The Analyst

Preparation of a Macrocyclic Polyamine-bonded Column for the Electrophoretic Separation of Inorganic and Organic Anions

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A 24-membered macrocyclic polyamine based on the propylene-1,3-diamine unit that was able to form polyprotonated, highly charged species in the neutral pH region was selected to act as an anion complexone. It was synthesized and incorporated in a fused-silica capillary for the electrophoretic separation of inorganic and organic anions. This complexing agent can selectively modulate the mobility of anions by forming anion complexes with varying degrees of stability. Parameters which influence capillary electrophoretic separations such as applied voltage, choice of electrolyte anion, electrolyte pH and electrolyte concentration were investigated. A mixture of thiosulfate, chloride, sulfate, selenate, perchlorate, tungstate, carbonate and selenite could be separated in 5 mM sodium chromate at pH 10.0 within 13 min. A mixture of bromide, oxalate, malate, citrate, tartrate, maleate, succinate, acetate, lactate, butyrate, *p*-hydroxybenzoate, salicylate and octanesulfonate could be separated in 10 mM phthalate at pH 5.0 within 11 mins. Under the same conditions, even geometric isomers or mixtures of polycarboxylates and polyphosphates, such as ATP and ADP, could be well separated. The experimental results indicated that incorporating the chelating functional group in the inner wall of the capillary markedly enhances the selectivity of the system.

Keywords: *Macrocyclic polyamine; capillary electrophoresis; covalent surface modification; anion complexation*

Recognition and binding of ionic substrates by organic host molecules are of vital importance in analytical chemistry. Most studies so far have centered on the recognition of alkali and alkaline earth metal cations by such host compounds as macrocyclic polyethers and other types of ionophores. Over the last two decades, much attention has been devoted to the study of anion coordination chemistry. Polyammonium entities have been shown to be good coordinating agents for anions. The selectivity of complexation depends on both electrostatic and structural effects. Dietrich *et al.*[1] reported that polyammonium salts, based on the ethylenediamine pattern, require a more acidic pH for full protonation than those based on the propylene-diammonium unit, to which binding occurs in the neutral pH range. It is well known that some biogenic polyamines such as spermidine and spermine containing amine functions separated by three and four methylene groups bind strongly to nucleotides. Accordingly, a number of polyamines have been synthesized and studied as suitable receptors for biologically significant anions, such as adenosine triphosphate (ATP^{4-}), adenosine diphosphate (ADP^{3-}), adenosine monophosphate (AMP^{2-}) and polycarboxylates occuring in the catabolic tricarboxylate cycle.[2–7]

Currently, nearly 1500 publications in the field of capillary electrophoresis (CE) appear annually, in most of which there is greater emphasis on specific CE applications. Publications relevant to the creation of bonded or adhered phases along the capillary wall, except those in dynamic procedures involving the use of buffer additives, are scarce.[8,9] The chemical status of the surfaces in fused-silica capillaries determines, along with the pH of the running buffer, the electroosmotic flow (EOF) in electrophoretic systems. The EOF is a consequence of the electric double layers that are formed on the capillary surfaces by ionic interactions with the different types of ions contained in the buffer. The chemistry of the surface (*i.e.*, the presence of functional groups or its adsorptivity) strongly influences the formation of the double layers and the zeta potential, which causes the EOF.[8] For the determination of just anions, the separation time can be shortened by reversing the direction of the EOF and the polarities of the electrode. Various quaternary amines have been tested as EOF modifiers to give shorter separation times.[10–13]

We have reported the attachment of chelating functional groups to fused-silica capillaries for the electrophoretic separation of transition metal ions.[14] It would be interesting to know whether incorporating a macrocyclic polyamine with propylene-1,3-diamine unit to the surface of a fused-silica capillary would have any effect on the capillary electrophoresis system. In order to combine the interesting properties of anion coordination and CE, we have developed a method for the surface modification of fused-silica capillaries for the electrophoretic separation of organic and inorganic anions, especially some biologically important anions, such as ADP, ATP, citrate and other carboxylates relating to the catabolic tricarboxylate cycle.

Experimental

Apparatus

A high-voltage power supply with a 30 kV capacity (Model 890-CE, Jasco, Tokyo, Japan), a variable-wavelength UV/VIS detector (Jasco 870-CE) and an integrator (Jasco 807-IT) were employed for CE. The separations were carried out on a fused-silica capillary column with an external coating of polyimide (J & W Scientific, Folsom, CA, USA) and chemically modified with a macrocyclic compound as described below. The modified capillaries were of 100 μm id and were 50 cm long between the injection end and the detection window. Indirect UV absorbance detection was performed at 250 nm. Sample injection was achieved by electromigration.

Elemental analyses were carried out with a Perkin-Elmer (Norwalk, CT, USA) Model 2400 elemental analyzer performed by the Elemental Analyses Service Center of NSC at the National Taiwan University. IR spectra were obtained on a Perkin-Elmer Model 983 spectrophotometer. ^1H NMR spectra were measured on a Bruker (Karlsruhe, Germany) AC-200 spectrometer at 200 MHz.

Reagents and Chemicals

Most chemicals were of analytical-reagent grade from Merck (Darmstadt, Germany). Purified water (18 MΩ cm) from a Milli-Q water purification system (Millipore, Bedford, MA,

USA) was used to prepare all solutions. γ-Glycidoxypropyltrimethoxysilane was obtained from Aldrich (Milwaukee, WI, USA). All liquid reagents and solvents used in moisture-sensitive reactions were distilled and collected over type 4Å molecular sieves.

Synthesis of 1,5,9,13,17,21-Hexaazacyclotetraeicosane ([24]ane-N_6)

This compound was prepared by a procedure modified from that of Dietrich *et al*.[5]

Preparation of N,N′*, 4-tri(4-toluenesulfonyl)-4-azaheptanediamine* (***I***)

3,3′-Diaminodipropylamine (20 g) and triethylamine in dichloromethane (300 ml) were placed in a three-necked bottle and vigorously stirred at 50 °C, then 4-toluenesulfonyl chloride (116 g) in dichloromethane (200 ml) was added dropwise to the reaction mixture and refluxed for 6 h. The product was isolated by extraction twice with a mixture of ice–hydrochloric acid and pure water. The mixture was then neutralized with sodium hydrogencarbonate and further treated with the same extractant. The dichloromethane layer was dried with anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by recrystallization from ethanol gave white crystals of **I** (76 g, yield 85%).

Preparation of 4,8,12-tri(4-toluenesulfonyl)-4,8,12-triazapentadecane-1,15-diol (**II**)

The tritosyl derivative of 4-azaheptanediamine (**I**) was the starting material for the two linear parts used in the cyclization step. Compound **I** (20 g) was treated with 3-chloropropan-1-ol (10 g) in *N*,*N*-dimethylformamide in (DMF) in the presence of excess K_2CO_3 (25 g) at 110 °C for 20 h. The product was allowed to cool and isolated from unreacted K_2CO_3. The solvent was evaporated under reduced pressure and the residue was partitioned between water and dichloromethane. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. Purification by silica gel column chromatography with methanol–dichloromethane (1 + 99 v/v) as mobile phase gave a pale yellow oil (**II**) (15 g, yield 30%).

Preparation of 1,15-di(methanesulfonyloxy)-4,8,12-tri(4-toluenesulfonyl)-4,8,12-triazapentadecane (**III**)

Compound **II** (10 g) and triethylamine (8 g) in dichloromethane (200 ml) were placed in a round-bottomed flask and reacted at −18 °C with stirring. Methanesulfonyl chloride (4 g) in dichloromethane (20 ml) was added dropwise and stirring was continued for another 2 h. The material was allowed to come to room temperature and then washed with 1 M sulfuric acid and saturated sodium chloride solution. The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to leave a yellow oil (**III**) (12 g, yield 90%).

Preparation of 1,5,9,13,17,21-hexa(4-toluenesulfonyl)-1,5,9,13,17,21-hexaazacyclotetraeicosane (**V**)

In a 500 ml round-bottomed flask were placed **I** (7 g), sodium hydride (1.86 g) and DMF (150 ml). After stirring for 30 min, the unreacted sodium hydride was removed by filtration. The filtrate (**IV**) was heated at 100 °C, then **III** (10 g) in DMF (150 ml) was added dropwise over 1 h and heating was continued for further 2 h. The mixture was stirred overnight, the solvent was then evaporated under reduced pressure and the mixture was partitioned between dichloromethane and 1 M HCl. The organic layer was dried over $MgSO_4$. Evaporation gave the crude product. The product (**V**) was purified by chromatography on silica gel with methanol–dichloromethane (2 + 98 v/v). Recrystallization was from dichloromethane–absolute ethanol (5 g, yield 35%).

Preparation of 1,5,9,13,17,21-hexaazacyclotetraeicosane

Compound **V** (2 g), phenol (2.85 g) and a mixture of HBr and acetic acid (33%, 50 ml) were placed in a round-bottomed flask and heated at 80 °C for 14 h. The mixture was cooled to room temperature. After evaporation, the residue was partitioned between water and dichloromethane. The water layer was concentrated under reduced pressure. Purification by ion-exchange chromatography on Dowex 1-X8 followed by recrystallization from ethanol in hydrochloric acid solution gave white crystals of [24]ane-N_6 (0.8 g, yield 85%).

Coating of Capillaries with γ-Glycidoxypropyltrimethoxysilane

Fused-silica capillaries (100 μm id, approximately 80 cm long) were first rinsed with 1 M KOH (30 min), then pure water (15 min), 1 M HCl (15 min) and pure water (15 min). The capillaries were purged with nitrogen for 20 min, then dried at 110 °C overnight. For coating, the capillary was filled with a 10% w/v solution of γ-glycidoxypropyltrimethoxysilane in toluene. The capillary was kept for 3 h at 110 °C for silanization. After purging with toluene to remove unreacted reagent for several minutes, the capillaries were dried in a vacuum oven. The capillary was then filled with a 10% w/v solution of the macrocyclic compound [24]ane-N_6 in DMF. After standing for 10 h at 120 °C for functionalization, the dried capillaries were purged with ethanol and pure water for several minutes before equilibration with buffer solution. They were then ready for use.

Sample Preparation

Real samples were purchased at a supermarket. They were diluted to appropriate concentrations with pure water and passed through a 0.45 μm membrane filter.

Results and Discussion

Characterization

Procedures for preparing a new capillary column are outlined in Fig. 1. The presence of functional groups in the capillaries was confirmed from their IR data. For measuring IR spectra, the external coating of polyimide was removed prior to grinding, then a higher ratio of coated capillary to potassium bromide than

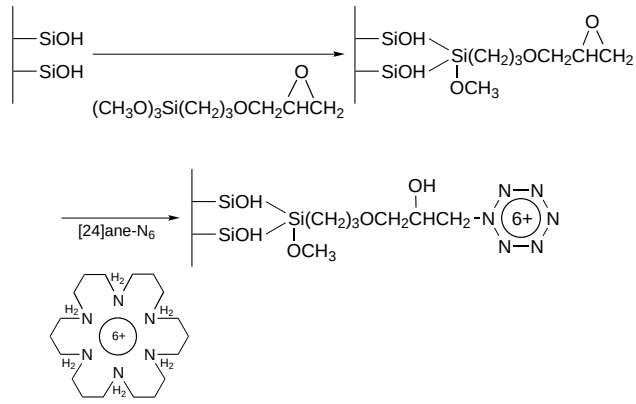

Fig. 1 Procedures for covalent surface modification of the fused-silica capillary column.

the conventional one was used for blending. Specific evidence of covalently bound [24]ane-N_6 is provided by IR absorption due to the N–H stretching (3450 cm^{-1}) and a more intense C–H bending (2926 cm^{-1}). Since the nature of the chemical group bound to the column surface, the degree of surface deactivation and the phase thickness determine the dependence of the EOF on pH, and also the direction of the flow, quantification of the EOF was used to measure the effectiveness of bonding procedures.[8]The EOF velocities of both modified and bare fused silica were determined according to the migration time of benzyl alcohol, which served as a neutral marker.[15] The results are shown in Fig. 2. At pH > 4, the numbers of surface silanols on the bare fused-silica capillary decreases as the pH increases. Consequently, the migration velocity of benzyl alcohol increases as the pH increases. By chemical modification, the direction of the EOF with capillary bonding with the [24]ane-N_6 group is reversed and the migration velocity increases as the pH decreases. Here the sample injection was made from the negative end. This can be accounted for by the finding from silica modification that mainly the amino groups of the macrocyclic compound are protonated at a lower pH value.

Separation Conditions

The aim of this study was to develop a system appropriate for the determination of nucleotides or carboxylates in biological samples with the aid of an anion coordination mechanism, so both inorganic and organic anions were investigated with the prepared capillary column. Preliminary tests showed that increasing the applied voltage only slightly improved the resolution and a smaller difference in migration time was observed, so an applied voltage of 15 kV was used throughout this work.

Sodium chromate was chosen as a background electrolyte, providing suitable UV absorbance and matching the ionic mobility of inorganic anions[16,17] (Table 1). As summarized in Fig. 3, the mobilities are significantly influenced by the electrolyte pH, although the variation at pH 5.7–7.0 is smaller than in other regions. The stabilities in this pH range may be attributed to full protonation,[6] since the protonation constants ($\log K_a$) of the monomeric [24]ane-N_6 are 6.60, 7.15, 7.90, 9.05, 10.35 and 10.45.[18]

The influence of the sodium chromate concentration on the mobilities of anions and their resolutions was studied over the range 2.5–10 mM at pH 9.0. At sodium chromate concentrations above 2.5 mM acceptable peak shapes were achieved. The mobilities of anions decreased when higher sodium chromate concentrations were used, but poorer resolutions were observed at concentrations more than 5 mM. Therefore, a sodium chromate concentration of 5 mM was used to achieve optimum resolution.

Table 1 Molecular masses, dissociation constants, effective electric charges and equivalent conductances of various analytes

Analyte	M_r*	pK_a	Effective charge pH 5.0	Effective charge pH 8.3	Equivalent conductance†
Oxalate	90	1.23, 4.19	1.866	2.000	74.1
Malate	134	3.40, 5.11	1.417	1.999	58.8
Citrate	192	3.14, 4.77, 6.39	1.655	2.988	70.2
Tartrate	150	2.98, 4.34	1.817	2.000	64.0
Maleate	116	1.91, 6.33	1.043		61.9
Fumarate	116	3.10, 4.60	1.709		61.8
Succinate	118	4.16, 5.61	1.073	1.997	58.8
Acetate	60	4.75	0.640	1.000	40.9
Lactate	90	3.86	0.988		38.8
Butyrate	88	4.82	0.602		32.6
p-Hydroxy-benzoate	138	4.48	0.724		31.4
Salicylate	138	2.97	0.991		36
Octanesulfonate	209		2		29
Thiosulfate	112		2		85.0
Bromide	80		1		78.1
Chloride	35.5		1		76.35
Sulfate	96		2		80.0
Selenate	143	−3, 1.66	2		75.7
Perchlorate	99.5		1		67.9
Tungstate	248	3.5, 8.1	1.988‡		69
Carbonate	60	6.36, 10.33	1.318‡		72
Selenite	127	2.64, 8.27	1.982‡		

* The molecular masses for organic acids are for acids in the hydrogen form. † Limiting equivalent ionic conductance (mho cm^2 $equiv.^{-1}$): data from refs. 16 and 17. ‡ At pH 10.0.

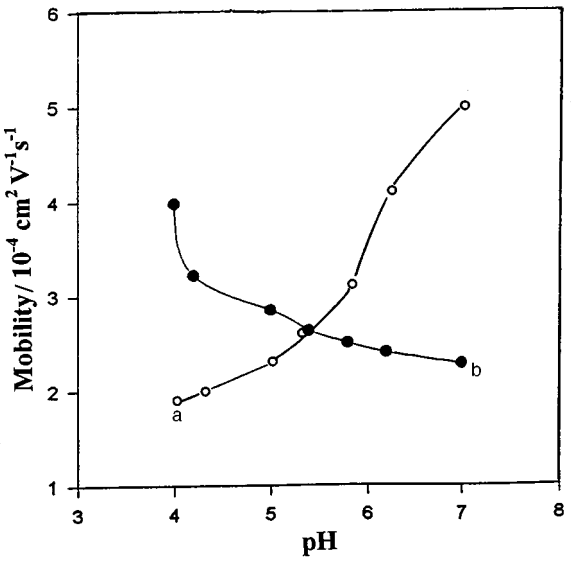

Fig. 2 Effect of pH on electroosmotic flow mobility in various capillary materials. Background electrolyte, sodium chromate (5 mM). Curve a, untreated fused-silica capillary; curve b, covalent surface modification with polyamine, [24]ane-N_6 fused-silica capillary.

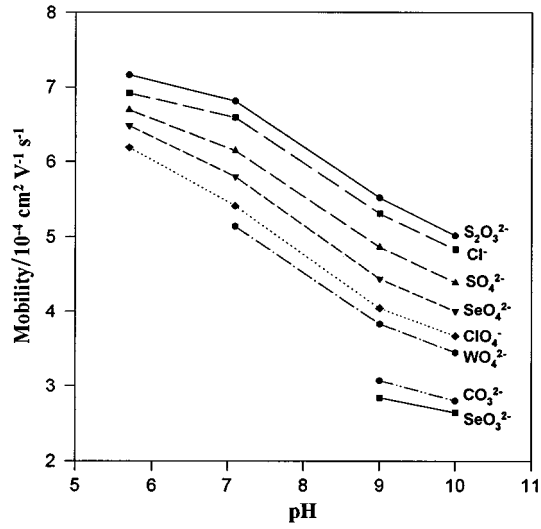

Fig. 3 Migration time of anions as a function of chromate electrolyte acidity. Column, covalent surface modification with polyamine, [24]ane-N_6 fused-silica capillary (50 cm × 0.1 mm id); electrolyte, sodium chromate (5 mM); applied voltage, −15 kV; sample concentration, 10^{-5} M each. Injection, electrokinetic for 5 s at −15 kV; detection: indirect UV at 250 nm.

Some important inorganic anions in biological systems, such as selenite, selenate, tungstate and carbonate, were also examined. As more than one species of these weak acids would exist at lower pH (Table 1), a background electrolyte pH greater than 8.5 was used to simplify the separation conditions. Fig. 4 shows a typical electropherogram of the inorganic anions in 5 mM chromate at pH 10.0. Under these conditions, the mobility of analytes decreases in the following order: thiosulfate > chloride > sulfate > selenate > perchlorate > tungstate > carbonate > selenite. With the untreated fused-silica capillary under the same conditions, no separation was observed. Only one peak appeared at around 5 min (with the sample injection from the positive to the negative end) within a working time of 40 min, whereas no peak was found (with the sample injection from the negative to the positive end) within a working time of 20 min. According to the published thermochemical radii of polyatomic ions,[19] chloride 181, sulfate 244, selenate 235, perchlorate 226, carbonate 164 and selenite 225 pm, the results showed that both electrostatic forces toward the macrocyclic ligand and ionic mobility of each anion (Table 1) determined the migration order.

Efficient separations in CE can only be achieved when the mobilities of samples and buffer components are similar.[20] Therefore, potassium hydrogenphthalate (KHP) was chosen as the background electrolyte for the separation of organic anions. At pH below 7, [24]ane-N_6 is fully protonated, but only above pH 4 are most carboxylates sufficiently ionized for the formation of anion–ligand complexes. In order to form a sufficiently stable anion complex, the separations were studied only over the pH range 4–7. However, curves with a zig-zag shape were obtained (Fig. 5). The reason might be due to the large ranges of pK_{a1} from 1.3 to 4.2 and of pK_{a2} from 5.2 to 6.2 for the carboxylates (Table 1), so various effective charges of these acids would exist under the same conditions.

Altering the concentration of KHP over the range 2–15 mM affected the migration times of most of the organic acids (Fig. 6). The results reveal that the migration velocity of all of the anions decreased with increasing concentration of KHP. Although a higher sensitivity was brought about by increasing the KHP concentration, there was also greater background noise. From the peak resolution calculated for different concentrations of KHP, the optimum approach to separating the organic anions was in 10 mM electrolyte buffer.

Fig. 7(*a*) shows a typical electropherogram of organic acids in 10 mM phthalate at pH 5.0. Where *p*-hydroxybenzoate and salicylate provide negative signal, the reason is due to both compounds displaying a UV absorbance at 250 nm which is larger than that of the background. Under these conditions, the mobility of the analytes decreases in the following order: bromide > oxalate > malate > citrate > tartrate > maleate > succinate > acetate > lactate > butyrate > *p*-hydroxybenzoate > salicylate > octanesulfonate. The faster migrating species are well separated. However, malate, citrate, tartrate, maleate and succinate are not completely baseline resolved, and the elution order does not correspond to the magnitude of the effective charge and molecular mass (Table 1). Here comparable separations with the untreated capillary and the macrocyclic polyamine-coated capillary were achieved. For the untreated column under the conditions in Fig. 7(*a*), only one peak appeared for the carboxylate mixture at 10.57 min within 30 min

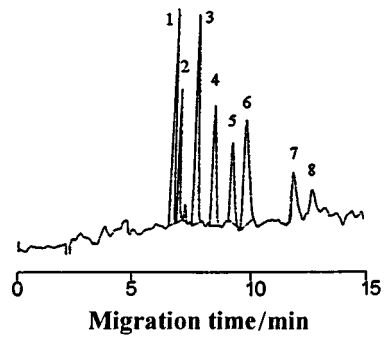

Fig. 4 Electropherogram for the separation of inorganic anions. Column, covalent surface modification with polyamine, [24]ana-N_6 fused-silica capillary (50 cm × 0.1 mm id); electrolyte, sodium chromate (5 mM, pH 10.0); applied voltage, −15 kV; sample concentration, 10^{-5} M each; injection, electrokinetic for 5 s at −15 kV; detection, indirect UV at 250 nm. Peaks: 1, thiosulfate; 2, chloride; 3, sulfate; 4, selenate; 5, perchlorate; 6, tungstate; 7, carbonate; 8, selenite.

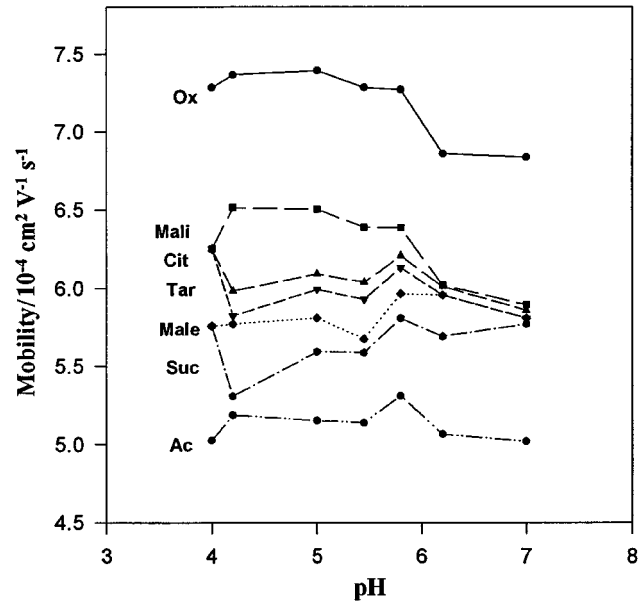

Fig. 5 Migration time of anions as a function of phthalate electrolyte acidity. Column, covalent surface modification with polyamine, [24]ane-N_6 fused-silica capillary (50 cm × 0.1 mm id); electrolyte, potassium hydrogenphthalate (10 mM); applied voltage, −15 kV; sample concentration, 10^{-5} M each. Injection, electrokinetic for 5 s at −15 kV; detection, indirect UV at 250 nm.

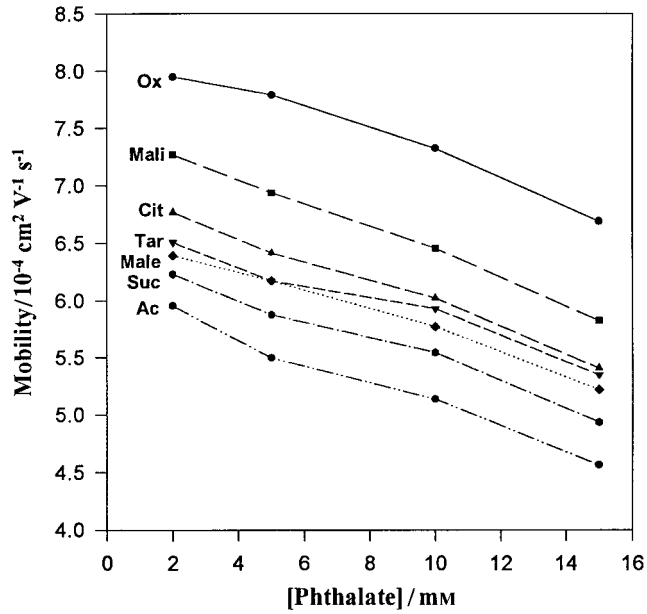

Fig. 6 Migration time of anions as a function of phthalate concentration. Conditions as in Fig. 5 except phthalate at pH 5.0.

of the separation time (with the sample injection from the positive end); only one peak appeared at 19.55 min within the same time with the sample injection from the negative end. According to the formation constants[1] listed in Table 2 and the charge density of the tested analytes, the assumption of anion complexation in addition to EOF in the separation mechanism seems rational.

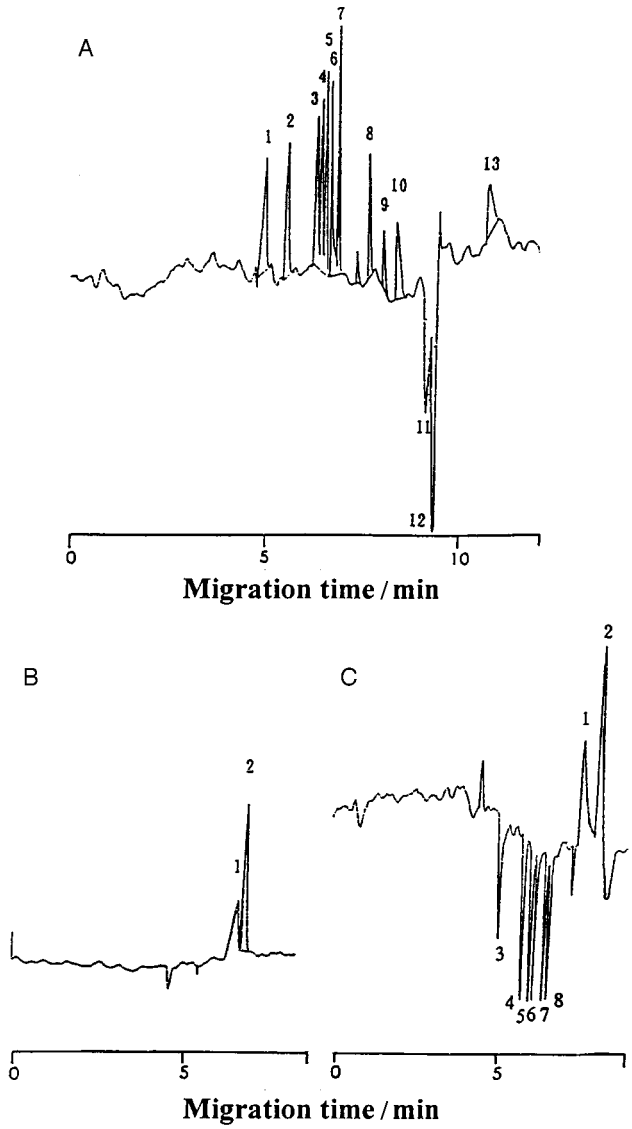

Fig. 7 Electropherograms for the separation of anions. Conditions as in Fig. 5, except electrolyte, potassium hydrogen phthalate (pH 5.0, 10 mM). Peaks: A 1, bromide; 2, oxalate; 3, malate; 4, citrate; 5, tartrate; 6, maleate; 7, succinate; 8, acetate; 9, lactate; 10, butyrate; 11, *p*-hydroxybenzoate; 12, salicylate; 13, octanesulfonate; B 1, fumarate; 2, maleate; C 1, adenosine triphosphate (ATP); 2, adenosine diphosphate (ADP); 3, oxalate; 4, malate; 5, citrate; 6, tartrate; 7, maleate; 8, succinate.

Table 2 Equilibrium constants for complex formation with monomeric polyammonium macrocycle ([24]ane-N_6) and anions (data from ref. 1)

Anion	Log K_f	Anion	Log K_f
$Sulfate^{2-}$	4.0	$Fumarate^{2-}$	2.2
$Oxalate^{2-}$	3.8	$Citrate^{3-}$	4.7
$Succinate^{2-}$	2.4	ADP^{3-}	6.5
$Tartrate^{2-}$	2.5	ATP^{4-}	8.9
$Maleate^{2-}$	3.7		

The geometrical isomers fumarate and maleate, with similar ionic mobilities, can also be separated with this system. The stronger binding of maleate than fumarate (Fig. 7B), corresponds to the order of stability constants between monomeric [24]ane-N_6 and the anions listed in Table 2.[1] Although the effective charge on fumarate is larger than that on maleate, the selectivity more or less might be due to the higher charge density of maleate (*cis* form) than that of fumarate (*trans* form). In other words, the structural effects obviously dominate electrostatic interactions between the analytes and the coated ligand.

The complexation of nucleotide phosphate polyanions toward the prepared capillary column was also studied, since they would form complexes of much higher stability with the present macrocyclic polyammonium salts than with the acyclic tetraammonium ligand spermine.[21] Under optimum conditions, separation of a polycarboxylate and polyphosphate anion mixture can be achieved within 8 min with 10 mM phthalate at pH 5.0. The separation is shown in Fig. 7C. The slower migration of ADP and ATP than that of the carboxylic acids might be due to the stronger binding of ADP and ATP toward the polyammonium macrocycle, [24] ane-N_6, bound to the capillary surface. Huang *et al.*[22] reported that the monophosphate ribonucleotide AMP requires more than 2 h, the diphosphate ribonucleotide ADP requires more than 1 h and ATP requires more than 45 min to leave an uncoated column under the conditions of 50 mM TRIS–100 mM phosphate (pH 6.0) and an applied voltage of −9 kV with a 57 cm × 75 μm id column, owing to the adsorption of the active group on the surface of untreated fused-silica capillary. In this work, both ATP and ADP can be separated and leave the macrocyclic polyamine-coated column within 8 min. This is further evidence of the successful coating.

Further, we tried to improve the separation efficiency with the addition of various amounts of ethanol in the range 0–15% v/v. However, it was found that the separations for both inorganic and organic anions did not vary significantly with organic modifier content, and the variation was within the experimental error.

Calibration and Detection Limits

The calibration graphs of peak area against anion concentration are given in Table 3 with the respective regression coefficients. Linearity applies over the range 10^{-5}–10^{-6} M and the detection limit based on three times the signal-to-noise ratio is 20–60 fmol. The relative standard deviations (RSD) of the migration times (five measurements) is < 2.0% and those of the peak area and peak height are both < 5.0%. The results from column to column exhibited larger RSDs than those within a single run (Table 3). The results are rational. Moreover, the RSDs are not larger than 6.5%. We attribute the success to there being two steps only in the preparation of the covalent surface-modified capillary column. Excellent resolution for the determination of both biologically and environmentally important selenium species was achieved utilizing 5 s electrokinetic injections at −15 kV. Calibration curves with selenate yielded a stronger detector response than selenite.

Analytical Application

In order to evaluate the quantitative performance of the proposed method, various organic acids from commercial beverage samples were analyzed. An appropriate dilution of the samples was made before CE analysis. The concentrations of the organic acids of interest for these samples are summarized in Table 4 based on the 90% confidence level.

Table 3 Precision and linearity for various analytes

	RSD (%)			Linearity		
Analyte	t_m/min	Area/10^3μV cm	Height/cm	$a/10^3$ μV cm μM^{-1}	$b/10^3$ μV cm	r
Oxalate	1.75* (4.15)†	4.30 (6.38)	4.50 (5.41)	2.25	0.10	0.9996
Malate	1.71 (3.98)	4.79 (6.12)	2.30 (4.57)	2.69	0.65	0.9985
Citrate	1.85 (5.12)	4.16 (5.84)	0.33 (2.31)	1.78	1.75	0.9980
Tartrate	1.54 (4.54)	3.96 (5.97)	1.25 (2.44)	1.87	0.11	0.9988
Maleate	1.95 (5.68)	4.92 (6.49)	4.96 (5.22)	1.41	0.33	0.9944
Succinate	1.68 (3.75)	2.65 (4.75)	3.52 (4.93)	1.96	0.79	0.9989
Acetate	1.76 (4.23)	2.87 (4.64)	3.65 (5.12)	2.15	0.78	0.9980
Thiosulfate				19.6	−3.3	0.9996
Sulfate				18.7	0.8	0.9990
Chloride				14.1	−2.0	0.9920
Selenate				5.7	−2.3	0.9989
Tungstate				2.9	−1.4	0.9987
Perchlorate				2.1	−0.7	0.9978
Carbonate				1.3	−0.3	0.9990
Selenite				0.8	0.1	0.9990

* Within-run; No. of measurements = 5. Sample concentration, 1×10^{-5} M. † Column-to-column; No. of measurements = 3. Sample concentration, 1×10^{-5} M.

Table 4 Determination of carboxylic acids in beverages by [24]ane-N_6 bonded-phase capillary electrophoresis. No. of measurements = 4; confidence level = 90%. Capillary column, 50 cm × 0.1 mm id; electrolyte, phthalate (10 mM, pH 5.0); separation voltage, −15 kV; electrokinetic injection, −15 kV, 5 s; detection, indirect UV at 250 nm; sample, 1 : 1000 dilution of apple juice and orange juice and 1 : 100 dilution of lemon tea.

	Concentration/M		
Analyte	Lemon tea	Orange juice	Apple juice
Citrate	$(3.22 \pm 2.24) \times 10^{-2}$	$(1.83 \pm 1.47) \times 10^{-2}$	$(1.74 \pm 1.59) \times 10^{-1}$
Tartrate		$(2.06 \pm 0.31) \times 10^{-1}$	
Malate		$(1.46 \pm 1.42) \times 10^{-2}$	$(3.10 \pm 2.30) \times 10^{-3}$*

* No. of measurements = 3.

Conclusion

The chemistry of polyazamacrocyclic ligands is of considerable interest since in addition to complexing cationic species, the protonated forms of these ligands can form complexes with anionic substrates. However, it must be noted that not all polyamines are potential candidates for anion complexation at neutral pH. Owing to the low pK_a values of most polyamines, for full protonation anion complexation would be restricted to acidic solutions. In this investigation, most of the data confirm the previous assumption that the selectivity of the system is mainly based on the anion complexation behavior.

For most isocratic anion-exchange separations, short-chain monocarboxylic acids co-elute with early eluting anions such as fluoride and chloride, whereas trivalent anions require gradient elution for a reasonable analysis time. For the CE separation of inorganic anions, an EOF modifier for the reversal of the direction of the EOF is usually needed. In this study, only a simple electrolyte buffer was used. Not only oxyanions but also polycarboxylates and polyphosphates, even species of selenium(IV) and selenium(VI), could be determined within a short time with the proposed system. Moreover, a higher separation efficiency and a wider field of application would be expected with the use of smaller diameter capillary columns.

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