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Review

Recent developments in the separation of inorganic and small organic ions by capillary electrophoresis

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Abstract

Which method should I use for ion analysis, ion chromatography (IC) or capillary electrophoresis (CE)? In terms of actual theoretical plates CE has a clear-cut advantage. The separation ability of IC is adequate for many sample types, and many separation scientists feel that IC offers greater reliability and confidence than CE. However, IC is a more mature technique and there has been more time to solve problems such as peak tailing and to improve reproducibility. The two techniques should be viewed as complementary. A number of recent developments in ion analysis by CE are discussed. These include some simple ways to control electroosmotic flow and improve reproducibility, separation of isotopes, improved methods of indirect photometric detection, a new contactless conductivity detector, separation of ions at low pH, and in solutions of high salt content. Progress in a new technique called IC–CE will be described in which a soluble ion-exchange polymer is added to the capillary electrolyte to separate anions based on differences in both electrophoretic mobility and ion-exchange interactions.

Keywords: Capillary electrophoresis; Ion chromatography–capillary electrophoresis; Reviews; Conductivity detection; Detection, electrophoresis; Inorganic cations; Isotopes; Inorganic anions; Organic acids

Contents

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1. Introduction

In terms of actual theoretical plates capillary electrophoresis (CE) is at least 10 times more powerful than ion chromatography (IC). Separations by CE are fast and it is relatively easy to find experimental conditions for an adequate separation of sample ions. One of the greatest assets of CE is its tolerance to variations in the sample matrix [1]. CE also has some well-known drawbacks that include an often poor reproducibility, a limited choice of detectors and a perception that CE is an exotic technique with principles that are more complicated than those of ion chromatography.

Scientific publications on CE [or high-performance capillary electrophoresis (HPCE) as it is called by some] have increased exponentially in number from 23 in 1987 to 1235 in 1997 [2]. However, sales of CE instruments have been disappointing and several companies have discontinued their line of instruments. Pernendu ''Sandy'' Dasgupta has stated that 90% of ion chromatography users are just that — users, not researchers. In contrast 95% of the people who use CE are researchers and 5% are users for routine analysis [3].

In comparing IC and CE it should be pointed out that with a 23-year head start most of the problems with IC have been worked out. CE is today a lively area of research. Drawbacks associated with CE are being addressed and truly exciting separation possibilities of this relatively new technique are becoming apparent. The past five years or so has seen many new developments in ion analysis by capillary electrophoresis.

A number of significant recent developments will be discussed in this paper.

2. Reproducibility

Perhaps the most serious criticism of capillary electrophoresis has been that migration times are not sufficiently reproducible. At best this is disturbing and at worst it can lead to errors in peak identification. In an effort to improve reproducibility it has become commonplace to resort to very frequent rinses of the fused-silica capillary with a base such as sodium hydroxide. Such rinses are time consuming and detract from the advantages of speed and resolution that CE often enjoys compared to conventional liquid chromatography.

This lack of reproducibility stems mainly from difficulties in maintaining a clean, reproducible inner surface on the capillaries used in CE. In particular, variations in the surface can affect the electroosmotic flow (EOF) and hence the analyte migration times.

Several factors may affect the condition of a capillary surface. A new silica capillary is usually conditioned by treatment with a base such as aqueous sodium hydroxide. This treatment hydrolyzes many of the siloxane groups of the silica to form silanol groups, $-SiO^-$, at alkaline pH values these groups are largely ionized: –SiOH. The degree of silanol group formation may vary along the length of the capillary, resulting in differences in surface charge and hence to differences in EOF.

Prior to making a CE run, the capillary is equilibrated with a background electrolyte (BGE) containing a solvent (usually water and perhaps some organic solvent), electrolyte ions to carry the electric current, a pH buffer, and in some cases an additional BGE additive. Then the sample is introduced into the capillary, a voltage is applied to the system, and the separation of sample analytes is carried out. During this operation every chemical in solution may undergo an equilibrium in which a certain amount is deposited on the silica surface. The amount deposited may be inconsequential for some species but the adsorbed surface layer may be significant for others. Adsorption may be uniform or only in certain spots along the column. The chances of adsorption are increased by the rather large ratio of surface to volume in the small capillaries (typically 50 μm I.D.). If any adsorbed substances are not cleaned off, the surface characteristics for the next run will be changed from the original run. In performing several repeated runs of the same sample, Cohen and Grushka found that the EOF decreased from run to run, suggesting a continuing change in the surface conditions [4]. This occurred with each of the several pH buffers they used.

There is a need for a simple and reliable way to maintain a constant, reproducible EOF and ensure reproducible migration times for sample ions. Polymer-coated capillaries have been used with some success to largely eliminate EOF, but an EOF is

often desirable in the separation of smaller ions. The simplest answer to this problem may be to include an additive in the BGE that will interact more strongly with the silica surface than any of the sample components.

Yeung and Lucy [5] were able to obtain monotonic variation in the electroosmotic flow from near zero to full reversal by adding a mixture of a cationic surfactant and a zwitterionic surfactant (CASU) to the (BGE) in varying proportions. Their system gave good reproducibility, but in general surfactants need to be used with some care to avoid excessive buildup of an adsorbed layer with continued use.

Addition of a low concentration of a smaller molecule to the BGE appears to be a simple yet effective way to obtain better reproducibility in CE. An amine additive, such as triethylamine (TEA), or an acidic additive, such as ethanesulfonic acid (ESA) or trimethylacetic acid, has proved to be effective. Most likely, a dynamic equilibrium exists between the additive in solution and adsorbed additive on the capillary surface. The adsorbed layer is apparently sufficient to inhibit interaction between the sample solutes and the capillary surface, yet the relatively low molecular weight of the additive prevents the buildup of an ever thicker adsorbed layer.

Cohn and Grushka [4] found that additives of 1.0 m*M* triethylamine (TEA) to the BGE shortened the migration time for the phenol marker and, more importantly, gave a constant migration time with a relative standard deviation of about 0.4%. At the same time the peak sharpness was markedly improved. Similar improvements in reproducibility were noted for certain other amines, amino acids and trimethylacetic acid when added to the BGE.

Thornton, Fritz and Klampfl [6] found that addition of octanesulfonic acid or ethanesulfonic acid to the BGE greatly improved peak shape and resolution in the separation of protonated amine acids by CE in acidic solution. At pH 2.8 no cathodic EOF was observed until an alkanesulfonic acid was added to the BGE. This was attributed to a thin adsorption layer of the sulfonic acid that imparted a negative charge to the silica capillary surface. The magnitude of cathodic EOF was a function of the alkanesulfonic acid chain length.

In the separation of basic drugs by capillary electrophoresis Ding and Fritz found that addition of either ethanesulfonic acid (ESA) or TEA to the background electrolyte improved both peak sharpness and symmetry [7]. The migration times of the drugs were also very reproducible, RSD=0.9% with ESA and 0.6% with TEA. Li and Fritz noticed similar improvements in peak sharpness and reproducibility for separation of protonated anilines by CE [8]. The RSD of migration times with ESA at pH 3.65 averaged 0.9% and those with TEA at pH 3.45 averaged 0.6%.

Experiments now suggest that frequent washing of a silica capillary with sodium hydroxide is no longer necessary when a suitable BGE additive is used. However, an initial treatment of a new silica capillary with base may be necessary to convert siloxane groups to silanol groups.

3. Sample introduction

In our earlier discussion of reproducibility attention was focused on capillary wall equilibration and on electroosmotic flow rate. But in order to obtain dependable and reproducible results from CE, attention must be paid to the sample injection step. In describing an inexpensive automated instrument for CE, Dasgupta and Surowiec [9] discussed the major techniques available for sample introduction. To obtain reproducible injection volumes it is necessary to avoid even minor variations in the timing between sample insertion into the capillary and reinsertion of the capillary into the BGE.

A novel technique for sample introduction by hydrodynamic injection [10] and by electromigration injection [11] has been described by the same authors. A wire loop is deployed at the tip of the electrophoresis system. When this loop is dipped into the sample solution a thin film of liquid is formed on the loop much like that with a child's soap bubble wand. A thin film of the same liquid is formed on the loop. The loop is then transferred to a sealed chamber and pneumatic pressure is applied to introduce the liquid into the capillary. A modification of this system permits transfer of the loop contents by electromigration injection. The loop injection technique appears to be a robust and reproducible alternative to the injection techniques in current use.

4. Separation of metal ions

The impressive power of CE to separate inorganic ions has been demonstrated by a number of authors. In 1991 Jones and Jandik [12] were able to separate some 30 anions in a total elapsed time of only 3.1 min. In 1992 two different research groups achieved a complete separation of all 13 of the lanthanides in <5 min [13,14]. This provided a definitive answer to one of the most difficult and long-standing problems in inorganic separations. In 1993 Shi and Fritz [15] were able to separate 27 metal cations, including the 13 lanthanides, by CE in only 6.0 min using lactate to partially complex the metal ions (Fig. 1). By adding a crown ether as a second complexing reagent they were able to resolve NH_4^+ and K^+ as well as other cations [16].

The successful separation of these metal cations was due largely to differences in their extent of complexation. Addition of a weak complexing reagent to the BGE gives a mixture of free metal cation and various complexed species. For example, the lanthanides form a series of complexes with a complexing ligand (LG) such as lactate or a α-hydroxyisobutyric acid (HIBA): M^{3+}, ML^{2+}, ML_2^+, ML_3, ML_4^G. The mobility is faster for species with a higher positive charge but rapid equilibria cause all species of a given metal to migrate as a single, tight zone. The electrophoretic mobility will be the various complexd species.

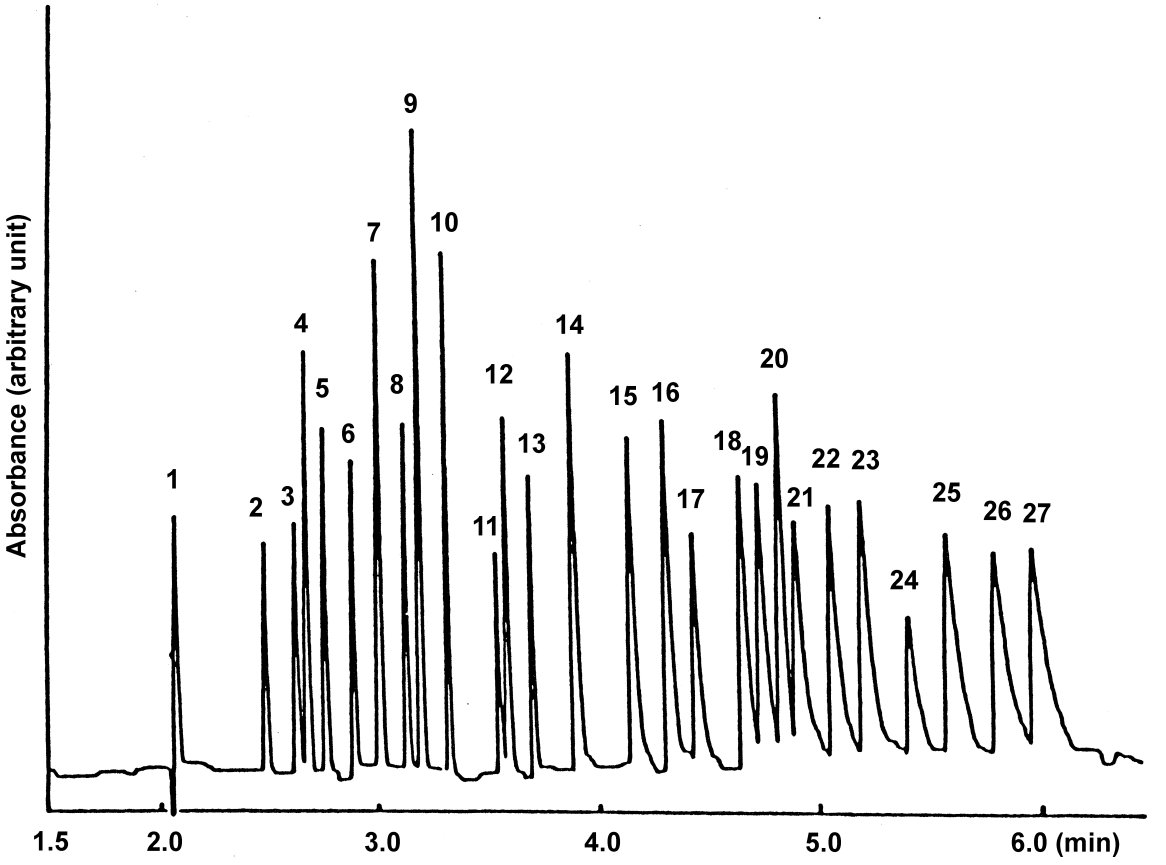

Fig. 1. Separation of 27 alkali, alkaline earth, transition, and rare earth metal ions in a single run using lactate. Eletrolyte: 15 m*M* lactic acid, 8 m*M* 4 methylbenzylamine, 5% methanol, pH 4.25. Applied voltage: 30 vK. Peaks: 1=K^+; 2=Ba^{2+}; 3=Sr^{3+}; 4=Na^+; 5=Ca^{2+}; 6=Mg^{2+}; 7=Mn^{2+}; 8=Cd^{3+}; 9=Li^+; 10=Co^{2+}; 11=Pb^{2+}; 12=Ni^{2+}; 13=Zn^{2+}; 14=La^{3+}; 15=Ho^{3+}; 16=Pr^{3+}; 17=Nd^{3+}; 18=S,m^{3+}; 19=Gd^{3+}; 20=Cu^{3+}; 21=Tb^{3+}; 22=Tb^{3+}; 23=Ho^{3+}; 24=Er^{3+}; 25=Tm^{3+}; 26=Yb^{3+}; 27=Lu^{3+}. (From Ref. [15] with permission).

$$\mu_{ep} = \alpha_M \mu_M + \alpha_{ML} \mu_{ML} + \alpha_{ML2} \mu_{ML2} + \ldots \quad (1)$$

For any given ligand, the various α values will depend on the metal ion, the concentration of ligand and the solution pH. The migration times of lanthanides with HIBA as the ligand were a linear function of the mean ligand number ($\bar{n}$) which ranged from 0.48 for lanthanum to 1.95 for lutetium [15].

5. Separation of isotopes

Is it better to separate ions under conditions of comigration (electrophoretic and electroosmotic vectors in the same direction) or counter migration (vectors in the opposite direction)? Comigration gives faster separations and the peak resolution is quite adequate in many instances. Counter migration conditions result in slower separations but the separation power is much higher. The greatest separation power is obtained when the electrophoretic and electroosmotic vectors are almost counter balanced so that the net mobility is very slow. This is evident from the equation:

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\Delta V}{V} \quad (2)$$

where R_s is the resolution, N is the average number of theoretical plates as defined in chromatography, ΔV is the difference in peak velocities and V is the average peak velocity. The key to obtaining ultra-high resolution is to adjust conditions so that the electrophoretic and electroosmotic vectors are almost equal and ΔV becomes very small.

This principle has been applied successfully to perhaps the most challenging separation problem of all — the separation of isotopes. The ability is to achieve precisely the desired EOF. In an untreated capillary the electroosmotic flow coefficient is given by:

$$\mu_{EOF} = \frac{\sigma_{SiO^-} K^{-1}}{\eta} \quad (3)$$

where σ_{SiO^-} is the surface charge density, η is the viscosity and K^{-1} is the double layer thickness which is inversely related to the square root of the buffer ionic strength.

McDonald and Lucy [17] were able to achieve a baseline resolution of $^{35}Cl^G$ and $^{37}Cl^G$ isotopes in ~45 min. The EOF was controlled by adjusting the pH of a chromate buffer (altering σ_{SiO^-}) and the ionic strength (altering K^{-1}).

Yeung and Lucy have successfully separated ^{14}N and ^{15}N isotopes of aniline [18] and ammonia [19,20]. These separations were based on the isotopic effect on pK_a. The K_a for the solute with the lighter isotope $^{14}NH_4^+$ or $C_6H_5NH_3^+$ is slightly larger. To fully utilize the isotopic effect on ionization the separation should be performed at the pH where the difference in the degree of ionization between the two isotopes is the greatest. According to Terabe et al. [21] the optimum pH for cations is 0.3 pH unit higher than the average pK_a. This was borne out experimentally. The optimum pH for separation of aniline isotopes was 4.96 (4.92 theory), and was 9.45 (9.55 theory) for the separation of ammonium isotopes.

To achieve these separations it was also necessary to precisely control the EOF in the counter-migration system. This was accomplished by adjusting the composition of a zwitterionic surfactant and a cationic surfactant. The separation of the ammonium isotopes is shown in Fig. 2. As predicted, the ^{15}N retains its hydrogen more strongly and so has a slightly higher effective charge than the ^{14}N isotope, causing the $^{15}NH_4^+$ to migrate more quickly in the counter-migration system.

6. Indirect spectrophotometric detection

Indirect absorbance detection is often used for the analysis of inorganic and smaller organic anions. This technique uses a UV-absorbing ion, known as the probe ion or visualization ion, in the BGE. Displacement of the probe ion by a migrating sample anion results in a quantifiable decrease in the background absorbance. Much of the now classical work in anion CE was done with chromate as the probe ion for indirect detection [22–28].

The mobility of the probe ion should match the mobilities of the sample ions as closely as possible,

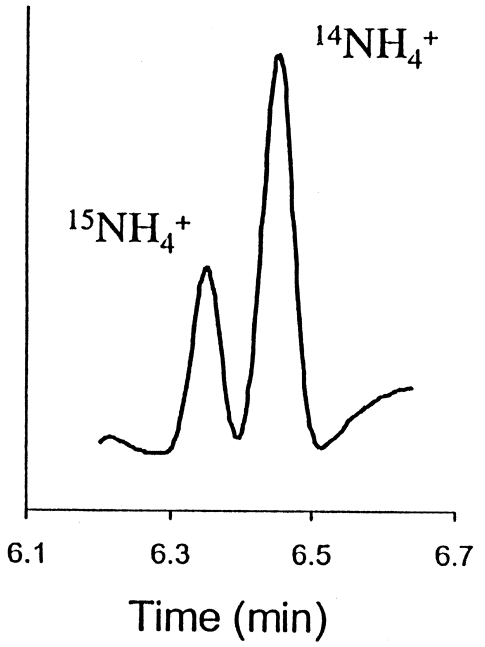

Fig. 2. Separation of isotopically labeled ammonium ($^{15}NH_4^+$) from natural ammonium ($^{14}NH_4^+$). Experimental conditions: applied voltage, −15 kV; capillary, 47 cm of 50 μm I.D. (40 cm to detector); temperature, 25°C; detection, indirect at 214 nm; buffer, 10 m*M* borate with 0.5 CASU with pH adjusted to 9.55 using benzyltributylammonium hydroxide; sample, 0.1 m*M* ($^{15}NH_4^+$) and 0.2 m*M* ($^{14}NH_4^+$). From Ref. [18] with permission.

otherwise the sample peaks become increasingly fronted or tailed as the discrepancy between the mobilities become larger. Shamsi and Danielson found that either naphthalenedisulfonic acid (NDS) or naphthalenetrisulfonic acid (NTS) worked well for the 22 inorganic and organic anions tested [24].

Doble and Haddad added more than one probe anion to the BGE as a means to control peak symmetries and improve the sensitivity of indirect detection [29]. It was found that an analyte anion mainly displaced the probe ion to which its mobility was closest and exclusively displaced the BGE component with the same mobility. Of several combination probes investigated, a mixture of chromate and 4-hydroxybenzenesulfonic acid gave the best peak shapes and sensitivities for the sample ions.

In comparing the electrophoretic mobilities of several UV-absorbing anions and analyte anions (Fig. 3), it is apparent that chromate is a reasonably good match for fast-moving inorganic anions but is not so good for the other analytes listed. Lau showed convincingly that molybdate is a much better probe ion than chromate for indirect detection of common anions [30]. Sensitivity is better with molybdate molar absorptivity (5650 at 230 nm compared to 3180 at 254 nm for chromate), molybdate solutions are more stable, and peak shapes are better. Systemic studies resulted in the following optimal conditions: 5 m*M* molybdate as the UV-absorbing ion, 0.15 m*M* cetyltrimethylammonium hydroxide (CTAH) as an electroosmotic flow modifier, 0.01% polyvinylalcohol as an additive to solve the co-migration problem of fluoride and formate, and 5 m*M* tris(hydroxymethyl)aminomethane as a buffer to maintain a pH of 7.9. A separation of a standard anion

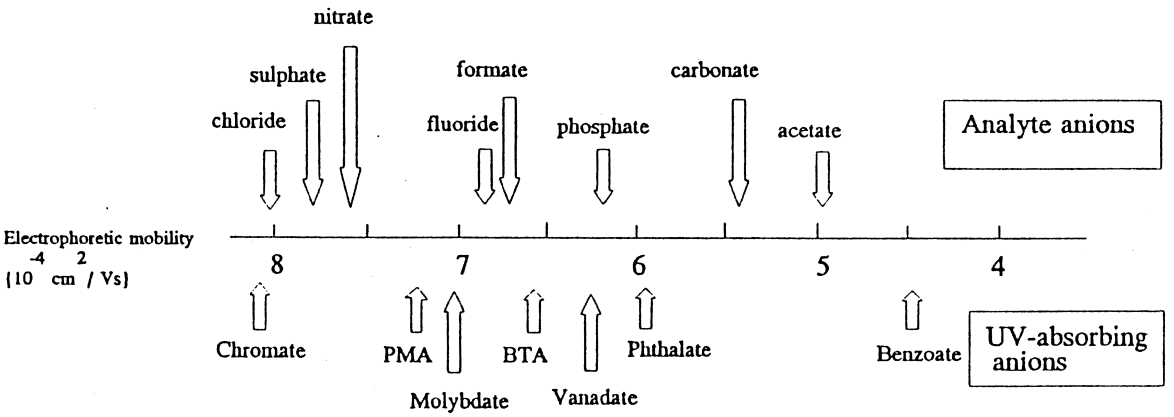

Fig. 3. The electrophoretic mobilities of common UV-absorbing anions and analyte anions. From Ref. [30] with permission.

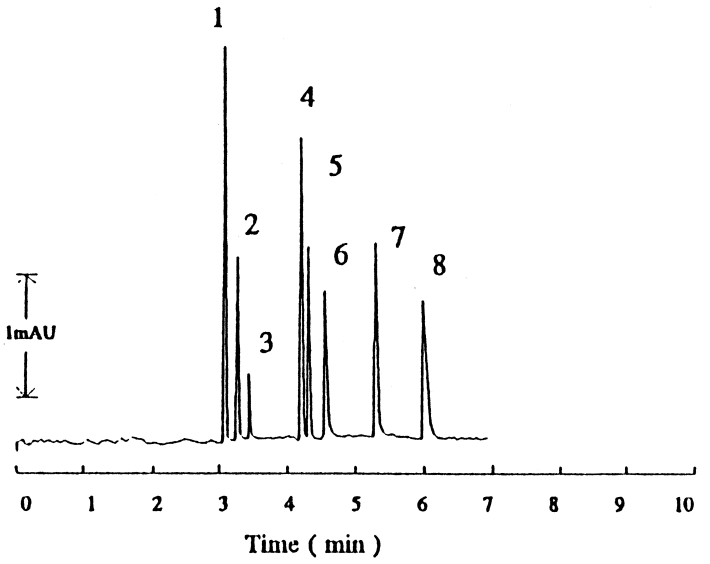

Fig. 4. Separation of a standard anion mixture [30]. Conditions: BGE: 5 m*M* Molybdate, 0.15 m*M* cetyltrimethylammonium hydroxide (CTAH), 5 m*M* Tris buffer at pH=7.9; 0.01% polyvinylalcohol (PVA); Capillary: 65 cm×0.075 mm I.D. fused silica; run: −20 kV; current: 12 uA; injection: 8 cm for 20 s; detection: 230 nm. Anion standards: 2 ppm each: 1=chloride; 2=sulphate 3=nitrate; 4=fluoride; 5=formate; 6=phosphate; 7=carbonate; 8=acetate.

mixture is shown in Fig. 4. A good electropherogram of the same mixture could still be obtained at a sample concentration of 200 ppb of each analyte.

7. Conductivity detection

The success of suppressed conductivity detectors in ion chromatography has no doubt inspired researchers to develop a similar system for capillary electrophoresis. Dasgupta and Bao [31] described a suppressed conductivity capillary electrophoresis separation system called SUCCESS. Another system was devised by workers at Dionex [32]. Direct conductivity detection of anions and cations is possible with a novel sensor design developed at ThermoCapillary Electrophoresis (Franklin, MA, USA) [33].

More recently, a capacitively coupled contactless conductivity detector (called a C4D) for CE was introduced by Zemann and his associates at Innsbruck, Austria [34,35]. The detector consists of two metal tube electrodes which are placed around the outer polyimide coating of the capillary (Fig. 5) with a 1-mm detection gap between the electrodes. When a high audio or low oscillation frequency between 40 and 100 kHz is applied to one of the electrodes, a signal is produced when an analyte zone with a different conductivity passes through the retention gap. An amplifier and rectifier is connected to the other electrode and the signal is further processed. A thin piece of copper foil is placed perpendicularly between the electrodes to prevent a capacitive transition between the two electrodes that would bypass the detection gap and increase the background noise level.

Good separations of both anions and cations were obtained with the C4D electrode with limits of detection in the low ppb concentration range. Linear calibration curves were obtained over four orders of magnitude from −0.1 to 1000 ppm. Fig. 6 shows a separation of eight metal cations with a crown ether added to achieve resolution of the NH_4^+ and K^+ peaks. Organic ions with lower conductivities can be detected by indirect conductivity.

8. Separations at low pH

In the vast majority of publications, CE sepa-

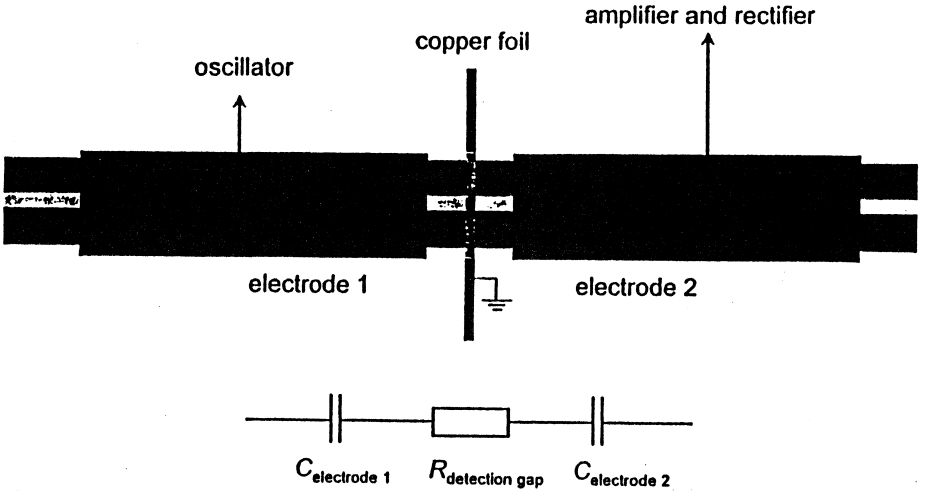

Fig. 5. Schematic drawing of the contactless capactively coupled conductivity detector. From Ref. [35] with permission.

rations of inorganic anions are carried out at an alkaline pH to ensure that the analytes will be in the ionic rather than the molecular form. However, a flow modifier must generally be used to reverse the direction of EOF. Thornton and Fritz found that excellent separations of anions are possible at pH values as low as 2.0 (HCl) or 1.8 with perchloric acid [36]. Special attention was paid to the anionic chloro complexes of gold(III) and the platinum group elements, which are more resistant to hydrolysis in more acidic solutions. By working at lower pH values, the capillary silanol groups are largely un-ionized and consequently the EOF is minimal.

Preliminary experiments were carried out by diluting stock solutions of $AuCl_4^-$, $PtCl_6^-$, and $RhCl_6^{3-}$ with water just before analysis by CE. Spectral measurements indicated that direct detection at 254 or 214 nm would be feasible. At first, buffers prepared by adding hydrochloric acid to β-alanine or glycine were used at pH values ranging from about pH 3.5 to 2.5. No peak for $AuCl_4^-$ was observed with the β-alanine buffer. Peaks were obtained for the sample ions in the glycine buffer, but they were broad and the baseline was noisy. Much better results were obtained when only hydrochloric acid was added to the aqueous electrolyte solution. The best results were obtained at a pH of 2.4, although the sample anion peaks were almost as sharp at pH 2.0.

Sharp peaks were obtained for gold(III) (Fig. 7) and for each of 24 species of chloro complexes of platinum group elements [36]. The theoretical plate number for the peak in Fig. 7 was 300 000. An applied potential of −10 kV was employed to reduce the high currents that occurred at −20 kV.

Sharp peaks were obtained for 14 other inorganic anions in hydrochloric acid or in perchloric acid (both pH 2.4) with direct detection at 214 nm. The migration times were very reproducible (average RSD = 0.6%). The test anions included MnO_4^-, VO_3^-, ReO_4^-, ferricyanide and ferrocyanide-ions for which chromatographic separations are seldom possible.

Conditions similar to those used for CE separation of anions in acidic solution were used to separate metal cations [37]. Methods for the detection of metal cations under acidic conditions, near pH 2, in capillary electrophoresis (CE) were investigated. Conditions for direct UV detection of UV absorbing metal cations such as Cr^{3+}, Cu^{2+}, Fe^{3+}, UO_2^{2+}, VO^{2+}, VO_2^+ were established with aqueous HCl or $HClO_4$ as the electrolyte carrier. The speciation of vanadium(IV) and vanadium(V) at pH 2.3 by CE was achieved with direct detection at 185 nm. With the strong absorbance at 85 nm, no complexation was needed to detect the metal cations.

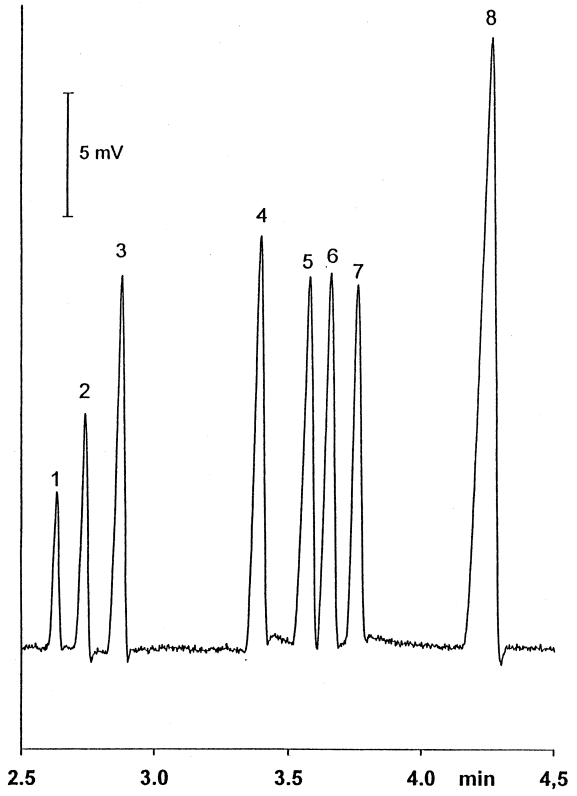

Fig. 6. Separation of inorganic cations with contactless conductivity detection. Conditions: capillary, 60 cm (effective length 47 cm)×50 μm I.D.; electrolyte, 20 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES), 20 m*M* His, 1 m*M* 18-crown-6, pH 6.1; injection, 10 s at 10 cm; sample concentration, 10 ppm (except magnesium and ammonium, 5 ppm); separation at +20 kV peak assignments, rubidium (1), ammonium (2), potassium (3), calcium (4), sodium (5), magnesium (6), manganese (7), and lithium (8). From Ref. [35] with permission.

The separation of amino acids has long been an important analytical problem. Usually the amino acids are first derivatized to provide a chromophore group for detection or the separated amino acids are detected after a post-column derivatization reaction. Amino acids exist as zwitterions over much of the pH range, but at low pH values the amino acids become protonated cations.

$$R\,CH(NH_3^+)CO_2^- + H^+ \rightarrow R\,CH(NH_3^+)\,CO_2H \quad (4)$$

With no sample pretreatment whatsoever, Thornton et al. [6] were able to separate amino acids at pH 2.8 with direct absorbance detection at 185 nm provided the BGE contained 50 m*M* ethanesulfonic acid. Most of the amino acids could be resolved within 20 nm at an applied voltage of +30 kV (Fig. 8).

9. Separations at high salt concentrations

It is commonly thought that even a moderately high ionic concentration in the BGE would lead to Joule heating and serious peak distortion. However, Ding et al. [38] found that very satisfactory separations of both inorganic and organic anions could be obtained in solutions as high as 5 m*M* sodium chloride using direct photometric detection.

The first experiments on the effect of high salt concentrations were run at pH 2.4 to almost eliminate EOF. A negative power supply (−10 kV) and a 75 μm I.D. fused-silica capillary were used. Both the sample and the BGE contained 0.5 *M* sodium chloride. The results for several inorganic anions under these conditions with direct photometric detection were poor. The peaks were badly shaped and there was almost no resolution of individual peaks. However, peak shape and resolution improved dramatically with increasing sodium chloride concentration in the BGE. At 1.5 *M* sodium chloride in the BGE, excellent separation was obtained for samples containing 0.5 *M* sodium chloride and low ppm concentrations of five inorganic anions. The salt content of the BGE needed to be at least three times that of the sample in order to provide sufficient peak focusing (electrostacking) during the sample introduction. These experiments demonstrated that the limits of salt concentration of both the sample and the BGE are much higher than had been expected.

A short study was conducted on the pH effect over a broad range, from pH 3.0 to 12.0. The sodium chloride concentration was 1.5 *M* in all buffers. There were no observed differences in either migration times or peak shapes for I^-, SCN^-, NO_3^-, IO_3^- between pH 3.0, 7.0, and 12.0. This effect strongly indicates that the electroosmotic flow is greatly suppressed, and the ionized silanol groups at the capillary surface are effectively shielded by the high

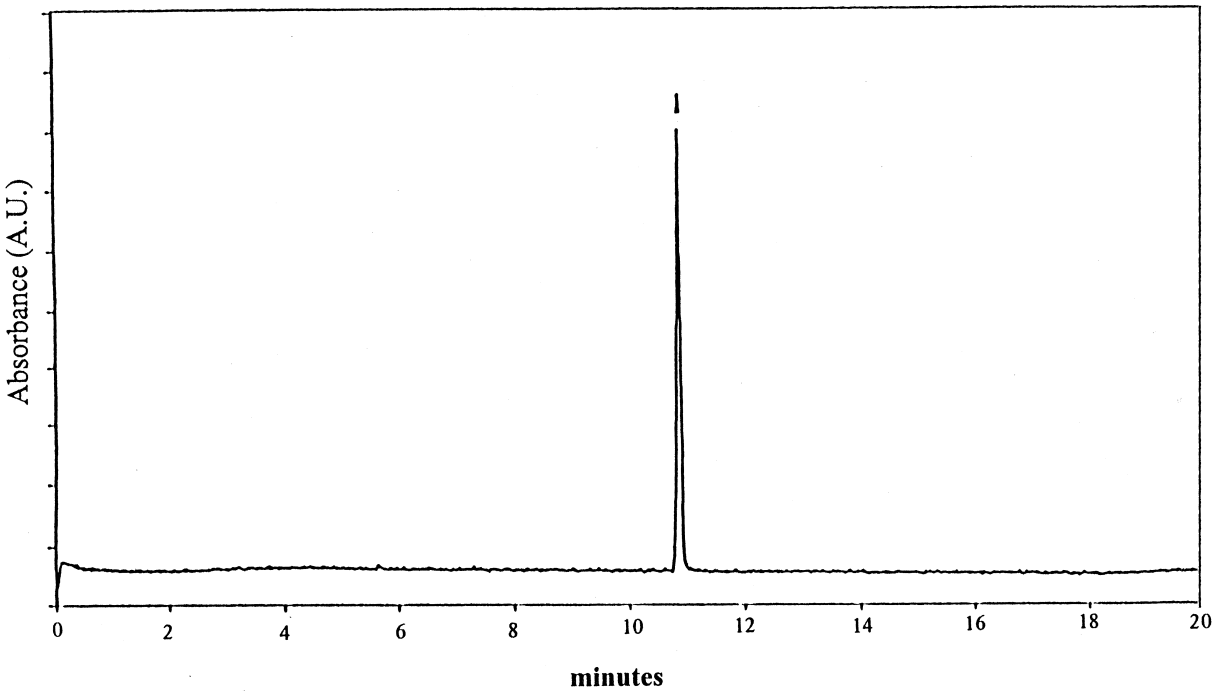

Fig. 7. Electropherogram of 10 μg ml^{-1} of gold (III) ($AuCl_4^-$). Conditions: fused-silica capillary, 60 cm (52.75 cm to detector)×75 μm I.D.; carrier solution, 4 m*M* H^+–25 m*M* Cl^-; applied voltage, −10 kV; UV detection at 214 nm; sampling time, 30 s. Peak: (1) $AuCl_4^-$. From Ref. [36] with permission.

concentration of cations, M^+, in the buffer solution [39].

Sample solutions containing low concentrations of several inorganic anions were run at pH 8.5 with increasing concentrations of sodium chloride or lithium sulfate in the BGE. The plots in Fig. 9 show several interesting effects. One is that the current increases rapidly with increasing salt concentration and levels out at 280 μA around 200 m*M* sodium chloride or lithium sulfate. This sharp increase in current can be attributed to less electrical resistance. The maximum current that can be obtained in our instrument is set at 280 μA. In order to maintain this current, the voltage was automatically lowered as the salt concentration in the BGE continued to increase. The full power of the instrument's power supply was then being used. The electrophoretic mobilities of the sample anions increased at the same time the current was increasing between 0 and 200 m*M* of added sodium chloride lithium sulfate (Fig. 9). Actually, a decrease in electrophoretic mobility is predicted with increasing salt concentration. The initial increases in Fig. 9 can be explained by Joule heating. Under the conditions used a temperature of 49°C was calculated for the capillary at high salt concentrations [38].

From Fig. 9 the greatest difference between electrophoretic mobilities and electroosmotic mobility occurs around 200 m*M* salt in the BGE. Fig. 10 shows an excellent separation of inorganic anions at pH 8.5 in 220 m*M* sodium chloride. The high salt concentration suppresses the EOF sufficiently that no flow modifier is required, even at pH 8.5.

CE of anions in solutions of high salt content has a number of practical applications. Bromide and nitrate in seawater were detected without any pretreatment or dilution of the samples. Anions in other high-salt samples can also be analyzed directly. Fig. 11 shows peaks for 10 ppm bromide and 2 ppm nitrate on 0.50 *M* sodium sulfate. The BGE contained 1.5 *M* pure sodium sulfate. It was also possible to determine both bromide and nitrate in 0.5 *M* sodium perchlorate by using 1.5 *M* sodium perchlorate in the

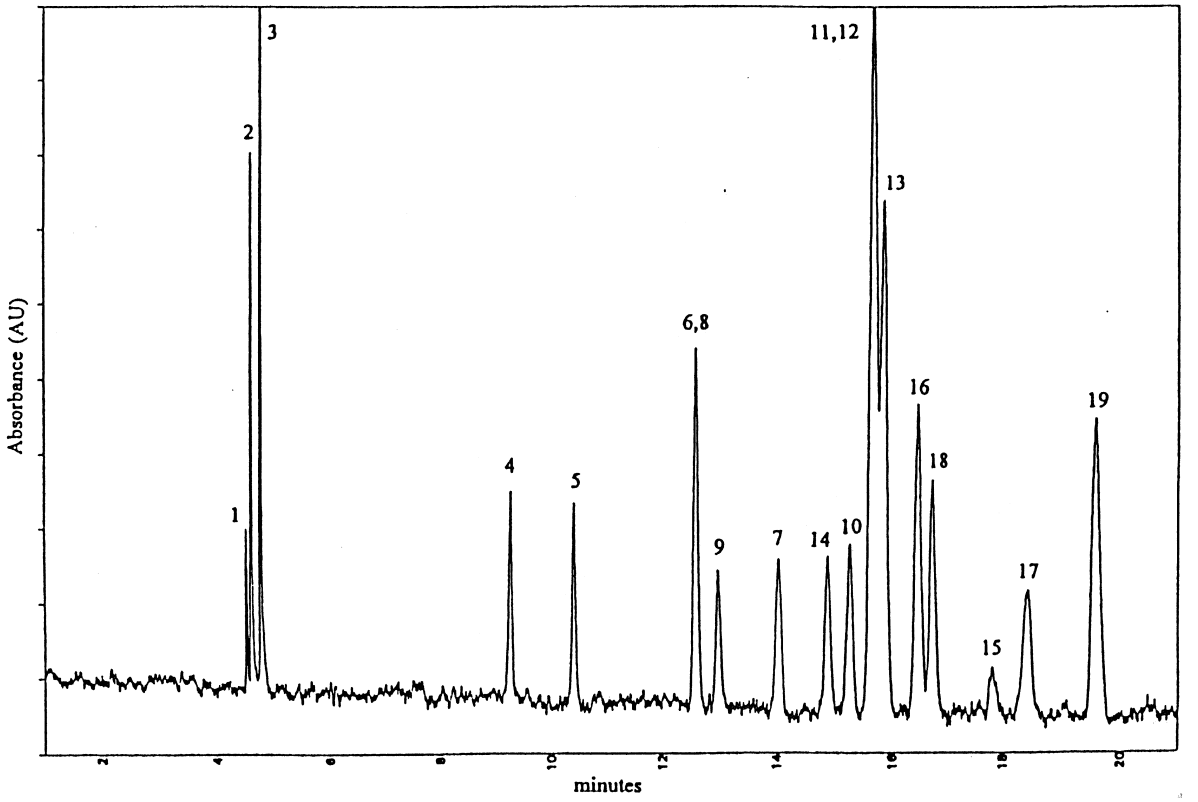

Fig. 8. Electropherogram of 20 common amino acids. 50 m*M* Ethanesulfonic acid, pH 2.8; applied voltage, 30 kV; injection time, 10 s. Peaks: 1=Lys; 2=Arg; 3=His; 4=Gly; 5=Ala; 6=Val; 7=Ser; 8=Ile; 9=Leu; 10=Thr; 11=Asn; 12=Met; 13=Gln; 14=Trp; 15=Glu; 16=Phe; 17=Pro; 18=Tyr; 19=Cys From Ref. [36] with permission.

BGE. With 1.5 *M* sodium chloride in the BGE, perchlorate and nitrate coeluted and only bromide could be measured.

10. Combined ion chromatography–capillary electrophoresis

Perhaps the ultimate method for separation of ions would be one that combines the principles of ion chromatography and capillary electrophoresis into a single technique. CE has unusually high efficiency for separation of ions with at least some difference in ionic mobility. IC is based on differences in the sample ions for ion-exchange sites in competition with the eluent ion. The migration order of ions through a capillary or ion-exchange column may be different. Thus in CE the migration order of the halides is: I^-, $Br^- > Cl^- > F^-$. In IC the migration order is reversed: $F^- > Cl^- > Br^- > I^-$.

An easy way to separate ions on the basis of both their electrophoretic and ion-exchange behavior is simply to add a water-soluble ion-exchange polymer to the BGE in a conventional CE setup. This idea was originally proposed by Terabe and Isemura [40] and was extended somewhat by Stathakis and Cassidy [41,42].

A comprehensive paper by Li et al. [43] used poly(diallyldimethylammonium chloride) (PDDAC) as the soluble anion-exchanger in a BGE containing a relatively high concentration of a salt such as sodium chloride or lithium sulfate for the separation of inorganic and organic anions. The name ''IC–CE'' was coined for separations of this type.

The ion-exchange equilibrium between a sample

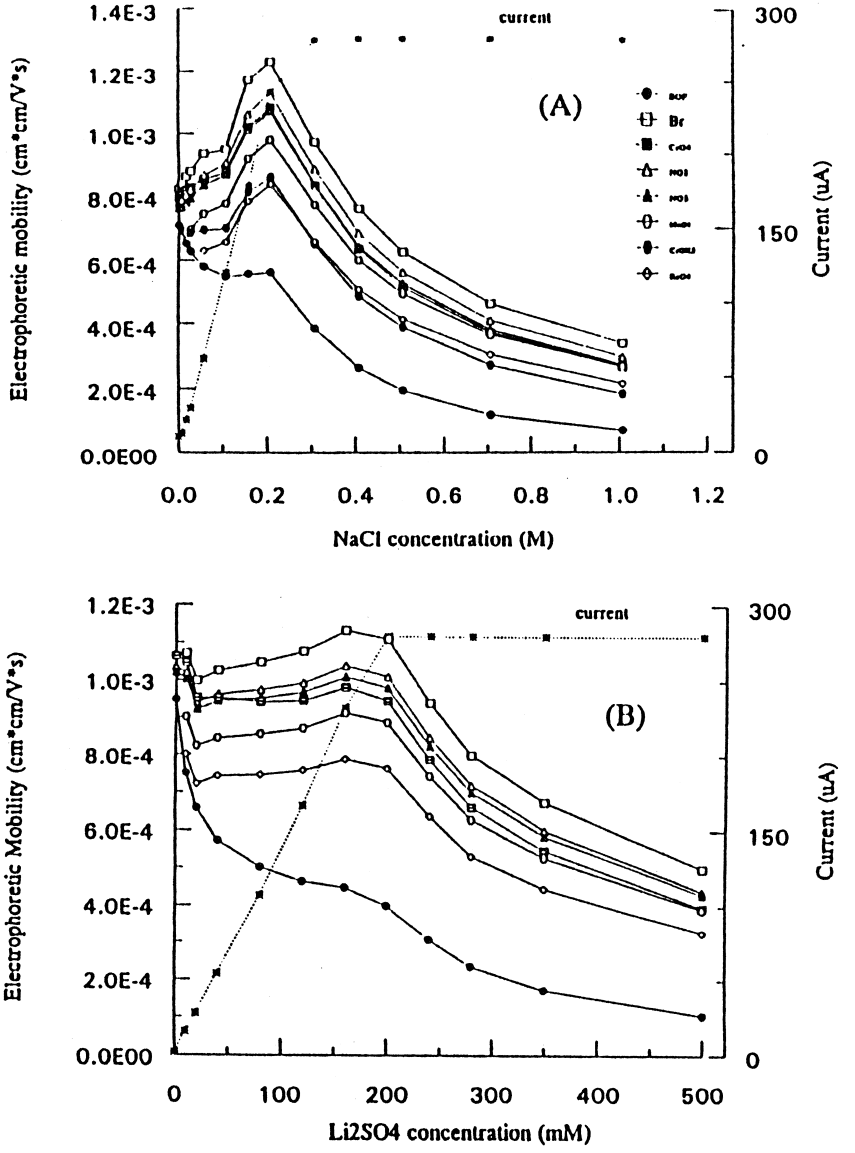

Fig. 9. Plots of electrophoretic mobility and current against the concentration of the salts: (A) sodium chloride, and (B) lithium sulfate. Ions (top to bottom): Br^-, Cr_4^{2-}, NO_2^-, NO_3^-, MnO_4^-, CrO_{x3}^{3-}, ReO_4^-. From Ref. [38] with permission.

anion (A^-) and the polymer ion-exchanger (P^+Cl^-) is given by the following equation:

$$P^+Cl^- + A^- \rightleftharpoons P^+A^- + Cl^- \quad (5)$$

for which the equilibrium constant (K) is:

$$K = \frac{[P^+A^-][Cl^-]}{[A^-][P^+Cl^-]}. \quad (6)$$

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

$$K' = K[P^+Cl^-], \quad (7)$$

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

$$\frac{[A^-]}{[P^+A^-]} = \frac{[Cl^-]}{K'}. \quad (8)$$

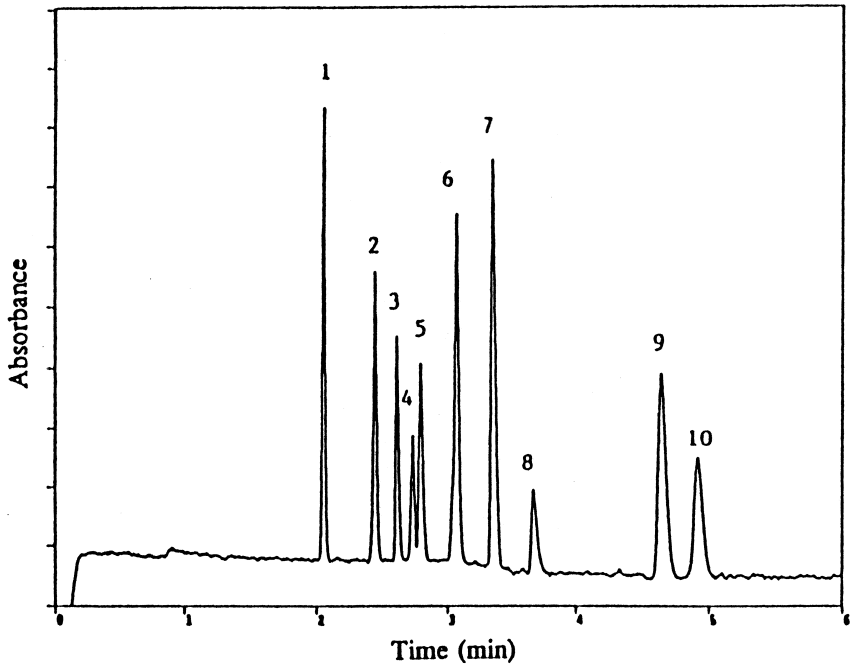

Fig. 10. CE separation of ten inorganic ansions. The 200 m*M* NaCl was added in the carrier electrolyte. Peaks: 1=Br^- (10 ppm); 2=NO_2^- (20 ppm); 3=$S_2O_3^-$ (80 ppm); 4=NO_3^- (2 ppm); 5=N_3^- (40 ppm); 6=$Fe(CN)_6^{4-}$; 7=MoO_4^{2-} (40 ppm); 8=WO_4^{2-} (40 ppm); 9=CrO_{x3}^{3-} (40 ppm); 10=ReO_4^- (40 ppm). From Ref. [38] with permission.

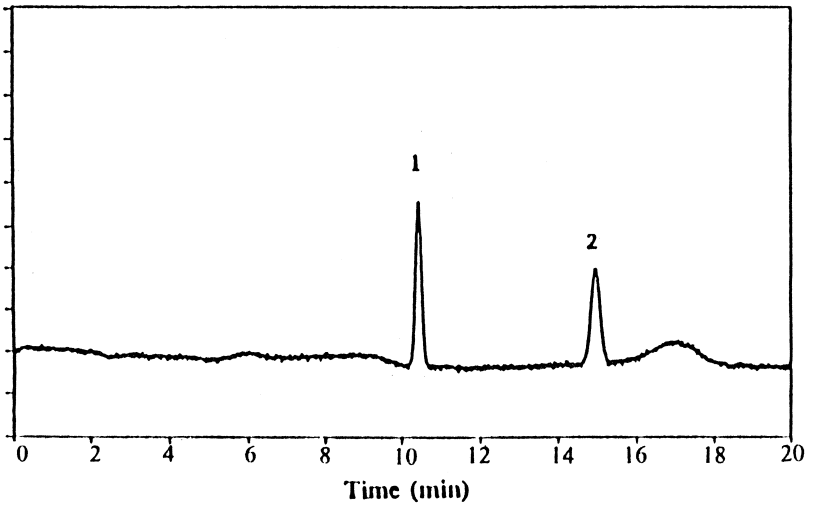

Fig. 11. CE analysis of other high salt samples. Sample was in 0.5 *M* sodium sulfate. 1.5 *M* sodium sulfate was added to the electrolyte. Peaks: 2, Br^- (10 ppm); 2, NO_3^- (2 ppm). From Ref. [38] with permission.

The migration rate of a sample anion will be proportional to the ratio of $[A^-]$:$[P^+A^-]$. The fraction of sample anion present as the free anion (AG) will migrate rapidly toward the anode, while the fraction associated with the ion-exchanger (P^+A^-) will move but slowly in the opposite direction. These equations show that salt concentration in the BGE (Cl^- in this example) will have a major effect on sample analyte migration as well as the polymer ion concentration and the equilibrium constant, *K*.

A negative power supply (−10 kV) is used for separation of anions. The BGE typically contains 0.05–0.30% PDDAC (1.9–11 m*M*), up to 150 m*M* sodium chloride or lithium sulfate and a 20 m*M* borate buffer at pH 8.5. A thin layer of PDDAC is apparently adsorbed on the capillary walls, giving an anodic EOF; the electrophoretic vectors of the sample anions are in the same direction.

The mobility of a sample anion will be a function of the fraction present as the free anion (α_A), the fraction associated with the polymer (μ_{AP}), the mobilities of each species, and the electroosmotic flow (μ_{EO}):

$$\mu_{TOT} = \alpha_A \mu_A - \alpha_A \mu_{AP} + \mu_{eo} \quad (9)$$

The μ_{AP} term is in the opposite direction and appears to be much smaller than μ_A. Thus the total mobility will depend primarily on the first and last term in this equation.

A separation of 17 inorganic and organic anions by IC–CE is shown in Fig. 12 using direct photometric detection. Baseline resolution was obtained

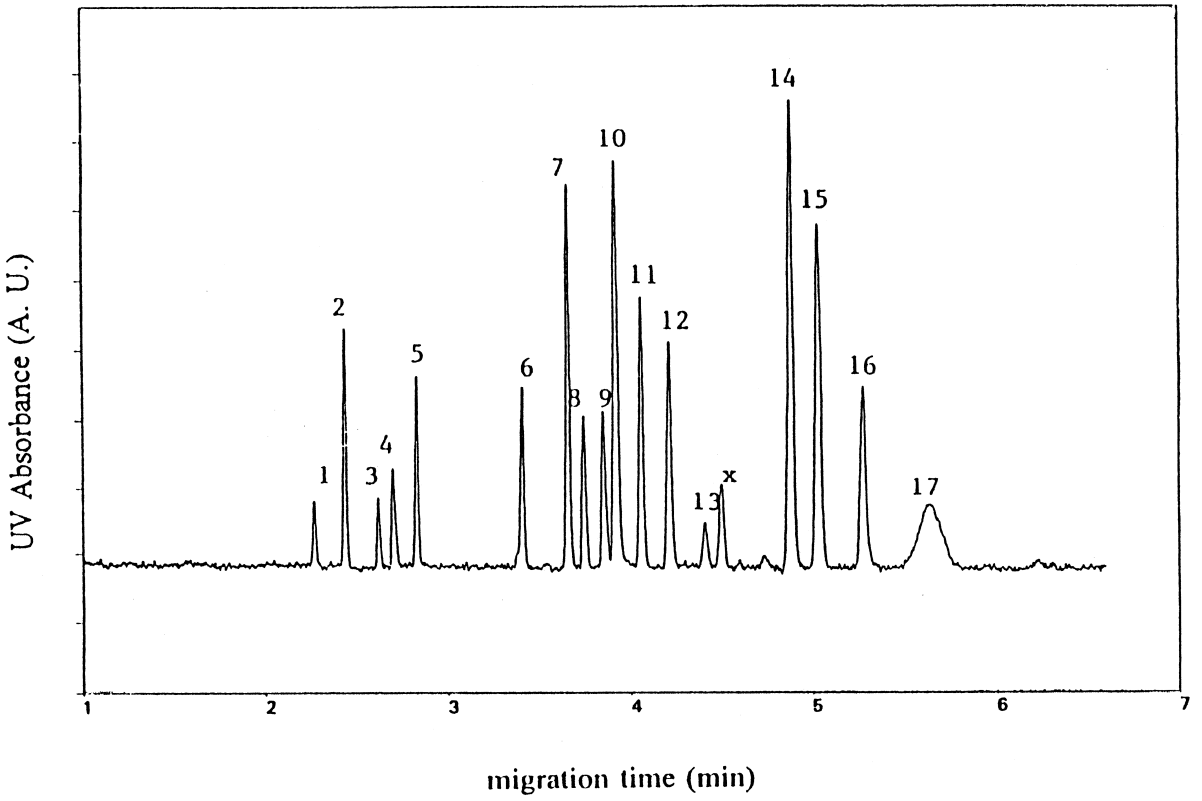

Fig. 12. Separation of 17 inorganic and organic anions. Peaks: 1=bromide; 2=nitrate; 3=chromate; 4=iodide; 5=molybdate; 6=phthalate; 7=1,2,3-tricarboxylate; 8=1,2-benzendisulfonate; 9=terephthalate; 10=isophthalate; 11=benzoate; 12=*p*-toluenesulfonate; 13=1,3,5-tricarboxylate; 14=2-naphthalensulfoante; 15=1-naphthalensulfonate; 16=3,5-dihydroxybenzoate; 17=2,4-dohydroxybenzoate; x=unidentified impurity.

for the bromide and iodide peaks and also for 1- and 2-naphthalenesulfonic acid anions. These separations were possible only because of the ion-exchange component. Bromide and iodide have almost identical electrophoretic mobilities, as do the two naphthalenesulfonic acid isomers.

11. Conclusions

At least two major conclusions can be drawn from the topics discussed here:

1. Capillary electrophoresis has a truly remarkable power to separate complex mixtures of cations and anions. Separations are fast and require only a small amount of sample.
2. The problems associated with CE are being solved. Simple methods are being developed to control EOF and assure reproducible migration times. A broader range of detection methods is available.

Perhaps the ultimate test of any analytical method is the extent to which it is used to solve practical problems. Professional users rightly demand that an analytical technique be effective, simple and highly reliable. Ion analysis by CE is now very close to meeting these criteria.

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