce(NO_3)_3$ as an eluent, linear curves were obtained for both Ca^{2+} and Cl^- over the mass range studied (182 to 3630 ng for Ca^{2+} and 324 to 6460 ng for Cl^-) in a 20-μl injection volume. Correlation coefficients were greater than 0.998 for each. Detection limits for Ca^{2+} and Cl^{2-} using IPD at 240 nm and a Spectra Physics M770 absorbance detector was about 72 and 39 ng, respectively, for a signal-to-noise ratio of 3:1.

CONCLUSIONS

The experiments indicate that indirect photometric detection is feasible for the simultaneous separation of cations and anions using a mixed-bed ion-exchange column. Since analyte cations and anions can coelute, peak purity must be carefully established. Mobile phase chromophoric electrolyte eluent concentration can be altered within detector offset limits in order to affect analyte retention. If a detector capable of monitoring two different wavelengths is used then chromophoric electrolyte eluents composed of a cation and anion that absorb at different wavelengths can also be used. This provides additional flexibility in manipulating eluent strength for elution of cations and anions and their detection.

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