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## Separation of anions by ion chromatography–capillary electrophoresis

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### Abstract

Capillary electrophoresis (CE) with a water-soluble ion-exchange polymer in the background electrolyte is very efficient for the separation of organic and inorganic anions because the ion-exchange selectivity, as well as differences in electrophoretic mobility, can be used for separating sample ions. Poly(diallyldimethylammonium chloride) (PDDAC) was employed for this purpose. A very stable electroosmotic flow was obtained between pH 2.3 and 8.5 due to the strong adsorption of PDDAC onto the capillary wall. The effect of ion exchange on the migration of sample anions and their separation was controlled by varying the concentration of PDDAC, the concentration and the type of salt used in the CE background electrolyte. Addition of organic solvent (e.g., acetonitrile) could also modify the sample migration and the separation. Baseline separations were obtained for anions with very similar mobilities, such as bromide and iodide, naphthalenesulfonates, and bi- and tricarboxylic acids. Typical separation efficiencies were between 195 000 and 429 000 theoretical plates per meter. Ten replicate separations gave an average RSD of 1.0% for migration times of the sample anions studied. Excellent separations were obtained for a variety of samples, including a separation of 17 inorganic and organic anions in less than 6 min. © 2000 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Since its introduction in the late 1970s, ion chromatography (IC) has become the dominant analytical method for separating and determining inorganic anions and small organic anions [1–8]. Separations in IC are based on differences in the affinity of sample anions for the exchange sites on a solid anion-exchange material in the separation

column. Movement of the sample anions along the column is caused by pumping a mobile phase containing a competing anion through the column. Samples containing several anions can usually be resolved satisfactorily by IC.

Capillary electrophoresis (CE) offers significantly higher separation power for anions [8–15]. As many as 36 anions have been separated within a very few minutes by CE [9]. These separations are based on differences in the electrophoretic mobilities of the sample anions. Anions with almost identical mobilities, such as bromide and iodide, cannot usually

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be separated by CE. A number of background electrolyte (BGE) additives have been used to modify the mobilities of anions and thereby improve their separation. These include cationic surfactants [10,16–18], polyvalent metal cations [19] and various cyclodextrins [20].

Polymers with ionic groups as well as surfactants have been used to modify CE behavior by coating the inner walls of fused-silica capillaries. For example, a capillary coated with polybrene gives the capillary surface a positive charge and thus impedes interaction of positively charged proteins with the walls. Wang and Dubin [21] found that an aqueous solution of poly(diallyldimethylammonium chloride) (PDDAC) of sufficiently high molecular mass could be used to give a stable polymeric coating to fused-silica capillaries. The positive surface reversed the direction of electroosmotic flow (EOF) in CE and gave a flow that was reproducible to  $\pm 2\%$  for 25 successive runs. The EOF was essentially constant over a broad pH range. The main thrust of this research was to prevent surface adsorption of proteins in CE separations.

Nutku and Erim [22] described a simple way to form a stable coating of polyethyleneimine (PEI) on fused-silica capillaries. At pH values where the PEI is protonated the surface becomes positive and the polymer can act as an anion exchanger. The ion-exchange properties of the coated capillary were demonstrated by a CE separation of inorganic anions in which the migration behavior was different from that attributable to electrophoretic mobilities alone. Interestingly enough, the separation of anions was not nearly as good when 0.1% PEI was added to the BGE as it was with the coated capillary alone.

Terabe and Isemura [23,24] described a separation method which they called ion-exchange EKC in which a soluble ionic polymer was added to the BGE in CE. Of several polymers tried PDDAC and polybrene proved to be the most effective. The usual electrophoretic migration was modified by the analyte anions forming ion-pairs of varying stability with the ionic polymer. These authors developed the basic theory of this technique and demonstrated its effectiveness in separating various organic anions. Cassidy and coworkers [25–27] applied a similar technique for the separation of several inorganic anions using indirect photometric detection.

The ability to combine ion-exchange and electro-

phoretic components into a single separation method is attractive because these components often have different migration orders. Thus, in IC the migration order of the halides is  $F^- > Cl^- > Br^- > I^-$ , while the migration order in CE is  $I^-, Br^- > Cl^- > F^-$ . To realize the full potential of the method it must be possible to vary the ion-exchange component anywhere from a strong effect to a weak effect. Modulation of the ion-exchange effect can be achieved by varying the concentration of ion-exchange polymer in the BGE or by manipulating any of several other experimental parameters.

We believe that the incorporation of a soluble ionic polymer into the BGE of a CE setup holds great promise for ion analysis and is therefore a technique worthy of further development. Although previous publications laid out the basic framework of IC–CE, the effect of BGE salt concentration on the ion-exchange equilibrium was apparently not considered. By varying the anion concentration in the BGE, the interaction of the sample anion with the anion-exchange polymer can be either increased or decreased so that migration times may be kept within a desired range. Contrary to previous expectations, Ding and Fritz [28] reported that practical CE separations are possible in BGE salt solutions as high as 5 M. A moderately high salt concentration (e.g., 100–250 mM) in the BGE was shown to significantly improve peak sharpness, apparently by providing conditions favorable for electrostacking.

The present work describes the separation of both inorganic and organic anions using a cationic polymer, poly(diallyldimethylammonium chloride), abbreviated as PDDAC or simply as  $P^+$ , and an added salt at a moderately high salt concentration. The effects of polymer concentration, pH, type and concentration of added ions, and organic solvents are studied. The scope of separations is more thoroughly delineated. Ion chromatography–capillary electrophoresis (IC–CE) is suggested as a name for this new technique because the separations are the result of both IC and CE components.

## 2. Experimental

### 2.1. Reagents

PDDAC, hexadimethrene bromide (HDM) (also

called polybrene), organic acids and poly(sodium 4-styrene sulfonate) were purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile, boric acid and all inorganic salts, except lithium sulfate, were supplied by Fisher Scientific (Fairlawn, NJ, USA). Lithium sulfate was the product of Sigma (St. Louis, MO, USA).

## 2.2. Separation conditions

All separations were performed on a Waters Quanta 4000 CE system (Milford, MA, USA). Unless otherwise specified, the following conditions were used: uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 40 cm long (effective length 32.5 cm × 50 μm I.D.). Separations were obtained at ambient temperature with a voltage of –10 kV. Direct UV detection was at 214 nm. Hydrostatic injection was 40 s at 10 cm height. Electropherograms were collected at a speed of 15 points/s and plotted by a Chromperfect data acquisition system (Justice Innovations, Mountain View, CA, USA).

All standard and electrolyte solutions were prepared with 18.2 MΩ deionized water from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). Stock analyte solutions were made with deionized water at 5000 ppm, and diluted in 10% buffer solutions to the desired concentration prior to injection. Electrolyte solutions were prepared by mixing lithium sulfate, PDDAC, 20 mM boric acid, and acetonitrile when needed, and adjusting the pH with 2 M sodium hydroxide or 1 M hydrochloric acid. All pH values were measured with a Corning 440 pH meter (Corning, NY, USA) calibrated immediately prior to use.

Each new capillary was conditioned with 0.1 M sodium hydroxide and deionized water for 1 h each. Prior to use, capillaries were rinsed with 0.1 M sodium hydroxide and deionized water for 10 min each, followed by a 30-min rinsing with buffer solution. Between injections, capillaries were automatically purged with buffer for 3 min.

## 3. Theory

Terabe and Isemura [23] derived an equation for the difference in velocity of two ions ( $\Delta v$ ) when a

soluble polymeric anion exchanger is added to the BGE:

$$\Delta v = \frac{(K_2 - K_1)[P^+](v_{\text{free}} - v_p)}{(1 + K_1[P^+])(1 + K_2[P^+])} \quad (1)$$

In this equation,  $K_1$  and  $K_2$  are ion-pair constants,  $[P^+]$  is the polymer ion concentration, and  $v_{\text{free}}$  and  $v_p$  are the electrophoretic velocities of the free analyte ion and polymer ion, respectively. The magnitude of  $\Delta v$  is affected by the differences in  $K_1$  and  $K_2$ , and by the concentration of polymers,  $P^+$ .

The ion-exchange equilibrium between a sample anion ( $A^-$ ) and the polymer ion exchanger ( $P^+Cl^-$ ) is given by the equation



for which the equilibrium constant ( $K$ ) is

$$K = \frac{[P^+A^-][Cl^-]}{[A^-][P^+Cl^-]} \quad (3)$$

At a fixed concentration of  $P^+Cl^-$ , a conditional constant,  $K'$ , may be written as follows:

$$K' = K[P^+Cl^-] \quad (4)$$

Combining Eqs. (3) and (4), and rearranging:

$$\frac{[A^-]}{[P^+A^-]} \frac{[Cl^-]}{K'} \quad (5)$$

The electrophoretic migration rate will depend primarily on the fraction of sample anion that is present as the free anion. This is true because the free anion ( $A^-$ ) will migrate rapidly toward the anode, while the fraction associated with the ion exchanger ( $P^+A^-$ ) will move only very slowly in the opposite direction. The fraction present as  $A^-$  will depend both on the total anion concentration ( $[Cl^-]$  in Eq. (5)) and on the value of  $K'$ , which will be different for each sample anion.

## 4. Results and discussion

### 4.1. Choice of polymer

Preliminary experiments were performed with each of several polymers added to the BGE at a pH value of 8.5. A relatively high concentration (120–150 mM) of a salt, such as sodium chloride or

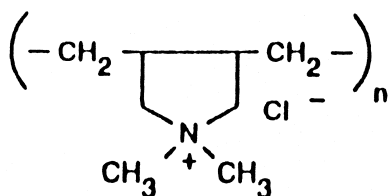


Fig. 1. Structure of PDDAC. Molecular mass: low, 100 000–200 000; medium, 200 000–350 000; high, 400 000–500 000.

lithium sulfate, was found to markedly improve the sharpness of sample anion peaks. PDDAC at a concentration of 0.05 or 0.3% was the most satisfactory of the polymers tested. Its structural formula is given in Fig. 1. A mixture of bromide, iodide, nitrate, nitrite, chromate, thiocyanate and molybdate was baseline resolved in a BGE solution containing 150 mM lithium sulfate, 20 mM borate and 0.05%

PDDAC at pH 8.5 (Fig. 2). This is an exceptionally good and quick separation and shows that the soluble polymer method applies to inorganic as well as organic anions. In the absence of PDDAC it is not possible to separate bromide and iodide because their electrophoretic mobilities are almost identical.

The other polymers tested did not perform as well as PDDAC. Polyethyleneimine (PEI), polyacrylamide and polyvinylpyrrolidone provided incomplete resolution of the seven inorganic test anions. The latter two polymers also gave a rather high background absorbance at the wavelength used.

#### 4.2. Effect of BGE pH

Cationic polymers are known to adsorb strongly onto the capillary wall so that EOF can be reversed from positive to negative [25]. For the present work,

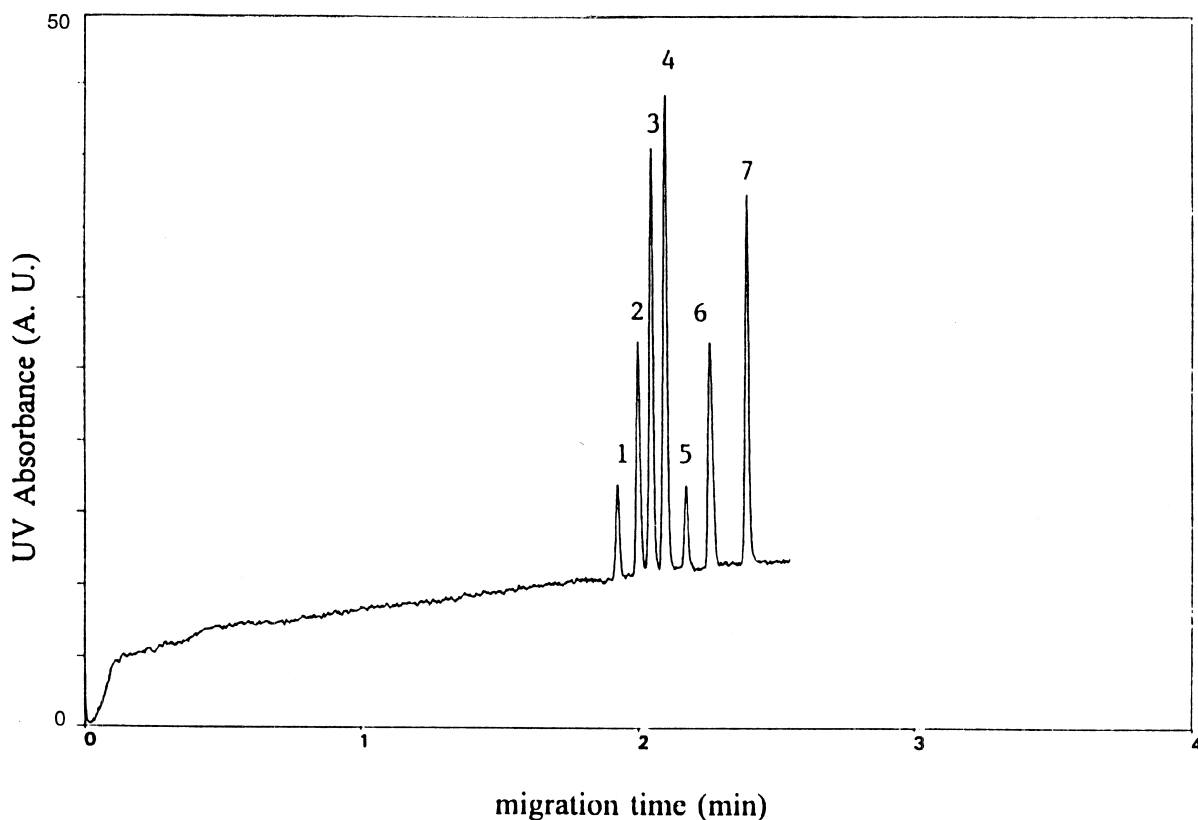


Fig. 2. Separation of inorganic anions. Electrolyte: 150 mM  $\text{Li}_2\text{SO}_4$ , 20 mM borate, 0.05% PADDAC, pH 8.5; other conditions as specified in Experimental section. Peaks: 1 =  $\text{Br}^-$ , 2 =  $\text{I}^-$ , 3 =  $\text{NO}_2^-$ , 4 =  $\text{NO}_3^-$ , 5 =  $\text{CrO}_4^{2-}$ , 6 =  $\text{SCN}^-$ , 7 =  $\text{MoO}_4^{2-}$ .

the EOF reversal was also observed after the addition of PDDAC in the BGE. To check the stability of PDDAC adsorption on the silica surface, the effect of pH on EOF was studied with the PDDAC concentration controlled at 0.05%, and the results are summarized in Table 1. As the pH was increased an increase in the degree of ionization of silica silanol groups occurred, yet there was only a minor change in EOF, indicating a strong and stable adsorption of PDDAC. Our average value of  $2.7 \cdot 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$  for  $\mu\text{EOF}$  is somewhat lower than the value of  $3.0 \cdot 10^{-4}$  reported by Wang and Dubin [21] because of the higher salt concentration in our BGE. Table 1 shows an average decrease in migration time of  $\sim 3\%$  from pH 2.3 to 8.5 for the anions tested. Thus, an exceptionally broad pH range is available for separation of anions.

#### 4.3. Effect of PDDAC concentration

The effect of the PDDAC concentration on both EOF and the electrophoretic mobility of nine sample anions is shown in Fig. 3. There is some increase in the ionic concentration as the PDDAC concentration is increased from 0.1 to 1.0% (2 to 20 mN), but the change in total ionic concentration is rather small because of the 150 mM lithium sulfate in the BGE. The EOF remains almost constant over the entire concentration range, as do the electrophoretic mobilities of peaks 1 (benzoate) and 4 (*p*-aminobenzoate). The electrophoretic mobilities of peaks 2, 3 and 5 show some decrease as the PDDAC concentration is increased, and peaks 6 through 9 show a sharp decrease due to stronger ion-exchange interactions. These results follow the predictions of Eqs. (2) and (3) that an increasing concentration of PDDAC

will result in a higher fraction of a sample ion ( $A^-$ ) being associated with the ion-exchange polymer. This in turn will result in a slower rate of migration for the sample anion (Eq. (5)).

The data in Fig. 3 predict that resolution of this mixture of anions would be very poor without the ion-exchange effect of the PDDAC. Separation of 1- and 2-naphthalenesulfonic acids is, for example, not possible by CE alone. Complete resolution of all nine anions except for *p*-hydroxybenzoic acid and *p*-aminobenzoic acid in a solution containing 0.3% PDDAC is shown in Fig. 4A. Baseline resolution of all nine anions was obtained with 1.0% PDDAC, as shown in Fig. 4B, although the separation takes longer and the baseline is noisier. Comparison of Figs. 4A and 4B shows several changes in elution order as predicted by crossovers for anions 1 and 2, and 3, 4 and 5 in Fig. 3.

#### 4.4. Effect of polymer molecular mass

PDDAC of high molecular mass (400 000–500 000), medium molecular mass (200 000–350 000) and low molecular mass (100 000–200 000) were compared for use in IC–CE. In the initial experiments, a better separation of inorganic anions was obtained with the material of high molecular mass. However, later experiments gave similar results for inorganic and organic anions with the high-molecular-mass PDDAC and that of lower molecular mass. Hexadimethrene bromide (polybrene), a material with quaternary ammonium groups and a considerably lower molecular mass than any of the PDDAC, gave much poorer separations. For example, 1- and 2-naphthalenesulfonates were incompletely separated with 0.3% polybrene ( $\alpha = 1.02$ ), but were baseline separated with 0.3% PDDAC ( $\alpha = 1.035$ ). The EOF was also lower with polybrene, suggesting a thinner surface coating on the capillary surface.

#### 4.5. Effect of added salt

Increasing concentrations of a salt added to the BGE will decrease the ion-exchange effect and cause sample anions to migrate more rapidly (Eq. (5)). This is demonstrated in Fig. 5 where higher NaCl concentrations are shown to repress ion-exchange interactions and lead to more rapid migration rates.

Table 1

Effect of electrolyte pH on the reversed EOF and migration time of inorganic anions. Electrolyte solution contains 150 mM  $\text{Li}_2\text{SO}_4$ , 0.05% PDDAC and 20 mM borate or acetate or hydrochloric acid for desired pH. EOF marker: water. Other conditions as described in Experimental section

pH	EOF ( $\text{cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ )	Migration time (min)			
		$\text{Br}^-$	$\text{I}^-$	$\text{NO}_3^-$	$\text{SCN}^-$
2.3	$-2.46 \cdot 10^{-4}$	1.98	2.06	2.17	2.34
5.0	$-2.65 \cdot 10^{-4}$	1.95	2.03	2.13	2.30
8.5	$-2.74 \cdot 10^{-4}$	1.93	2.00	2.10	2.26

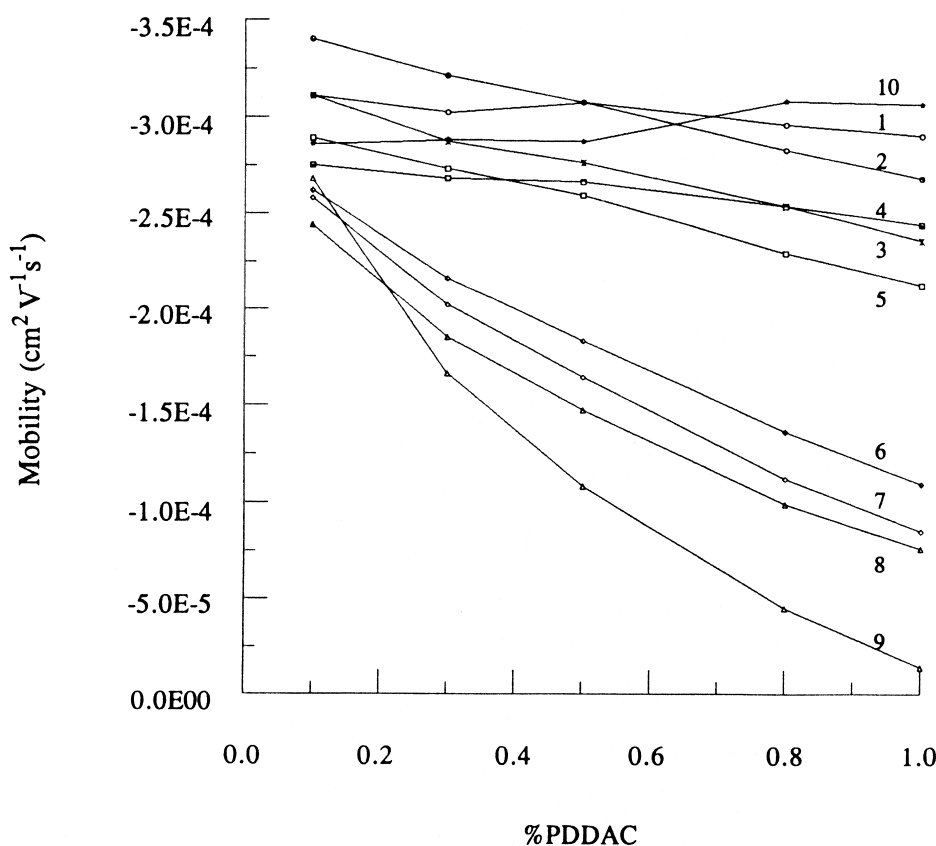


Fig. 3. Effect of PDDAC concentration on EOF and electrophoretic mobilities of organic anions. Electrolyte contains 150 mM  $\text{Li}_2\text{SO}_4$ , 20 mM borate and PDDAC at pH 8.5. Other conditions as specified in Experimental section. Samples: 1=benzoate, 2=benzenesulfonate, 3=*p*-toluenesulfonate, 4=*p*-aminobenzoate, 5=*p*-hydroxybenzoate, 6=2-naphthalenesulfonate, 7=1-naphthalenesulfonate, 8=3,5-dihydroxybenzoate, 9=2,4-dihydroxybenzoate, 10=water for EOF. The amount injected was 20 ppm for each anion.

The change in electrophoretic mobility is more pronounced for iodide, which undergoes a stronger ion-exchange effect than bromide. Similar effects were noted for other salts added to the BGE.

The effect of salt concentration was also studied by comparing separation of several inorganic anions in 50, 100 and 150 mM lithium sulfate in the BGE. While the EOF was virtually unchanged at different lithium sulfate concentrations, the best resolution was obtained at 150 mM concentration (Fig. 2). In 50 mM lithium sulfate, the sample anions migrate more slowly (2.2 to 2.7 min compared to ca. 1.9 to 2.4 min in 150 mM), but resolution of nitrite, iodide and nitrate was very poor. Increased salt concentration also favored the separation efficiency. Theoretical plate numbers for several of the sample anions

on a 40-cm capillary in 50 and 150 mM lithium sulfate were compared as follows: bromide 80 000 in 50 mM vs. 100 500 in 150 mM; thiocyanate 49 000 vs. 86 000; chromate 69 000 vs. 153 000; molybdate 60 000 vs. 152 000. More peak focusing through electrostacking is a likely reason for the better peak efficiency at the higher salt concentrations.

The type of salt, as well as its concentration, can have a major effect on the migration of sample anions. In ion chromatography, sulfate is known to have a much stronger affinity for a solid quaternary ammonium anion exchanger than acetate, for example. In IC-CE, acetate will have a much smaller inhibiting effect on the ion exchange of sample anions with PDDAC than the same concentration of sulfate. The migration times of bromide and iodide

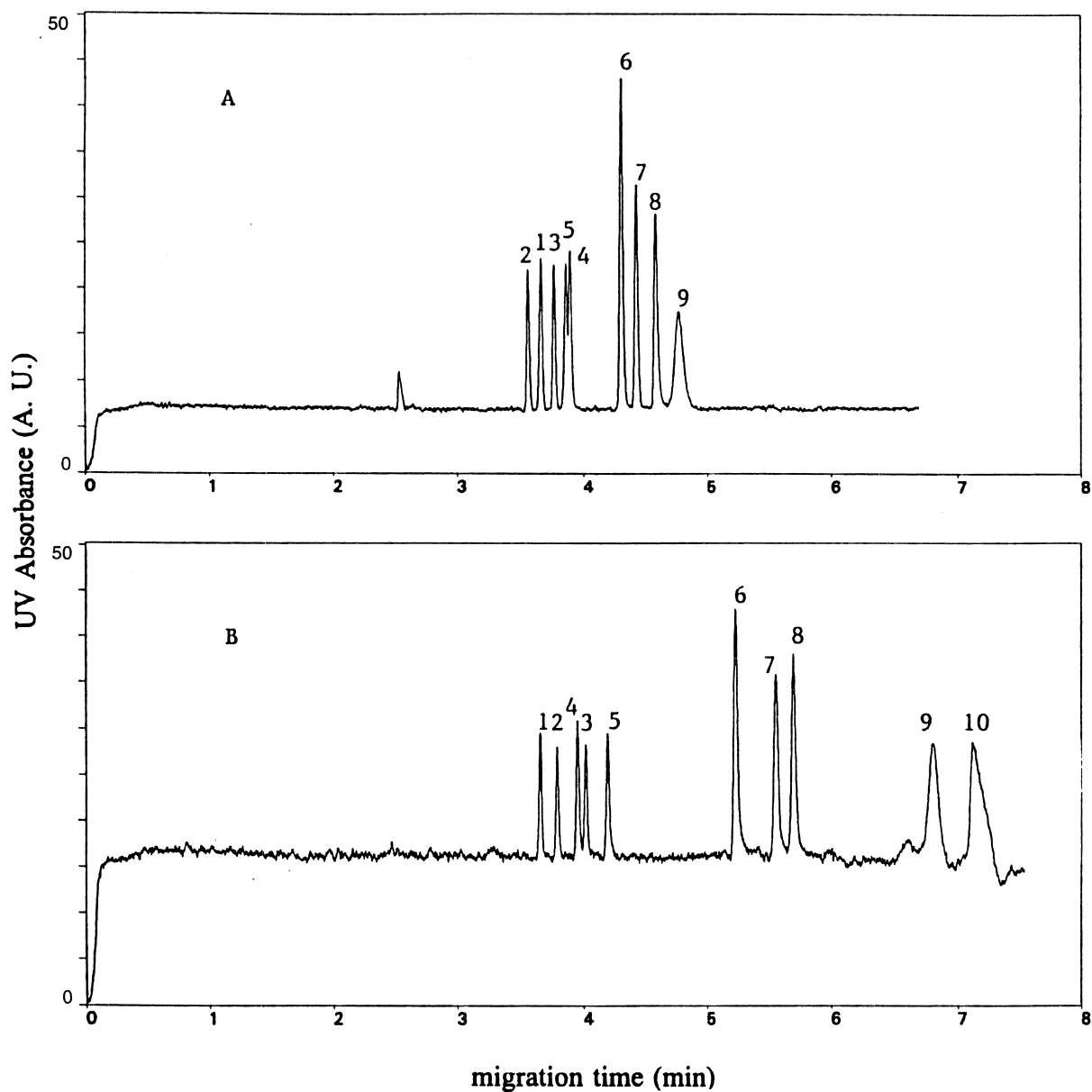


Fig. 4. Separation of nine organic anions. Electrolyte contains 150 mM  $\text{Li}_2\text{SO}_4$ , 20 mM borate, 0.3% (A) or 1.0% (B) PADDAC at pH 8.5; other conditions as specified in Experimental. Peak numbers are the same as in Fig. 3 The amount injected was 20 ppm for each anion.

in 150 mM lithium sulfate are 5.74 and 6.88 min, respectively ( $\alpha = 1.20$ ). In 150 mM sodium acetate the migration times are 6.08 min for bromide and 8.77 min for iodide ( $\alpha = 1.44$ ) (other conditions: 0.3% PDDAC, pH 9.0, injection-to-detection length 52.5 cm). The stronger ion-exchange interactions of

bromide and iodide in sodium acetate lead to longer migration times and a larger separation factor for bromide and iodide.

The counter ion of the BGE salt, as well as the particular anion, can also affect sample ion migration in IC–CE. Examples are shown in Fig. 6. Migration

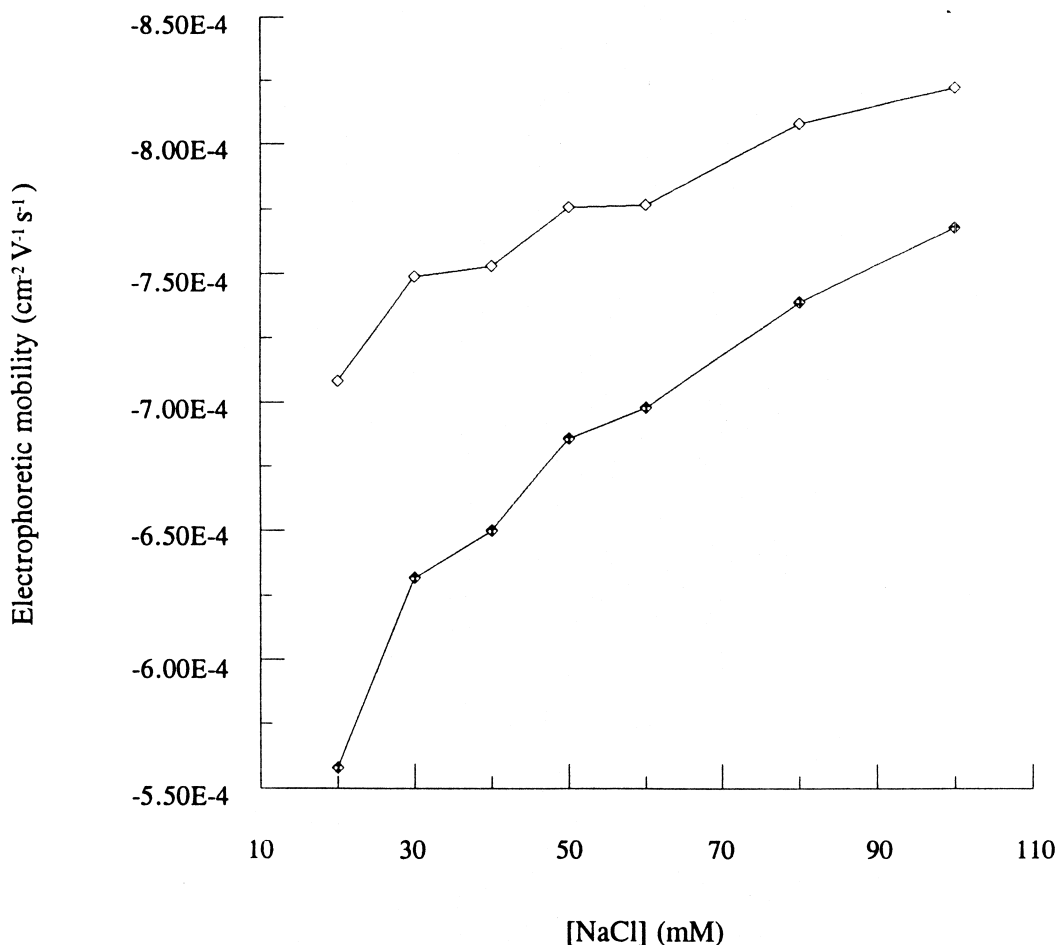


Fig. 5. Effect of NaCl concentration on EOF and electrophoretic mobilities of bromide and iodide. Electrolyte contains NaCl, 20 mM borate, 0.05% PADDCC at pH 9.0; other conditions as specified in Experimental section. EOF marker: deionized water. Symbols: ( $\diamond$ )  $\text{Br}^-$ ; ( $\blacklozenge$ )  $\text{I}^-$ .

of anions 6–9 is slower in chloride than in sulfate due to stronger ion-exchange interactions. However, a comparison of Fig. 6a with Fig. 6c, and Fig. 6b with Fig. 6d also shows longer migration times in the lithium salt than the sodium salt of the same anion. It is known that lithium salts can form weak complexes or ion-pairs with carboxylates. This would tend to reduce further the fraction of sample analytes present as the free anions.

#### 4.6. Effect of organic solvent

Ion-exchange selectivity on a solid ion exchanger for various sample anions appears to consist of at

least two components [29,30]. One might be termed the 'pure' ion exchange that stems from the attraction of sample anions for the ionic sites of opposite charge on the ion exchanger. Another component is the hydrophobic attraction of the sample anions for the organic matrix of the ion exchanger. The presence of an organic solvent in the liquid phase can reduce the latter interaction substantially. The question to be answered is whether an organic solvent will have a similar effect on the present system where the ion-exchange polymer is totally soluble in the liquid phase.

Introduction of organic solvents into the otherwise aqueous BGE is used frequently to alter both the



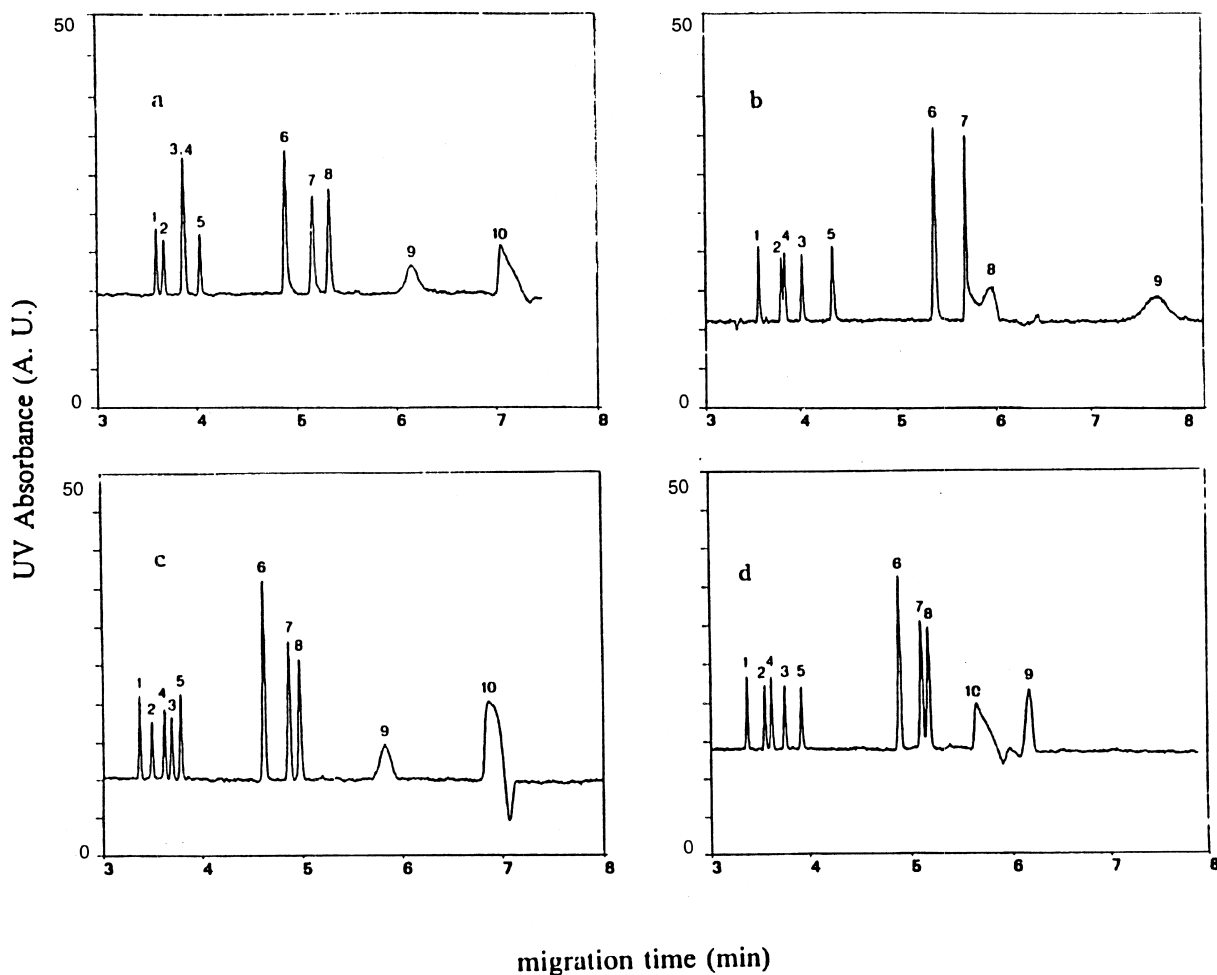


Fig. 6. Separation of nine organic anions with different salts. Besides 20 mM borate and 0.8% PDDAC, electrolyte also contains the following salts at 150 mM for each and pH 8.5: (a)  $\text{Li}_2\text{SO}_4$ ; (b)  $\text{LiCl}$ ; (c)  $\text{Na}_2\text{SO}_4$ ; and (d)  $\text{NaCl}$ ; other conditions as specified in Experimental. Peak numbers are the same as in Fig. 3. The amount injected was 20 ppm for each anion.

selectivity of a separation and the EOF [26,31,32]. In the current work acetonitrile was added to the BGE to determine its effect on the separation and, more importantly, to clarify the ion-exchange mechanism between the analytes and PDDAC. PDDAC has hydrophobic moieties in its backbone, so it should be capable of interacting with analytes via hydrophobic interactions [33] besides the evident ion-exchange interaction. Since hydrophobic interactions are affected more by the solvent than ion exchange, acetonitrile should substantially reduce migration times for analytes where hydrophobic interactions are predominant.

Fig. 7 shows the effects of adding acetonitrile to the BGE at concentrations of 15 and 30%. Concentrations much above 30% caused PDDAC to partially separate from the liquid phase. Some reduction in the EOF is generally observed in CE when acetonitrile is added to an aqueous electrolyte, but the sharper reduction between 15 and 30% acetonitrile is likely the result of a decreased adsorption layer of PDDAC on the capillary surface.

The effect of acetonitrile on the electrophoretic mobility is rather small for anions 1–5 in Fig. 7. However, anions 6 and 7, which contains bulkier naphthalene groups rather than phenyl groups, have

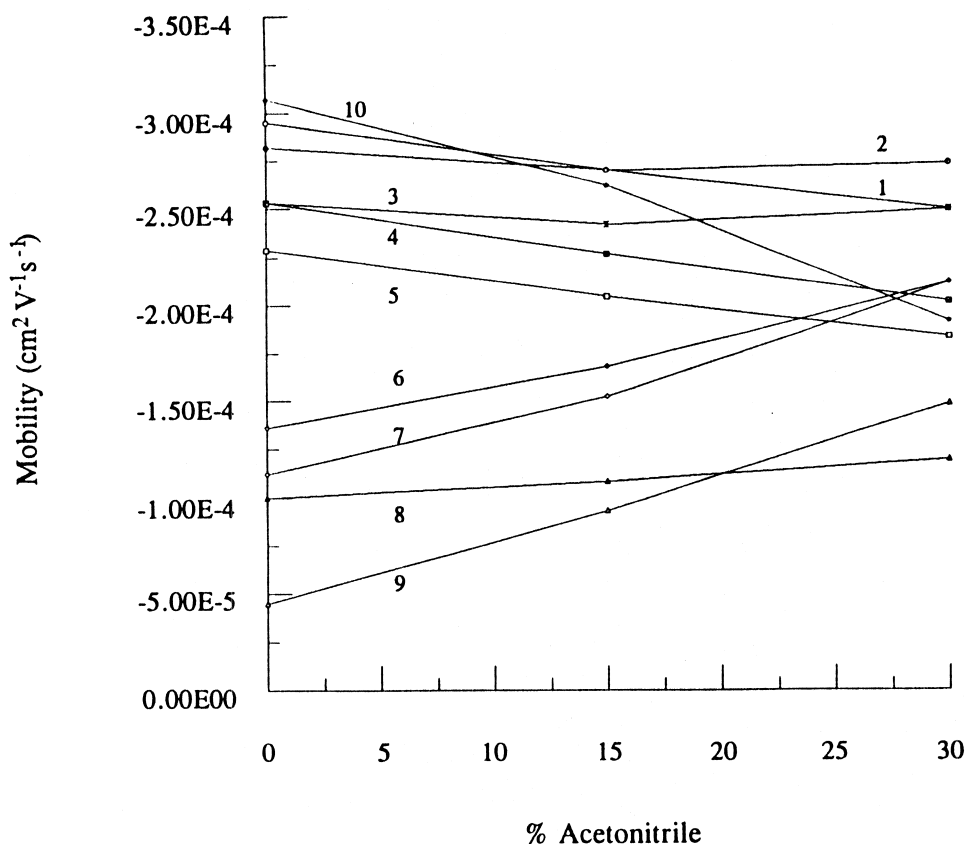


Fig. 7. Effect of acetonitrile concentration on EOF and electrophoretic mobilities of organic anions. Electrolyte contains 150 mM  $\text{Li}_2\text{SO}_4$ , 20 mM borate, 0.8% PDDAC and acetonitrile at pH 8.5; other conditions as specified in Experimental. Sample numbers are the same as in Fig. 3.

significantly higher electrophoretic mobilities in the solutions containing acetonitrile. This would be the result of decreased ion exchange and/or hydrophobic interactions with PDDAC. The ability to resolve the peaks of these two anions has in fact been destroyed by the incorporation of 30% acetonitrile in the BGE.

Addition of some acetonitrile to the BGE in IC-CE can be beneficial in some cases. In aqueous solution (0.8% PDDAC, pH 8.5) 3- and 4-hydroxycinnamic acids could not be separated, but addition of 7.5% acetonitrile to the liquid phase gave an excellent separation with resolution of approximately 10.

#### 4.7. Scope and reproducibility of IC-CE separations

Inorganic anions tend to have significantly shorter

migration times than the larger aromatic carboxylate and sulfonate anions. Mixtures of both of these general types can be separated in a single run by IC-CE, as shown in Fig. 8. All 17 peaks were well resolved and, with the exception of 2,4-dihydroxybenzoate, the peaks were very sharp and narrow. Data from 10 consecutive runs gave actual plate numbers ranging from 63 000 to 140 000 (Table 2). The average plates per meter for the anions in this table ranged from 195 000 for iodide to 429 000 for phthalate, with an overall average of 292 000. The reproducibility of each and every peak was excellent with a RSD of 1.0 or 1.1%. The RSD of peak areas had an overall average of 5.5%. However, no special precautions were taken to ensure that a precise amount of sample was introduced each time.

Several additional sample mixtures of organic anions were separated under a variety of conditions

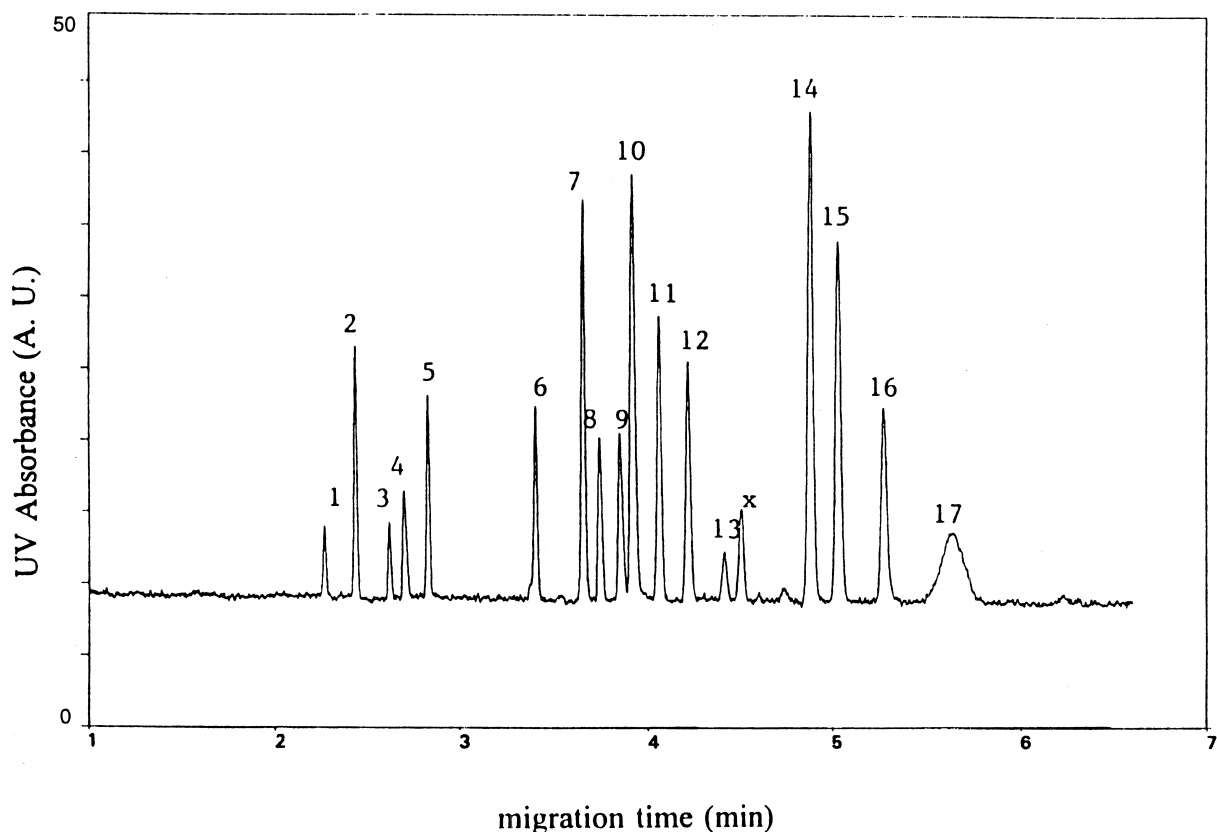


Fig. 8. Separation of 17 inorganic and organic anions. Electrolyte: 120 mM  $\text{Li}_2\text{SO}_4$ , 20 mM borate, 0.3% PADD, pH 8.5; other conditions as specified in Experimental. Peaks: 1 = bromide, 2 = nitrate, 3 = chromate, 4 = iodide, 5 = molybdate, 6 = phthalate, 7 = 1,2,3-tricarboxylate, 8 = 1,2-benzenedisulfonate, 9 = terephthalate, 10 = isophthalate, 11 = benzoate, 12 = *p*-toluenesulfonate, 13 = 1,3,5-tricarboxylate, 14 = 2-naphthalenesulfonate, 15 = 1-naphthalenesulfonate, 16 = 3,5-dihydroxybenzoate, 17 = 2,4-dihydroxybenzoate; x = unidentified impurity. The amount injected was 20 ppm for each anion.

Table 2

Reproducibility study for inorganic and organic anions. Conditions same as Fig. 8. Data from 10 consecutive injections ( $n = 10$ ) were for calculation of RSD

Anion	Plate number per meter ( <i>N</i> )	RSD (%)		
		Migration time	Peak area	Peak height
Bromide	263 000	1.1	9.6	5.1
Nitrate	276 000	1.1	4.3	7.4
Iodide	194 500	1.1	3.1	4.7
Benzoate	362 400	1.1	6.2	8.7
<i>p</i> -Toluenesulfonate	331 700	1.0	6.2	9.1
Phthalate	429 400	1.0	7.2	10.4
Terephthalate	313 000	1.1	6.2	6.1
Isophthalate	278 200	1.0	3.2	10.7
1,2-Benzenedisulfonate	363 000	1.0	5.0	7.8
1,2,3-Tricarboxylate	408 000	1.1	4.6	10.9
1-Naphthalenesulfonate	333 700	1.1	6.2	7.6
2-Naphthalenesulfonate	357 500	1.1	3.7	7.6

to illustrate the broad scope of IC–CE. Three isomeric phthalic acids were baseline separated in about 3.8 min using an electrolyte solution containing 0.3% PDDAC, 120 mM lithium sulfate and 20 mM borate at pH 8.5. A separation of three isomeric benzenetricarboxylic acids was also obtained under the same conditions, with 1,2,3-benzenetricarboxylic acid eluted at 3.47 min followed by 1,2,4- at 3.81 min and 1,3,5- at 4.36 min. A mixture of six different cinnamic acids, two pairs of which are positional isomers, was completely resolved provided 7.5% acetonitrile was added to the BGE that contained 0.8% PDDAC and 150 mM lithium sulfate at pH 8.5. Separations of valine and norvaline, and of leucine and norleucine are considered to be difficult, yet these were completely resolved within a few minutes by IC–CE using a 33-cm capillary and 0.3% PDDAC in the BGE. A mixture of six nucleotides, i.e. UMP, CMP, AMP, ADP, GMP and ATP, was also separated successfully with 0.3% PDDAC on a 60-cm capillary. In general, the resolution of these compounds of biological interest requires careful choice of electrolyte pH and added salt. For instance, while valine and norvaline were best separated with 80 mM potassium fluoride at pH 9.53, and leucine and norleucine were near baseline separated with 60 mM lithium sulfate at the same pH, the nucleotides were resolved at pH 9.0 using 80 mM lithium sulfate.

All of the separations reported in this work have used direct photometric detection. Separations with indirect photometric detection are also feasible, as demonstrated by Cassidy et al. [25,26], but the higher salt concentrations used here make indirect detection more difficult.

## 5. Conclusions

The IC–CE system described here is a nearly ideal system for separation of anions in solution. Electroosmotic pumping results in a square flow front rather than the hyperbolic flow profile encountered with mechanical pumping. Electrophoretic migration of sample ions can be modified in a controlled manner by their ion-exchange interaction with the ionic polymer. In conventional ion-exchange chromatography partitioning of analytes between a liquid

mobile phase and a solid stationary phase inevitably causes a certain amount of peak broadening. This is largely avoided in IC–CE because the interaction of analyte ions with the polymeric ion exchanger occurs within a single phase.

Finally, IC–CE is a very simple technique. In capillary electrochromatography, a popular emerging separation method, considerable care is required to fabricate suitable frits and properly pack microbore columns with a solid stationary phase. By contrast, separations in IC–CE take place within a single phase that is easily prepared by incorporation of a low concentration of a soluble polymer into the BGE of a CE capillary.

IC–CE need not be limited to the separation of anions. The same principles should apply to the separation of sample cations using a soluble polymer containing sulfonate or other anionic groups to make the polymer a cation exchanger. The hydrophobic parts of ionic polymers may also be useful for separation of nonionic sample components based on their difference in interaction in solution.

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