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New electrolyte systems for capillary zone electrophoresis of metal cations and non-ionic organic compounds

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New electrolyte systems for capillary zone electrophoresis of metal cations and non-ionic organic compounds

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To my wife, Lin, for her love and understanding

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GENERAL INTRODUCTION

High-performance capillary electrophoresis has become the most attractive separation technique since its introduction in the early to middle 1980's. In comparison with other early developed electrophoretic techniques, modern capillary electrophoresis uses smaller diameter capillaries and higher voltages which results in much higher separation efficiency.

At the present time, conventional liquid chromatographies (reverse-phase, normal-phase and ion chromatographies) are still dominant separation techniques in the liquid phase but capillary zone electrophoresis (CZE) promises even better separation efficiency. CZE has other advantages over conventional HPLC techniques. It is easy to change the conditions for CZE separations due to no requirement of stationary phase. CZE is capable of separations very small amount of analytes, and needs much less solvent. Of course, CZE also has disadvantages. CZE is not suitable for preparative separations in large scale and has difficulty in reaching a low detection limit because of very small volume and short optical path length of capillary. As CZE is still at its early stage of development, some unsolved problems remain, such as unstable electroosmotic flow which influences reproducibility and quantitative aspects of analysis. In general, CZE is not just a separation technique in competition or simply as an alternative to HPLC, but as an additional method complementary to HPLC.

Several new electrolyte systems which can be used to separate metal cations and

non-ionic organics with high separation efficiency are described and many separation parameters are discussed in the dissertation.

Dissertation Organization

The present dissertation contains six chapters. The first chapter, GENERAL INTRODUCTION, gives background information about capillary electrophoresis and a literature review. The following two chapters are two papers published in *Journal of Chromatography*, which contain research work on development of new electrolyte systems of capillary zone electrophoresis of metal cations. The next two chapters are a paper published in *Journal of High Resolution Chromatography* and a paper submitted to *Analytical Chemistry*, which discuss CZE electrolytes for separation of non-ionic organic compounds. The GENERAL CONCLUSIONS is the final chapter. The major professor, Dr. James S. Fritz, is the only co-author of all four publications.

There is a reference list for each of the first five chapters. Except for the GENERAL INTRODUCTION and the GENERAL CONCLUSIONS, each chapter contains a summary or abstract and conclusions.

Historical

Although modern capillary electrophoresis was developed early to the middle 1980's, it is the descendant of numerous electrophoresis and chromatography techniques. The discovery of fundamentals by a large number of researchers over the course of the past 200 years has permitted very rapid development of capillary electrophoresis is very

quickly. After Faraday stated his laws of electrolyte in 1933 and 1934[20], researchers began to study electrophoretic migration and electrophoresis in late 19th century[21-27]. Among these researchers, Whetham[25] obtained "moving boundary"separation of solution of colored ions in vertical tubes, Picton and Linder[26] studied movement of colloids in tube with electric field and Hardy[27] demonstrated globulin movement in "U" tube with electric current. The works on development of electrophoresis were continued[28-32] during this century and it was proved that electrophoretic methods are very useful for biomolecular separations in the early of 1990's[30-33]. Tiselius received the Nobel Prize for his contribution for development of the moving boundary method with which he studied the electrophoresis of proteins[30,31].

In 1923, Kendall and Crittenden[34] preparatively separated rare earth metal ions by "ion migration method" which was the first isotachophoresis. In the late 1930's and the early 1940's, Svenson[35] and Longsworth[36] developed zone and displacement electrophoresis. Martin[37] reported the first displacement electrophoresis in narrow bore tubes. In 1963, Konstantinov and Oshurkova[38] detected the presence of separated zones in moving boundary electrophoresis by photographic methods. At the same time Martin and Everaert[39] commenced work on a book describing the technique of displacement electrophoresis, or isotachophoresis, as it now is called, which was based on work presented in Everaerts' doctoral thesis[40]. This book laid the foundation for performing electrophoretic separations in capillaries and also described methods of in-line detection of the separated zone.

In 1967, Hjertén[41] published his dissertation in which he suggested that zone

electrophoresis could be carried out in narrow bore tubes and demonstrated separations with 3-mm i.d. tubes using UV detection to specify the separated zone. He also demonstrated a separation of bismuth and copper cations in a free solution with a narrow tube. Virteman[42] extended this work with 200-500μm capillaries using potentiometric methods of detection. The separation efficiency obtained in these studies was not very high, because the inner diameters of the capillaries were still large and relatively low voltage was used.

In 1979, Mikkers *et al.*[43,44] reported high performance capillary zone electrophoresis of 16 organic acids in PTFE capillaries with inner diameter of 200μm. Separated zones were detected by means of a UV and a conductivity detector. The separation were excellent, but the peak shape was poor due to the limitations of the equipment, which had been designed for capillary isotachophoresis. The detector also had insufficient sensitivity, and thus required relatively large sample loads. This led to a potential drop across the separation zone, giving the asymmetric peak shapes.

For the first time, Jorgenson and Lukacs[45,46] experimentally achieved and theoretically explained high resolution electrophoresis in glass capillaries. They described a separation in a 100-cm, 75-μm i.d. silica capillary with a driving potential of 30 kV using in-capillary fluorescence detection. They studied the effect of electroosmosis. These workers exploited electroosmotic flow in their separations and, with the inherent sensitivity of the detection system, symmetric peaks were obtained. A simple theory describing the separation processes was also presented.

The appearance of high performance of capillary electrophoresis (HPCE) showed

that capillary electrophoresis was a promising new general method for analytical and micropreparative separations of ionic species, especially for biochemically important samples. However, capillary electrophoresis would be a limited technique if it were useful only for charged species. Fundamental studies on micellar electrokinetic capillary chromatography (MECC or MEKC), have been provided by Terabe[47-49], while data pertaining to geometrical parameters, column efficiency, wall treatment and velocity profiles have been developed as well[50-53]. MECC provides a way to resolve non-ionic molecules. The breakdown of micelles in electrolyte solution containing high concentration of organic solvent and separation of neutral organic molecules by solvophobic association with tetraalkylammonium ion by CZE was investigated by Walbroehl and Jorgenson[54].

During the development of CZE and MECC, another important subtechnique of capillary electrophoresis, capillary gel electrophoresis (CGE) attracted several researchers attention[55,56]. This technique is based on the new high performance capillary electrophoresis and traditional slab-gel electrophoresis. Gels are potentially useful for electrophoretic separations because they are an anticonvective media; they minimize solute diffusion, which contributes to zone broadening; they prevent solute adsorption to the capillary walls; and they eliminate electroosmosis, allowing maximum resolution in short lengths of column. CGE has been successfully used for protein separation[56,57], oligonucleotide separation[58,59] and DNA sequencing[60,61].

Chiral capillary electrophoresis has been developed with CZE, MECC and CGE[62-66], which is especially useful for bioseparation and pharmaceutical separation.

Now, more and more analytical chemists are involved in capillary electrophoresis research, and more and more commercial instruments are available in the market place.

Instrumental

A basic configuration of a capillary electrophoresis instrumental system is illustrated in Figure 1. Capillary electrophoresis is generally performed between two electrolyte buffer reservoirs. A bare or coated capillary with 25 to 100μm i.d. is usually used and a high voltage of 5 to 30 kV is applied to generate an electric field to drive electrically charged species to migrate towards an appropriate electrode. On- column detection is quite usual although sometimes an off-column is used. Electropherograms may be recorded with a data acquisition device (a chart record or a computer acquisition system).

Column

Packed columns Before the development of high-performance capillary electrophoresis, in 1974, Pretorius[67] *et al* applied high voltages over columns (1mm i.d.) packed with a microparticulate chromatographic phase (75 - 125μm Partisil). He demonstrated that a typical reverse-phase eluents (MeOH-H_2O) could be driven by electroosmosis instead of pumping using on octane-coated silica gel. This method reduced plate height and increased separation efficiency, but the absence of suitable injection and detection systems prevented analytical application. In 1981, Jorgenson and Lukacs[46] solved the instrumental problems and showed the separation of methylanthracene

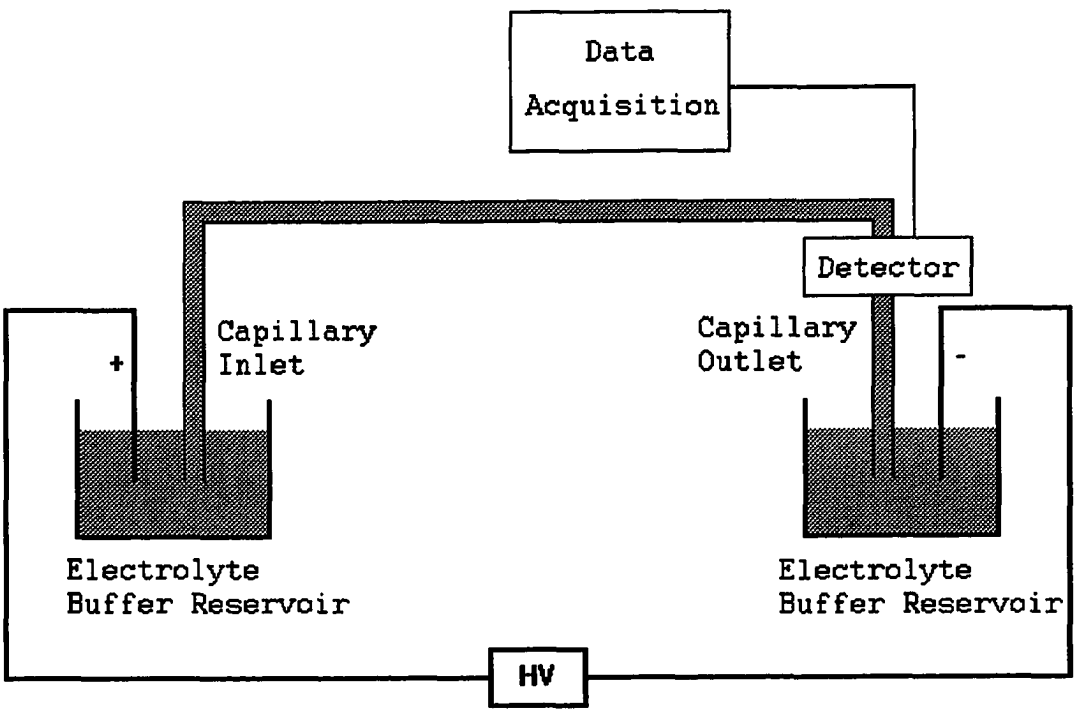

Figure 1. Basic configuration of capillary electrophoresis system.

and perylene on a 175μm i.d. column packed with 10μm octadecylated silicagel (ODS), with acetonitrile as the mobile phase and using an on-column fluorescence detector. In 1987, Knox[68] reported similar separation of polycyclic aromatic hydrocarbons (PAH) in a 50μm i.d. column with 5μm ODS packing. Combining pressure and voltage, Tsuda[69,70] separated compounds with similar capacity factors but different charges.

The effect of particle size on the flow profile and the influence of the mobile phase compositions on electroosmosis have been studied[68,71]. The contributions from the packing particles to the plate height have been discussed and it has been suggested that, using sub-micron particles, all other contributions than diffusion should become insignificant[72]. This has, however, not yet been realized.

Gel-filled columns These columns are used for capillary gel electrophoresis. Most gel-filled capillaries used today have diameters between 50 to 100μm, which represents a compromise between minimizing thermal gradients across the capillary, maximizing the detection path length, and practicalities involved in gel fabrication. Gel materials can be the traditional gels used for slab-gel electrophoresis (polyacrylamide[58,60] and agarose[73,74]) or entangled polymer solutions[75,76], which have a low viscosity and are pumpable. Gel-filled columns are mainly used for biological macromolecular separations and will not be discussed here in detail.

Open columns High-performance CZE and MECC are performed in open-tubular capillaries. Although borosilicate glass[45,46], Teflon[77] and other material-made capillaries have been used, in recent years, almost all capillary electrophoresis separations have been performed in polyimide-coated fused silica capillaries. The main advantages of

fused silica capillaries over other kinds of capillaries available today include their flexibility, good thermal and optical properties in UV range, and most importantly, the availability of high-quality fused silica tubings with internal diameters below 100μm. The drawbacks with use of fused silica capillaries are hydroxy groups existing on the surface, which causes interaction with charged molecules and limits detection sensitivity, especially when on-column optical detection is used. The later disadvantage is caused by use of small inner diameter capillaries in order to minimize heating effect.

In order to eliminate the electrostatic interactions, which is a significant problem for separations of charged macromolecules like proteins, polymer-coated capillaries have been used. A great variety of coating materials has been proposed. Resistance to hydrolysis is important as the working pH-range in CE analysis is larger than in LC: values can be as low as 2 but also as high as 12. Operating principles also differ: non-ionic coatings are used for shielding of the fused silica surface from polar interaction and ionic coatings used for charged repulsion between coating and analyte. They can gave contrary effects to the one intended if the analyte is hydrophobic or has a charge opposite to the coating. Therefore, it is not likely that a single, universal coating will solve all problems. Table I lists coating materials used for fused silica capillaries.

As mentioned above, one of the main problems associated with the use of small bore cylindrical capillaries is the limitation on detection sensitivity when on-column optical detection is employed. The causes of the problem include short path length, and distortion and scatter of light caused by the rounded capillary walls. Use of rectangular capillaries can alleviate this problem. Rectangular borosilicate glass capillaries were

TABLE I. Capillary coatings for CE

Coatings	Reference
1. Non-ionic coatings	
Methylcellulose	41, 78
Maltose	79
Polyacrylamide	80, 81
Polyarylic acid	82
Polyvinylpyrrolidone	82
Siloxanes	41, 83, 84, 85
Polyols	82, 86
Polyethylene glycol	78, 87
Epoxyldiol	79
Glyceroglycidoxypropyl	82
Aryl pentafluoro	88
2. Ionic coatings	
Aminopropylsilation	89, 90
Sulfonic acid based cation exchangers	91
Polyethylene imine	92
Physically adsorbed cationic polymer	93

chosen to replace cylindrical capillaries by Tsuda *et al*[94]. Typical dimensions of rectangular capillaries ranged from 16μm x 195μm to 50μm x 1mm. Detection across the long cross-sectional axis provides a significant increase in sensitivity of detection techniques which depend on path length.

Use of rectangular capillaries provides another advantage. The advantage of rectangular profiles of capillaries were first pointed out in a theoretical study[95] which indicated that temperature gradient between the center of the capillary and the outside capillary wall are five to ten times lower for rectangular capillaries than for cylindrical capillaries due to higher surface area-volume ratio which is favorable to heat dissipation. However, rectangular capillaries available today (Wilmad Glass Co., Buena, NJ) are only in borosilicate glass with no protective coating. Therefore, they tend to be much more fragile than polyimide-coated fused silica tubings and are not suitable for hydrodynamic injection. Tsuda *et al.*[94] overcomed the problem by utilize a split-flow injector. Nevertheless, rectangular capillaries will not be widely accepted until protective coating on the tubings is available.

When on-column optical detection is applied on polyimide coated capillaries, the coating material must be removed before using. Removal of coating material will leave a fragile detection window. However, replacement of polyimide with an optical transparent capillary coating for silica can solve the problem and help to make CE columns much easier to handle during change of column and everyday use. Recently, fused silica tubings with UV transparent coating has become available commercially (Polymicro Technologies, Inc., Pheonix, AZ).

Injection

In capillary electrophoresis, like in HPLC, injection causes extra-column contribution to peak dispersion[96-99]. Therefore, it is important to ensure that the sample injection method employed is capable of delivering small volumes of sample (typically several naroliters) into the column efficiently and reproducibly[99-101]. Hydrodynamic injection[99,102,103] and electrokinetic injection[45,46,103] are two common injection modes used in capillary electrophoresis. In both injection modes, one end of the capillary is used as the sample injector directly to eliminate the zone broadening caused by connection with sample valves[45,46,98,103-105].

Hydrodynamic injection Hydrodynamic injection can be accomplished in four ways:

1. by elevating capillary at the sample end, permitting sample introduction by siphoning[106];
2. by applying pressure on the individual sample vial[107];
3. by applying vacuum on the detection end of the capillary[102, 103]; or
4. by injecting with a syringe and employing a splitter to reduce the volume introduced into the capillary[108].

Hydrodynamic injection is generally useful for capillaries with i.d. between 25 to 100μm. Below 25μm, it is difficult to inject sufficient sample into the capillary. Above 100μm, hydrodynamic flow are far too fast to permit a small, reproducible injection.

Electrokinetic injection This injection technique uses electrophoretic and/or electroosmotic migration to inject samples into the capillary by applying a voltage for a

short of period. High-mobility solutes will be enriched compared to low-mobility solutes because of different mobilities. Electrokinetic injection can be useful if selective injection of anions or cations is required.

In comparison with electrokinetic injection method, hydrodynamic injection has no inherent discrimination of the solutes injected. Thus, in most of cases, hydrodynamic injection is preferred.

Sample overloading Separation efficiency of capillary electrophoresis can be reduced by sample volume overloading[100] and sample concentration overloading[101].

The maximum number of theoretical plates (N_{max}) of the overall system is proportional to the square of the ratio of the volume of the injected sample (q_{inj}) to the volume of the column (q_c)[100] or

$$N_{max} = 12 (q_c/q_{inj})^2 \qquad (1)$$

From equation (1), it can be seen that increase of injected sample volume will cause decrease of the maximum number of theoretical plates.

Concentration overloading is caused by the difference in electrical conductivity of the sample and the electrophoretic medium. At high sample concentration, system efficiency can be degraded due to perturbation in potential field gradient by the sample within the capillary and severely distorted peaks may result. On the other hand, if the sample injected has a slightly lower conductivity than the electrophoretic buffer, sampling can be achieved with improved peak shape and hence increased efficiency.

Sample stacking When the conductivity of the injected sample is lower than that of the surrounding buffer, the analyte zone will be narrowed[109-111]. This phenomenon

is called sample stacking. The electric field depends inversely on the specific conductivity, or higher field strength at lower conductivity. Therefore, the electric field strength increases at the higher field and hence the ions in the analyte zone are compressed into a narrow band. This effect can be utilized in both hydrodynamic injection and electrokinetic injection.

Investigation of the dispersion processes by Vinther and Soeberg[109,110] indicated that moderate stacking conditions should be employed during injection, that is, the sample solution should have a specific conductivity only sightly lower than that of the buffer solution. The reason is that radial dispersion increases if there is a large difference in conductivity between the sample solution and the buffer solution.

Detection

Nearly all detection method used for liquid chromatography are suitable for CE. Table II lists some detection methods which have been employed for capillary electrophoresis. On-column UV absorbance and fluorescence are the most popular detection methods and the only ones that have been implemented in commercial instruments at present time. In this dissertation, UV absorbance is the exclusive detection method chosen for CZE studies due to the lack of selections of the commercial instrument. Therefore, the following discussion is limited to UV absorbance.

On-column UV detection is the easiest to achieve in practice and UV detectors are the most common detectors in commercially available CE instruments. UV absorbance is relatively universal by comparison with other methods. Commercial UV detectors are

Table II. Detection methods employed in capillary electrophoresis

Detection method	Reference
1. Optical detection	
UV absorbance	112-120
Fluorescence	83,121-127
Refractive-index	128-130
Raman	131,132
Chemiluminescence	133
Circular dichroism	134
2. Electrodetection	
Potential gradient	135-137
Conductivity	43,138-141
Amperometry	142-148
Potentometry	149,150
3. Mass spectrometry	
Ion spray	151,152
Electrospray	153-157
Coaxial CF-FAB	90,158-161
4. Radio-active	162-164
5. Ion Mobility Spectrometer	165

readily available at low cost. Because the optical path length of capillaries is limited, only moderate sensitivity can be achieved in capillary electrophoresis.

Generally UV detection requires chromphores in analyte, but indirect UV detection can be used for analytes without chromphores. For example, most of metal cations have no sufficient UV absorbance and, thus an indirect UV detection can be selected, a strongly UV-absorbed cation, called UV co-ion is added to electrolyte buffer. Therefore, the electrolyte has high background. When an analyte, metal cations, passes the detection window, it displaces the UV co-ion, causing a decrease of absorbance background and giving a negative peak (Figure 2).

Theoretical

The electrophoretical mobility, μ_{ep}, of a particle is defined by the linear velocity, ν_{ep}, per unit of applied electric field, E:

$$\mu_{ep}(m^2/V.s) = \nu_{ep}(m/s)/E(V/m) \qquad (2)$$

The electrophoretic mobility is caused by electrostatic interaction of an ion in the electrolyte buffer solution and applied electric field. If an ion has charge of q and radius of a, its quantitative value of electrophoretic mobilty can be obtained by balancing electromotive force, qE, and frictional force, $6\pi a\eta\nu_{ep}$ (Stokes' law):

$$qE = 6\pi a\eta\nu_{ep} \qquad (3)$$

or

$$\mu_{ep} = \nu_{ep}/E = q/6\pi a\eta \qquad (4)$$

where η is the viscosity of the electrolyte buffer solution.

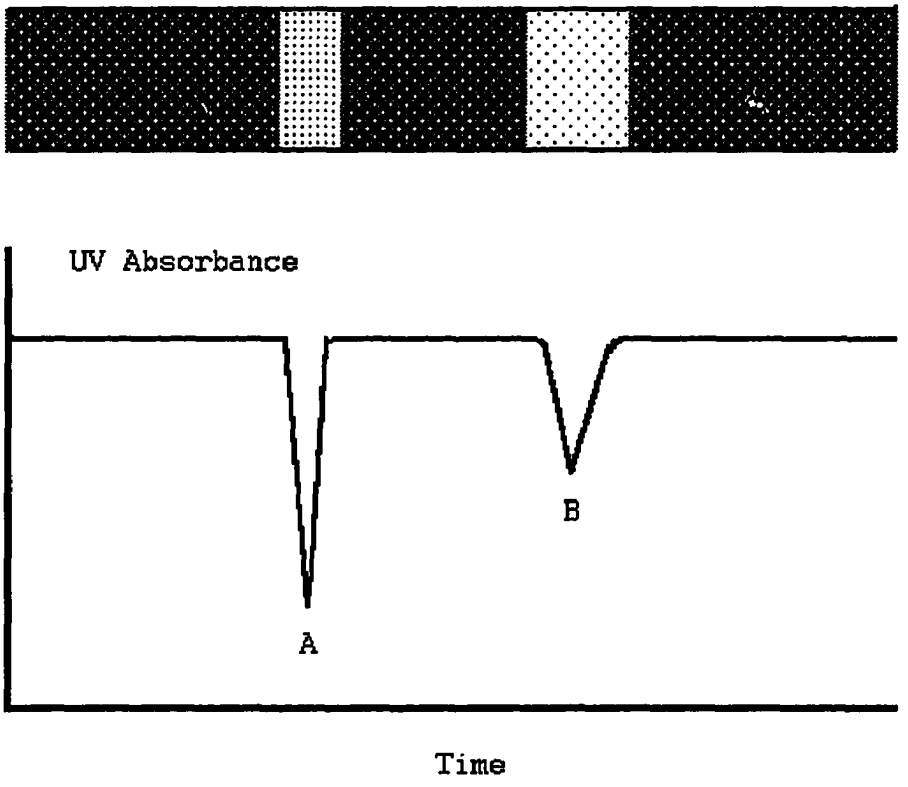

Figure 2. Indirect detection principle - displacement of UV co-ion causing a negative absorption peak.

Uncoated fused silica is commonly used for CE. Some of silanol groups inside of capillary deprotonize and result in a negatively charged surface. Cations from the buffer move toward this surface in order to neutralize the negative charges and form a positively charged double layer. The potential across the layer is called *zeta* potential, ζ. If a electric field is applied, the cations in the double layer migrate toward the cathode (negative end) and drag the whole solvent to move in the same direction. This migration of the whole solvent is denoted as electroosmotic flow (EOF). Similar to the electrophoretic mobility, the electroosmotic mobility, μ_{eo}, under the electric field strength E can be obtained by balancing electromotive and frictional forces and also considering the transition from curved geometry of an ion to flat geometry of the capillary wall:

$$\mu_{eo} = \epsilon\zeta/4\pi\eta \qquad (5)$$

The solvent in HPLC is driven by pressure, which results in a parabolic flow profile (Figure 3(b)). The flat flow profile of solvent flow originating at the wall is expected in CE (Figure 3(a)), because the double layer is very thin (up to several hundred nanometers) compared to the inner diameter of capillary. This flat flow profile and lack of need for a stationary phase give CE much higher separation efficiency than HPLC (theoretical plate number of CE: up to 1,000,000; that of HPLC: 10,000).

The apparent mobility of an ion, μ, is the sum of its electrophoretic mobility and electroosmotic mobility

$$\mu = \mu_{ep} + \mu_{eo} \qquad (6)$$

If ν is the apparent migration speed of ions, L_d the capillary length between injection end and detection window, L the total length of the capillary, V the applied voltage and t_M

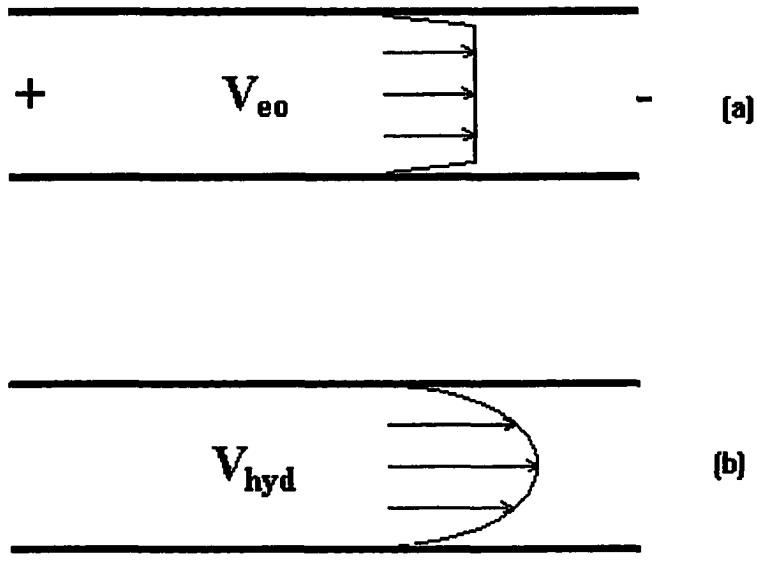

Figure 3. (a) Flat flow profile in capillary electrophoresis;

(b) parabolic flow profile in HPLC.

migration time of solute ion, the apparent mobility can be calculated as

$$\mu = \nu/E = L_d L/t_M V \quad (7)$$

or

$$t_M = L_d L/\mu V = L_d L/(\mu_{ep}+\mu_{eo})V \quad (8)$$

The migration order for cations, neutral species and anions is shown in Figure 4. The coelectroosmotic capillary electrophoresis of cations and counterelectroosmotic capillary electrophoresis of anions[5] make cations elute first, then neutal species, and finally anions. All neutral analytes have same apparent mobility because electrophoretic mobility of neutral species is zero.

The direction of electroosmotic flow can be changed by either dynamically[16,166-168] or permanently changing the surface charge[93], and, thus, the elusion order of cations, neutral species and anions can be reversed.

The most common and simplest CE technique is capillary zone electrophoresis, which separation is based on the difference in the electrophoretic mobilities of analytes. For successful separation analytes must have mutually different apparent mobilities resulting in different migration velocities of their zones during the analysis. The efficiency of CZE separation can be expressed by analogy with chromatography by the number of theoretical plates, N[169]:

$$N = t_M^2/\sigma^2 = t_M^2/(w/4)^2 \quad (9)$$

where t_M is the migration time of the analyte and σ^2 is the total variance of the concentration profile of a zone. The total variance of the concentration distribution of a migration zone can be expressed as the sum of the variances due to all independent

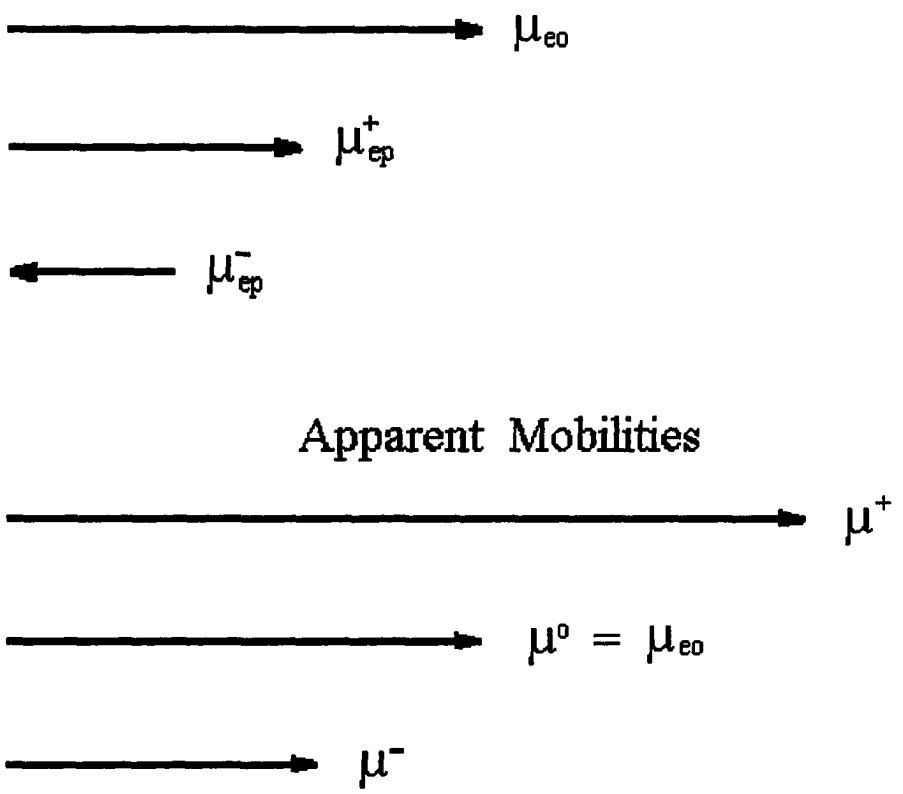

Figure 4. Electroosmotic and electrophoretic mobility vectors, and migration order for cations, neutral species and anions.

dispersion sources:

$$\sigma^2 = \sigma_I^2 + \sigma_D^2 + \sigma_T^2 + \sigma_{eo}^2 + \sigma_O^2 \qquad (10)$$

where the right-hand side terms represent the contributions of injection, diffusion, Joule heating, electroosmosis dispertion and other effects, respectively.

The resolution R_s between closely eluting peaks can also be defined by analogy with chromatography:

$$R_S = \Delta t_M / \bar{w} \qquad (11)$$

where Δt_M is the difference in migration time of two peaks and $\bar{w} = \frac{1}{2}(w_1 - w_2)$ the average peak width at base. Assuming that $\bar{\mu} \approx \mu_1 \approx \mu_2$ and using equation (7), the migration time difference can be written as:

$$\Delta t_M = (L_d L/V)(\Delta\mu/\bar{\mu}^2) \qquad (12)$$

Rewriting equation (9) and replacing $\bar{t}$ with equation (7) gives:

$$\bar{w} = 4\bar{t}_M/N^{1/2} = 4(L_d L/\bar{\mu}V)N^{-1/2} \qquad (13)$$

and the resolution can be written as:

$$R_S = (\Delta\mu/\bar{\mu})(N^{1/2}/4) \qquad (14)$$

Assuming that only factor contributing to peak dispersion is the diffusion of the analytes, the variance σ^2 can be related to diffusion coefficient D by the Einstein equation:

$$\sigma^2 = 2Dt_M \qquad (15)$$

Substituting N with equation (9) and assuming $L_d \approx L$, the equation (14) becomes:

$$R_S = (\Delta\mu/4)(V/2D\bar{\mu})^{1/2} \qquad (16)$$

The equation means that increasing the applied voltage inproves resolution.

However, when the applied voltage is too high, the Joule heating effect may become significant.

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SEPARATION OF METAL IONS BY CAPILLARY ELECTROPHORESIS WITH A COMPLEXING ELECTROLYTE

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Abstract

Excellent separations of metal ions can be obtained very quickly by capillary electrophoresis provided a weak complexing reagent is incorporated into the electrolyte to alter the effective mobilities of the sample ions. Indirect photometric detection is possible by also adding a UV-sensitive ion to the electrolyte. Separations are described using phthalate, tartrate, lactate or hydroxyisobutyrate as the complexing reagent. A separation of twenty-seven metal ions was achieved in only 6 min using a lactate system. A mechanism for the separation of lanthanides is proposed for the hydroxyisobutyrate system.

Introduction

Although ion chromatography continues to be the major method used to determine anions and many inorganic cations, capillary zone electrophoresis (CZE) holds the promise of even better separations. It is becoming more and more common to use the term

capillary electrophoresis (CE) instead of capillary zone electrophoresis. Some excellent separations of ions have been achieved by CE. A complete separation of all thirteen lanthanides has also been obtained (3).

Separations by CE are based on differences in the electrophoretic mobilities of the sample ions. In cases where the mobilities of the free cations are very similar, the reagent is often added to partially complex the sample cations and thereby increase the differences in effective mobilities. The separations are affected by the type and concentration of completing reagent and by other factors such as pH, ionic strength, and viscosity. When indirect photometric detection is used, the separation may also be influenced by the chemical nature and concentration of the UV-active reagent.

In the present work, some new reagents are evaluated for the separation of a number of inorganic metal ions. In the CE of the lanthanides, the distribution of free metal ion and metal-complex species is calculated and a mechanism is proposed for the excellent separation that is obtained.

Experimental

CE was performed with Waters Quanta 4000 Capillary Electrophoresis system (Millipore Waters, Milford, MA, USA), equipped with a positive power supply. Polyamide-coated, fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA), 60 cm in length with an I.D. of 75 μm, were used. A window for on-column detection was created 7.3 cm from the end of the capillary. Indirect UV detection was employed at 214 nm. The separation voltage was applied at 15 kV to 30 kV.

At the beginning of each experimental day, it was sufficient to rinse the capillary with deionized water for about 15 minutes. However, in order to keep the capillary clean, the capillary was flushed with 0.5M KOH solution after being used three or four days.

Hydrostatic sample mode was selected for injection and sample time was set at 30 seconds. Before each run, a two minute purge of capillary with electrolyte was programmed. A Curken 250-1B plotter (Curken, Danbury, CT, USA) was used for recording electropherograms. All standards and electrolytes were prepared using 18MΩ deionized waters by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). All reagents for preparing electrolytes were of analytical-reagent grade. HIBA and UV-Cat1 were obtained from Waters (Milford, MA, USA). Phthalic acid, p-toluidine and phenylethylamine were purchased from Aldrich (Milwaukee, WI, USA). Tartaric acid and 4-methylbenzylamine were supplied by Fisher Scientific (Fair Lawn, NJ, USA) and Fluka (Ronkonkoma, NY, USA), respectively.

Results and Discussion

General conditions for separation

CE separation of metal ions is based on differences in the mobility of the ions in an electric field. Unfortunately, in some cation groups the individual cations have almost the same mobility. The divalent transition metal cations and the lanthanide cations are examples. In such cases addition of a water-soluble complexing reagent to the capillary electrolyte is used to obtain larger differences in effective mobility. This occurs by complexing the metal ions to differing extents. Ions that are complexed to a greater

degree move more slowly through the capillary than those that have a lower fraction of the element in the complexed form. The effects of pH and concentration of complexing reagent have been previously pointed out (7).

Some way to detect the separated metal ions must be available. This problem was solved by indirect photometric detection (1,2). An organic cation is added to the electrolyte which absorbs in the UV spectral region. A proprietary reagent developed at Waters (UV-Cat1) was found to work well. We also tried a number of aryl derivatives of aliphatic amines, which form cations when protonated. Of those tried, phenylethylamine, benzylamine, p-toluidine and 4-methylbenzylamine were the most satisfactory. In order to optimize separation and detection efficiencies, it is important to choose a suitable combination of complexing reagent and UV-sensitive reagent. Some adjustment in the concentration of a UV-sensitive reagent is often required to obtain optimal separation conditions.

Separations using phthalate

Several complexing reagents were tried in connection with the attempted separation of several mono- and divalent metal cations. Only partial complexing of the metal cations is desired because complete complexing is apt to result in species that move at the same rate as the electroosmotic flow. The following complexing reagents were tried initially: hydroxyisobutyric acid (HIBA), phthalic acid, malonic acid and succinic acid. Of these, HIBA and phthalic acid were the most promising. HIBA had been used previously for the separation of several mono- and divalent metal ions (1). However, we obtained even

better separations with phthalic acid. A good separation of eight metal ions in about 6 min is shown in Figure 1.

Separations with phthalic acid were usually better when some methanol was added to the electrolyte. Methanol caused the moving times to be longer and resolution to be better. Elution times of the ions studied increased with added methanol up to about 20% methanol (by volume). With further increases in the proportion of methanol there was a plateau with no further changes in elution times.

Separations using tartrate

After optimizing conditions for CE with a phthalate system, it was found that even better separations of metal cations could be obtained with tartrate as the complexing reagent. Tartrate forms comparatively weak complexes with a number of metal ions and is therefore suitable for CE. However, copper (II) is more strongly complexed and elutes very late or not at all.

An excellent separation of twelve metal ions in a total time of only 4 min is shown in Figure 2. Resolution is excellent with a steady, flat baseline, as shown in Figure 2A. At the higher concentration in Figure 2B, the peaks are very narrow andwell shaped.

Some optimization of conditions was required to obtain separations of the quality shown in Figure 2. The concentration of tartrate and p-toluidine used, pH and applied voltage were the most important variables. Methanol (20%) was added to the electrolyte to improve resolution of the divalent metal ions.

Much of the literature on capillary electrophoresis does not adequately address the

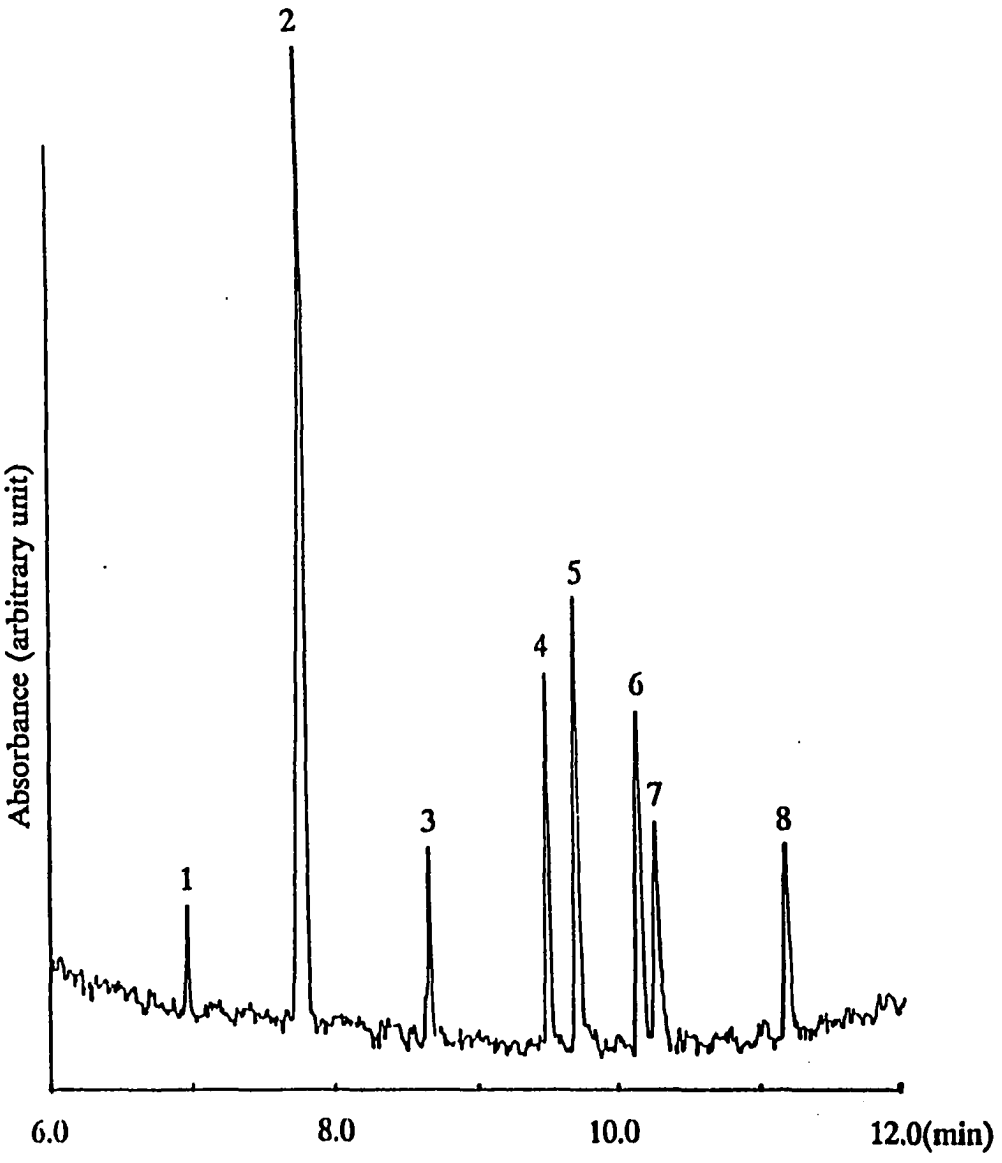

Figure 1. Separation of metal ions using phthalate. Electrolyte, 2 mM phthalic acid, 5 mM UV-Cat1, 20% methanol, pH 4.3; applied voltage, 15 kV. Peaks: 1=K^+; 2=Na^+; 3=Pb^{2+}; 4=Mn^{2+}; 5=Co^{2+}; 6=Ni^{2+}; 7=Zn^{2+}; 8=Cd^{2+}.

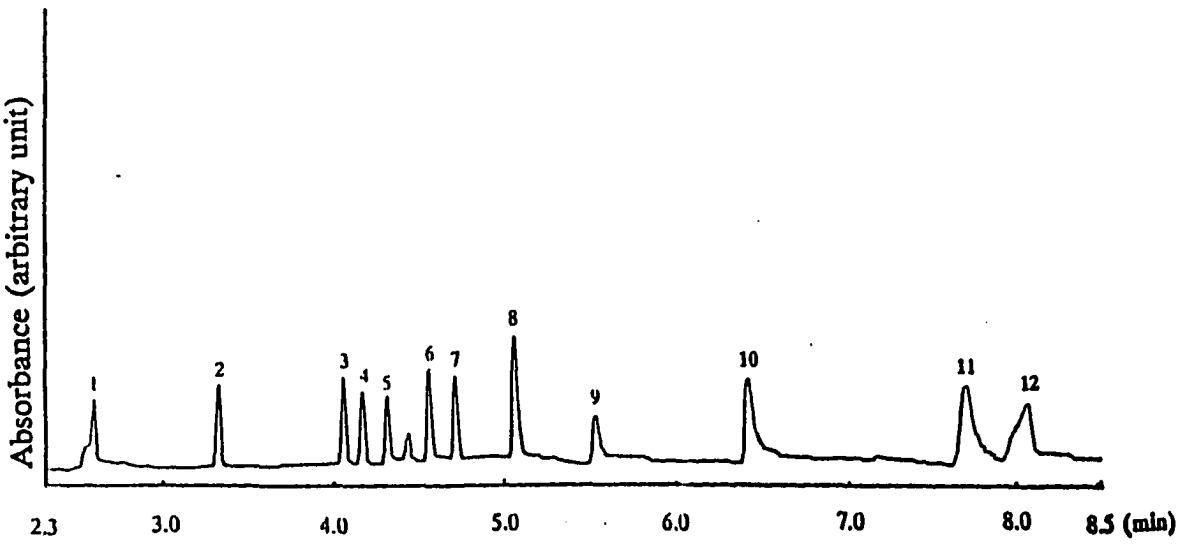

Figure 2A. Separation of 12 alkali, alkaline earth and transition metal ions using tartrate. Electrolyte, 2.5 mM tartaric acid, 6 mM p-toluidine, 20% methanol, pH 4.8; applied voltage, 30 kV. Peaks: 1=K^+ (1.3 μg/ml); 2=Na^+ (0.8 μm/ml); 3=Li^+ (0.2 μg/ml) 4=Mg^{2+} (0.3 μg/ml); 5=Ba^{2+} (1.3 μg/ml); 6=Sr^{2+} (1.3 μg/ml); 7=Mn^{2+} (0.8 μg/ml); 8=Ca^{2+} (0.8 μg/ml); 9=Cd^{2+} (1.3 μg/ml); 10=Co^{2+} (1.0 μg/ml); 11=Ni^{2+} (0.8 μg/ml); 12=Zn^{2+} (0.8 μg/ml).

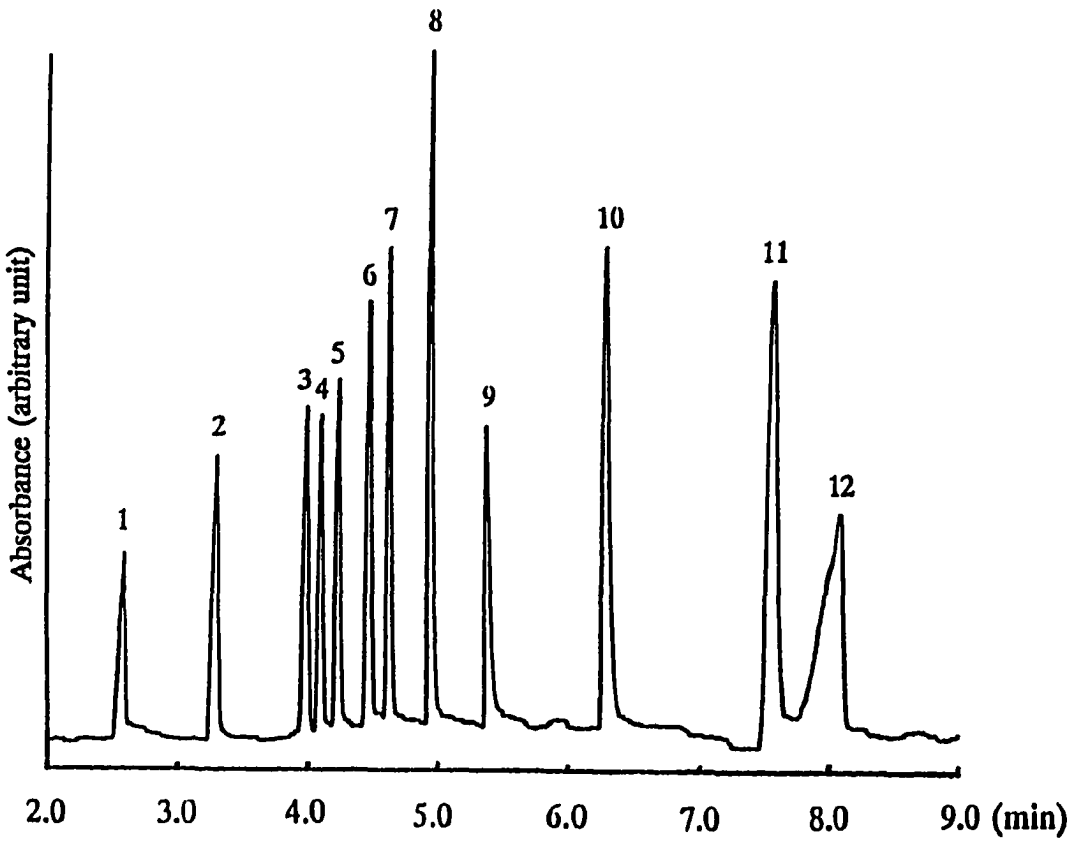

Figure 2B. Separation of 12 alkali, alkaline earth and transition metal ions using tartrate. Four times higher concentration of each analyte as in Figure 2A. Electrolyte conditions and applied voltage same as described in Figure 2A.

question of the quantitative aspects. We therefore prepared calibration curves for the metal ions studied in the tartrate system. Good linear calibration curves were obtained for the ions studied in the 0.4 to 10 ppm concentration range. The only exception was zinc (II) which gave a relatively poor correlation coefficient for linear regression.

Separations using lactate

Lactate has the same complexing groups as tartrate (carboxyl and hydroxy) but is a smaller molecule and forms somewhat weaker complexes with most metal ions. Preliminary experiments indicated that a lactate system might give good separations both for divalent metal ions and for trivalent lanthanides.

A brief optimization of major conditions was first carried out for separation of the lanthanides. These conditions included the concentrations of lactate and UV visualization reagent, and pH. A very good separation of the thirteen lanthanides was obtained. It was also found that excellent separations could be obtained under the same conditions for alkali metal ions, magnesium and the alkaline earths, and several divalent transition metal ions. All of these except copper (II) eluted before the lanthanides. An excellent separation of 27 metal ions was obtained in a single run that required only 6 min (Figure 3).

The detection limit for these metal ions was in the range 0.05—0.5 ppm. Light ions are in the low detection limit range, and heavy ions are in the high detection limit range. The average deviation in peak height from one run to another was approximately ± 5% or smaller at the ion concentrations used.

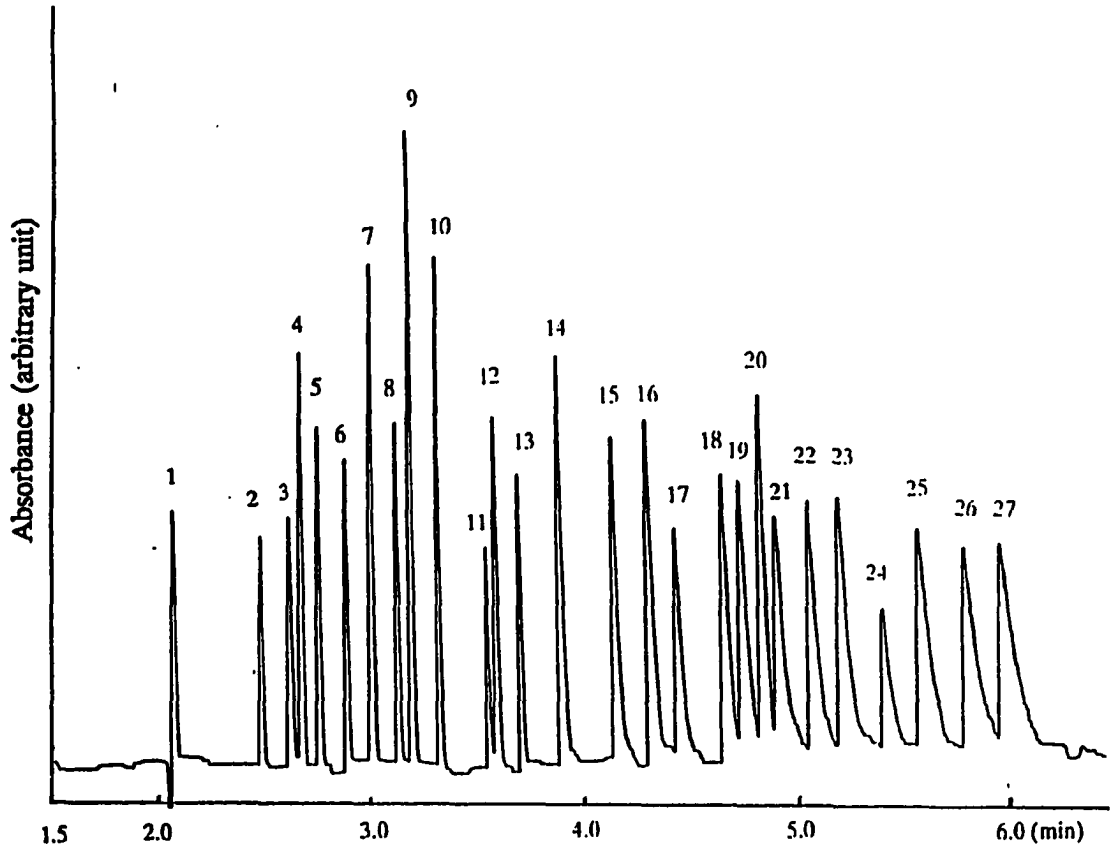

Figure 3. Separation of 27 alkali, alkaline earth, transition and rare earth metal ions in a single run using lactate. Electrolyte, 15mM lactic acid, 8mM 4-methylbenzylamine, 5% methanol, pH4.25; applied voltage, 30kV. Peaks: 1=K^+; 2=Ba^{2+}; 3=Sr^{2+}; 4=Na^+; 5=Ca^{2+}; 6=Mg^{2+}; 7=Mn^{2+}; 8=Cd^{2+}; 9=Li^+; 10=Co^{2+}; 11=Pb^{2+}; 12=Ni^{2+}; 13=Zn^{2+}; 14=La^{3+}; 15=Ce^{3+}; 16=Pr^{3+}; 17=Nd^{3+}; 18=Sm^{3+}; 19=Gd^{3+}; 20=Cu^{2+}; 21=Tb^{3+}; 22=Dy^{3+}; 23=Ho^{3+}; 24=Er^{3+}; 25=Tm^{3+}; 26=Yb^{3+}; 27=Lu^{3+}. The concentration of each lanthanide, Ba^{++}, Sr^{++}, and Pb^{++} was 5 μg/ml; the conentration of transition metals, K^+, Na^+, and Ca^{++} was 3—4 μg/ml, Mg^{++} was 2 μg/ml and Li^+ was 1 μg/ml.

Separations using HIBA

HIBA has been used extensively for CE. Very good separations of magnesium, the alkaline earths and several of the divalent transition metal ions have been reported (1). Excellent separation of all of the lanthanides have also been obtained (1,3). In one instance, thirteen lanthanides plus six other metal ions were separated in a single run, although the shapes of some of the earlier peaks were rather poor (1,3).

We experimented with separation of the lanthanides using HIBA as the complexing reagent. A brief optimization was employed to establish pH and suitable concentrations of HIBA and the UV-sensitive reagent. The concentration of HIBA needed was found to be considerably lower than lactic acid in the previous system. A truly excellent separation of the lanthanides was obtained (Figure 4). The conditions we established are quite similar to those previously reported.

The separation mechanism

In capillary electrophoresis it is now common to use a reagent to partially complex metal ions and improve the quality of their separation by altering their net flow characteristics. For example, addition of tartrate to a solution containing metal cations (M_1^{++}, M_2^{++} , etc.) may convert some fraction of the metal ions to tartrate complexes (M_1 tart, M_2 tart, etc.). The free cations move along the capillary at rates proportional to their ionic mobilities while the complexed metal ions move at a slower speed. Continuous equilibration between free and complexed metal ions causes each metal to move through the capillary as a tight zone. Separation occurs because of the different overall rates at

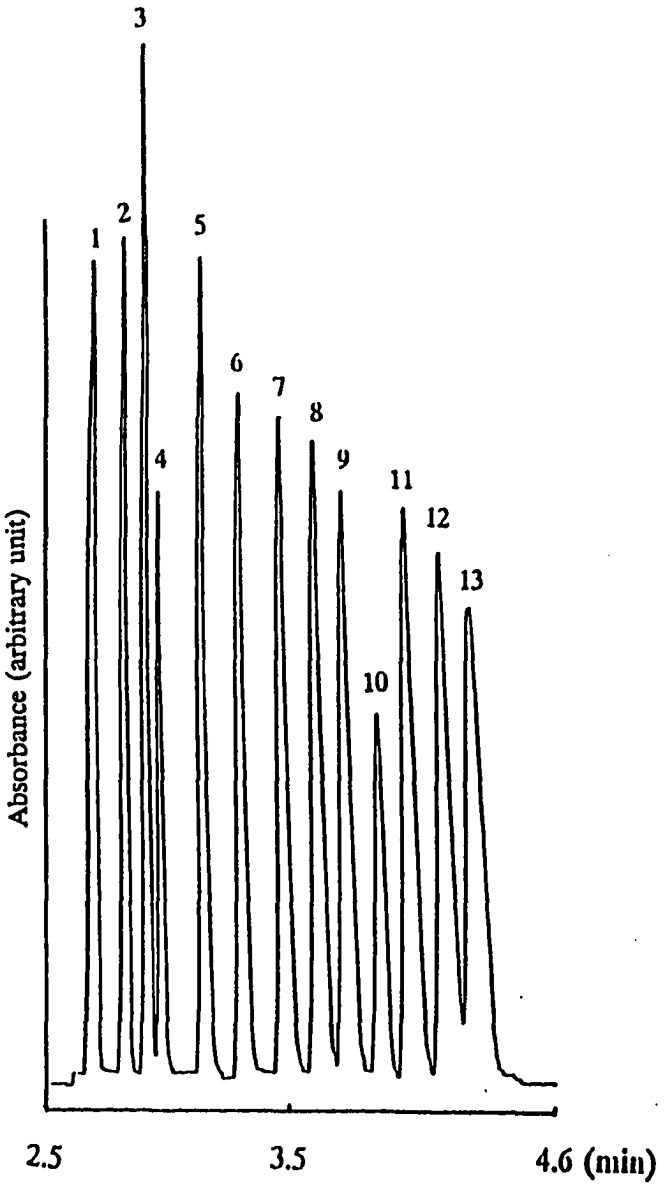

Figure 4. Separation of 13 lanthanides using HIBA. Electrolyte, 4 mM HIBA, 5 mM UV-Cat1, pH 4.3; applied voltage, 30 kV. Peaks: 1=La^{3+}; 2=Ce^{3+}; 3=Pr^{3+}; 4=Nd^{3+}; 5=Sm^{3+}; 6=Gd^{3+}; 7=Tb^{3+}; 8=Dy^{3+}; 9=Ho^{3+}; 10=Er^{3+}; 11=Tm^{3+}; 12=Yb^{3+}; 13=Lu^{3+}.

which the zones move. The situation is again somewhat analogous to HPLC except that in this case the "stationary phase" (metal tartrate complexes) is not a real phase and is not stationary, but moves at a slower speed than the free metal cations.

The more completely a metal ion is complexed, the slower will be its rate of movement. If the fraction of metal in the free cation is too small, it may take a long time for the metal to emerge from the capillary and no good separation will be obtained. However, if the various metal ions are not sufficiently complexed (too large a fraction of free metal ion), ions of similar ionic mobility may be poorly separated. Almost nothing has appeared in the literature concerning how strongly metal ions should be complexed for optimal separation by CE. However, Hirokawa et al. (9) computed the absolute mobilities of some lanthanide complexes used in isotachophoresis.

We considered the separation of rare earths using 4.0 mM hydroxyisobutyric acid (HIBA) at pH 4.3 as the complexing reagent (see Figure 4). Using published formation constants (10), the fraction of rare earths present in various chemical forms was calculated by a well-known method (11,12) under the same conditions of pH and HIBA concentration used for the CIE separation in Figure 4. The calculated distribution of chemical species for each rare earth is shown in Table I.

Some interesting conclusions can be drawn from the information in Table I. The predominating species are the free metal ion (M^{3+}), the 1:1 complex (probably ML^{2+}), the 2:1 complex (probably ML_2^{+}) and the 3:1 complex (probably ML_3). A small fraction of the higher rare earths is also present as the 4:1 complex. Another striking feature is that the average number of ligands associated with a rare earth ($\bar{n}$) increases rapidly with

Table I Fraction of free (α_M) and complexed (α_{ML_n}) rare earth metal ions and average number of ligands ($\bar{n}$) in 4 mM HIBA electrolyte solution at pH 4.3.

Metal	α_M	α_{ML}	α_{ML2}	α_{ML3}	α_{ML4}	n
La	0.578	0.360	0.612			0.482
Ce	0.448	0.496	0.052	0.004		0.612
Pr	0.407	0.496	0.093	0.005	0.000	0.679
Nd	0.333	0.572	0.085	0.010	0.000	0.772
Sm	0.296	0.520	0.170	0.013	0.001	0.903
Gd	0.250	0.481	0.244	0.024	0.001	1.045
Tb	0.181	0.470	0.307	0.040	0.002	1.212
Dy	0.141	0.384	0.387	0.084	0.004	1.426
Ho	0.122	0.365	0.413	0.093	0.006	1.494
Er	0.097	0.309	0.472	0.112	0.010	1.629
Tm	0.079	0.309	0.473	0.123	0.016	1.686
Yb	0.070	0.296	0.431	0.169	0.033	1.797
Lu	0.047	0.222	0.514	0.172	0.045	1.946

increasing atomic number. This occurs in a fairly regular manner as demonstrated by a plot of $\bar{n}$ against atomic number which has a linear regression correlation coeffient of 0.9958.

In CE the positively charged complexes, as well as the free metal cation, would be expected to move through the capillary by electrophoretic flow as well as by the electroosmotic flow that affects all species. However, the electrophoretic mobility should be slower for ML^{2+} than for M^{3+} and still slower for the larger ML_2^+. Even if the different species move at different rates, rapid equilibrium shifts should keep a tight zone for each of the rare earths. For example, as the faster moving M^{3+} starts to move ahead of the other species, it reequilibrates with the ligand (L) to form a larger fraction of the slower-moving species. At the back edge of the zone, the slower-moving complexes reequilibrate to give a greater fraction of M^{3+}. The average rate of movement should depend on the weighted average of the mobilities of the different species.

This last speculation was tested by plotting the moving time of the various rare earths (from Figure 4) against the $\bar{n}$ values in Table I. This plot is shown in Figure 5. It is essentially linear with a correlation coefficient of 0.9978.

Conclusions

Use of complexing electrolyte can achieve very high efficiency for separation transition metal cations and lanthanide cations. It was found that lactate is an excellent complexing additive for separation these metal cations with very similar electrophoretic mobilities. With using methanol as a electrolyte modifier and 4-methylbenzylamine as a

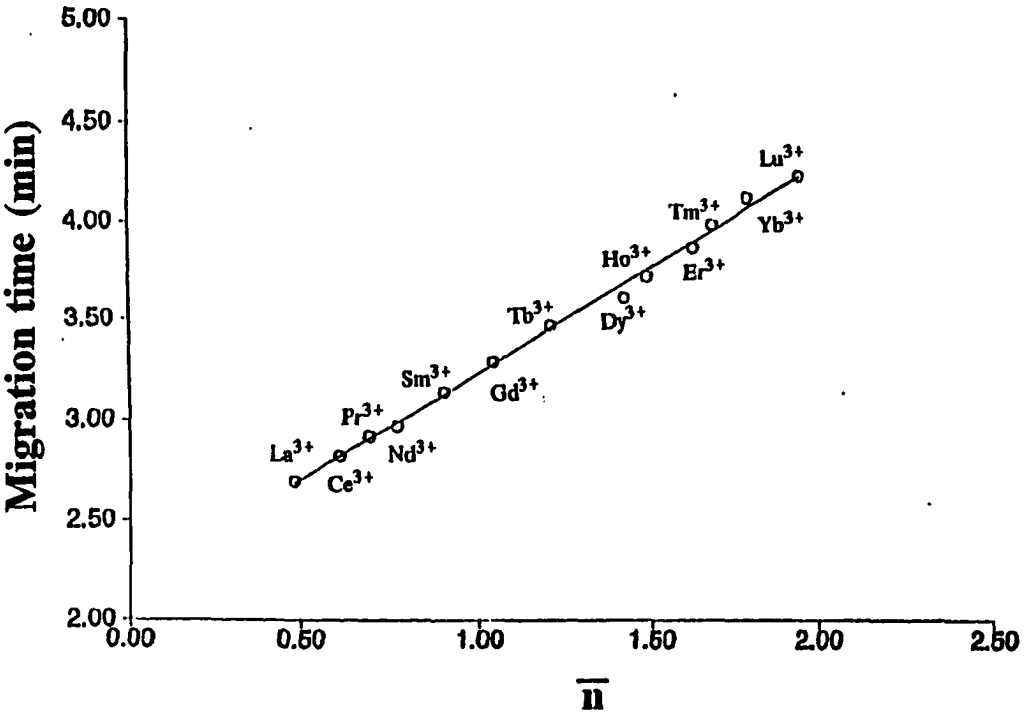

Figure 5. Plot of migration time *vs.* average number of ligands for 13 lanthanides. Electrolyte conditions and applied voltage same as described in Figure 4.UV co-ion, 27 metal cations can be separated in 6 minutes with baseline resolution.

Separation of metal cations using complexing electrolyte is based on the different complexing ability of metal cations which may have same mobility in a free electrolyte solution. More strongly complexed metal ions have large size and low electric charge, and migrate less fast. Plotting migration time *vs.* average number of ligands in HIBA electrolyte solution shows a linear relationship.

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We wish to thank Waters Division of Millipore for their gift of the Waters Quanta 4000 CE system and associated chemicals. We also thank Andrea Weston and Peter Jandik for valuable technical assistance in the initial stages of this research.

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NEW ELECTROLYTE SYSTEMS FOR DETERMINATION OF METAL CATIONS BY CAPILLARY ELECTROPHORESIS

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Youchun Shi and James S. Fritz

Abstract

Many +1 and +2 metal cations can be separated efficiently by CE using lactic acid as a complexing reagent. Addition of a crown ether, as well as lactic acid, permits the separation of K^+ and NH_4^+ in addition to the ions previously separated. The problem of determining trace amounts of metal ions in the presence of a very high concentration of another metal ion was also addressed. For example, a large Na^+ peak (1000 ppm) covers up the peaks of Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} (1 ppm each). However, addition of increasing concentrations of methanol to the electrolyte permits complete resolution of Ca^{2+} and Mg^{2+} from the Na^+. Further addition of a crown ether moves Sr^{2+} and Ba^{2+} to longer migration times and permits resolution of these ions as well. Separation of metal cations with slower complexation kinetics is possible under conditions where only the free metal ions are present. Aluminum(III) and vanadium(IV), along with several other metal ions, were separated at pH 3.2 using nicotinamide as a buffer and as a reagent for indirect detection.

Introduction

The art and science of separation of metal cations continues to develop at a fast pace. Indirect detection by means of a chromogenic cation (often called a visualization reagent) is quite common, although suppressed conductivity (1) and electrochemical detection (2) have recently been used for some inorganic cations. It has become quite common to include a weak complexing reagent in the electrolyte to enhance the separation of cations with very similar electrophoretic mobilities. Several authors have reported excellent separations based on these principles (3-11). In a recent publication we reported the separation of 27 metal cations in only 6.0 min using lactic acid as an auxiliary complexing reagent (11). However, it was not possible to resolve the potassium and ammonium peaks from one another.

In the present work an electrolyte containing both lactic acid and a crown ether was used to separate 16 of the most commonly determined metal ions. The effect of methanol on this system was also investigated. Quantitative aspects of the separations were studied and the problem of determining trace metal ions in the presence of a high concentration of another cation was addressed.

Experimental

A Waters Quanta 4000 capillary electrophoresis system (Millipore Waters, Milford, MA, USA), equipped with a positive power supply was employed to separate metal cations and generate all electropherograms. Polyamide-coated, fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA), were 60 cm in length with an

I.D. of 75 μm and a distance of 52.5 cm from the point of injection to the window of on column detection. Indirect UV detection was employed at 214 nm or 254 nm. A voltage of 15 to 30 kV was applied for all separations. A hydrostatic sample mode was chosen for the separations. A Servogor 120 flatbed recorder (GOERZ Instruments Ges.m.b.H., Austria) was used to plot electropherograms.

All standards and electrolytes were prepared with analytical-reagent grade chemicals and 18 MΩ deionized waters by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA).

Lactate electrolyte buffers were mixed with lactic acid (J. T. Baker, Phillipsburg, NJ, USA), 4-methylbenzylamine (Fluka, Ronkonkoma, NY, USA), methanol (Fisher Scientific, Fair Lawn, NJ, USA), and 18-crown-6 (Aldrich, Milwaukee, WI, USA). The 4-methylbenzylamine was used as protonated cations both for indirect detection of the sample cations and for pH-adjustment.This reagent is identical to Waters UV-Cat 1 which is patented for use as an indirection detection reagent in capillary electrophoresis (12).

The uncomplexing buffer was prepared with nicotinamide (Sigma, St. Louis, MO, USA) and other modifer. Formic acid (Aldrich, Milwaukee, WI, USA) was used to adjust pH.

Results and Discussion

Electrolytes containing lactic acid and a crown ether

Lactic acid makes possible the separation of metal ions with almost identical mobilities by complexing the individual metal ions to varying degrees. The divalent

transition metal ions and the lanthanides are two examples. However, ammonium and potassium(I) cations also have virtually identical mobilities and are not complexed by lactic acid. Previous investigators found that ammonium and potassium ions can be separated by CE if a suitable crown ether is incorporated into the electrolyte (13). The potassium ion is selectivity complexed and its mobility is reduced just enough to permit a good separation.

We found that an electrolyte containing both lactic acid (11 mM) and a crown ether (2.6 mM 18-crown-6) will permit an excellent electrophoretic separation of 16 metal ions, including NH_4^+ and K^+ (Figure 1). The electrolyte also contained 7.5 MM 4 methy- benzylamine as a reagent for indirect detection of the sample ions. Separations in which 12-crown-4 or 15-crown-5 was used in place of 18-crown-6 failed to separate NH_4^+ and K^+.

Incorporation of 18-crown-6 into the electrolyte containing lactic acid affects the migration of several metal ions other than K^+ and NH_4^+. The crown ether increases the migration time of Sr^{++} by 15%, Pb^{++} by 18% and Ba^{++} by 35%, apparently by complexation to form a bulkier, less mobile species. The effect of 18-crown-6 on the migration times of metal ions, compared to lactic acid alone, is summarized in Table I.

Effect of Methanol

Several investigators have studied the effect of organic solvents on CE (11,14-16). A non-ionic marker was used to measure the electroosmotic flow as a function of methanol in the electrolyte in metal ion separations. The electroosmotic flow coefficient decreases in a non-linear manner, as shown in Figure 2. The electrophoretic flow

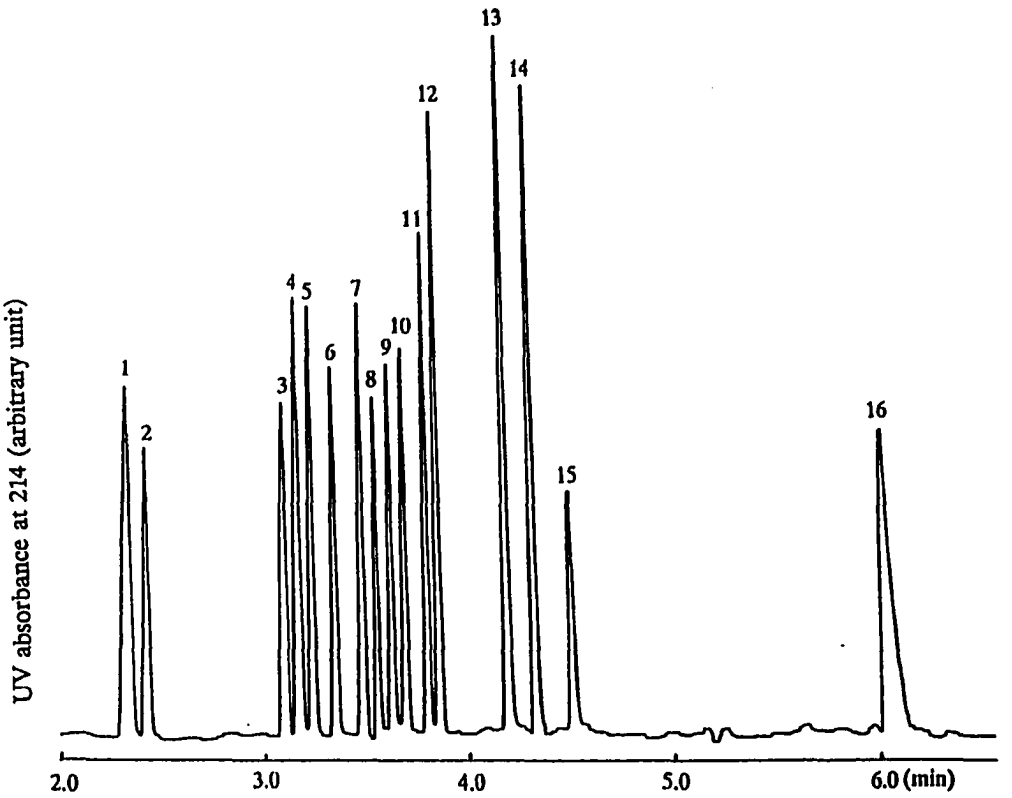

Figure 1. Separation of 16 common metal ions and ammonium. Electrolyte, 11 mM lactic acid, 2.6 mM 18-crown-ether, 7.5 mM 4-methylbenzylamine, 8% methanol, pH 4.3; applied voltage, 30 kV; injection time, 30 s. Peaks: 1=NH_4^+(5ppm), 2=K^+(5ppm), 3=Na^+(3ppm), 4=Ca^{2+}(3ppm), 5=Sr^{2+}(5ppm), 6=Mg^{2+}(1.5ppm), 7=Mn^{2+}(3.2ppm), 8=Ba^{2+}(5ppm), 9=Cd^{2+}(4ppm), 10=Fe^{2+}(3.2ppm), 11=Li^+(0.8ppm), 12=Co^{2+}(3.2ppm), 13=Ni^{2+}(3.2ppm), 14=Zn^{2+}(3.2ppm), 15=Pb^{2+}(5ppm), 16=Cu^{2+} (4ppm).

Table I. Effect of 2.6 mM 18-crown-6 on the migration times of metal ions in electrolyte also containing 11 mM lactic acid, 7.5 mM 4-methylbenzylamine and 8% methanol, buffered to pH 4.3.

Ion	lactic acid t_M, min	lactic acid + 18-crown-6 t_M, min	t_M increase, %
NH_4^+	2.33	2.37	1.7
K^+	2.33	2.54	9.0
Ba^{++}	2.78	3.75	35
Sr^{++}	2.96	3.41	15
Na^+	3.05	3.14	3.0
Ca^{++}	3.12	3.22	3.2
Mg^{++}	3.28	3.43	4.6
Mn^{++}	3.42	3.54	3.5
Cd^{++}	3.55	3.68	3.7
Li^+	3.70	3.85	4.1
Co^{++}	3.81	3.95	3.7
Pb^{++}	4.08	4.81	18
Ni^{++}	4.15	4.44	7.0
Zn^{++}	4.30	4.62	7.4
Cu^{++}	5.96	6.20	4.0

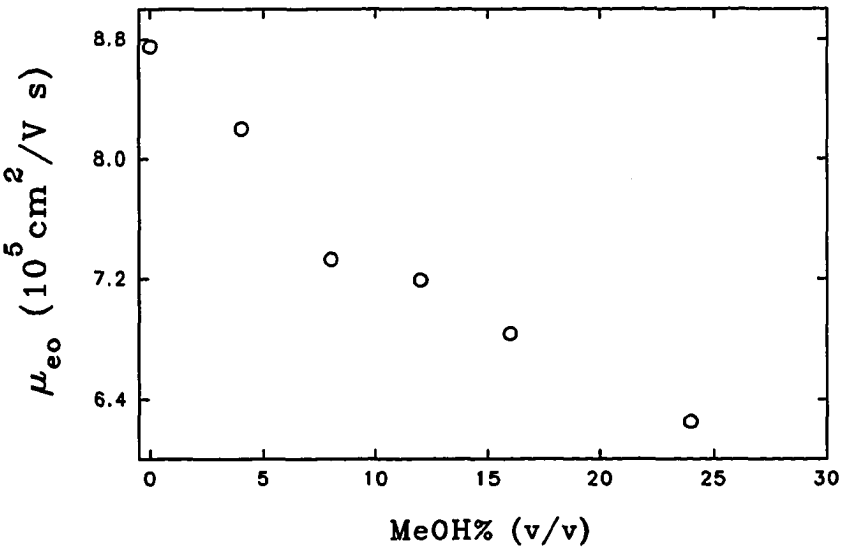

Figure 2. Change of electroosmotic mobility with methanol as a buffer modifier. Electrolyte, 15 mM lactic acid, 10 mM 4-methylbenzylamine, 0%-24% methanol, pH 4.3; applied voltage, 20 kV; injection time, 30 s; neutral marker, acetophenone.

coefficient decreases linearly as the percentage of methanol in the electrolyte is increased (Figure 3). The sum of these effects is a non-linear increase in migration times of the metal ions (Figure 4). In general, methanol in the electrolyte improves the separation ofmetal ions having adjacent migration times.

Effect of a high sodium(I) concentration

One of the shortcomings of CE compared to ion chromatography is that ion peaks in CE become very broad at higher concentrations. We investigated the determination of 1 ppm each of Mg^{++}, Ca^{++}, Sr^{++} and Ba^{++} in the presence of 75 ppm of Na^{+}. Figure 5 shows that only Mg^{++} can be separated from this concentration in 8% methanol, and only Mg^{++} and Ca^{++} can be determined in 16% methanol or 32% methanol. However if 18-crown-6 is added to the electrolyte, the migration times of Sr^{++} and Ba^{++} are slowed sufficiently that all four of the trace metal ions can be determined (Figure 6). By lowering the applied voltage and reducing the injection time from 30s to 6s, 1 ppm of each of the four trace metal ions can be determined in the presence of 1,000 ppm of sodium (Figure 7). It will be noted that increasing proportions of methanol in the electrolyte, as well as a lower voltage, result in significant increases in the migration times.

Calibration plots

Known concentrations of each of the metal ions were separated under the conditions used in Figure 1 to test the quantitative aspects. Lithium(I) was selected as an internal

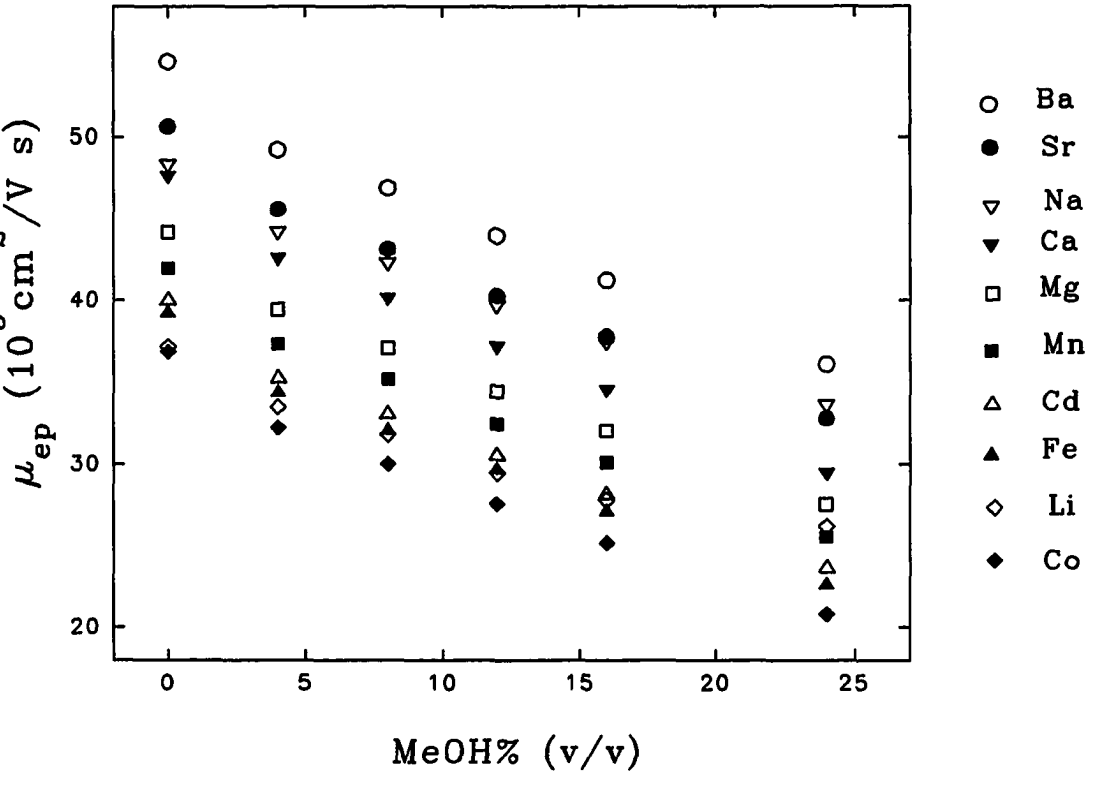

Figure 3. Change of electrophoretic mobility with methanol as a buffer modifier. Same conditions as described in Figure 2.

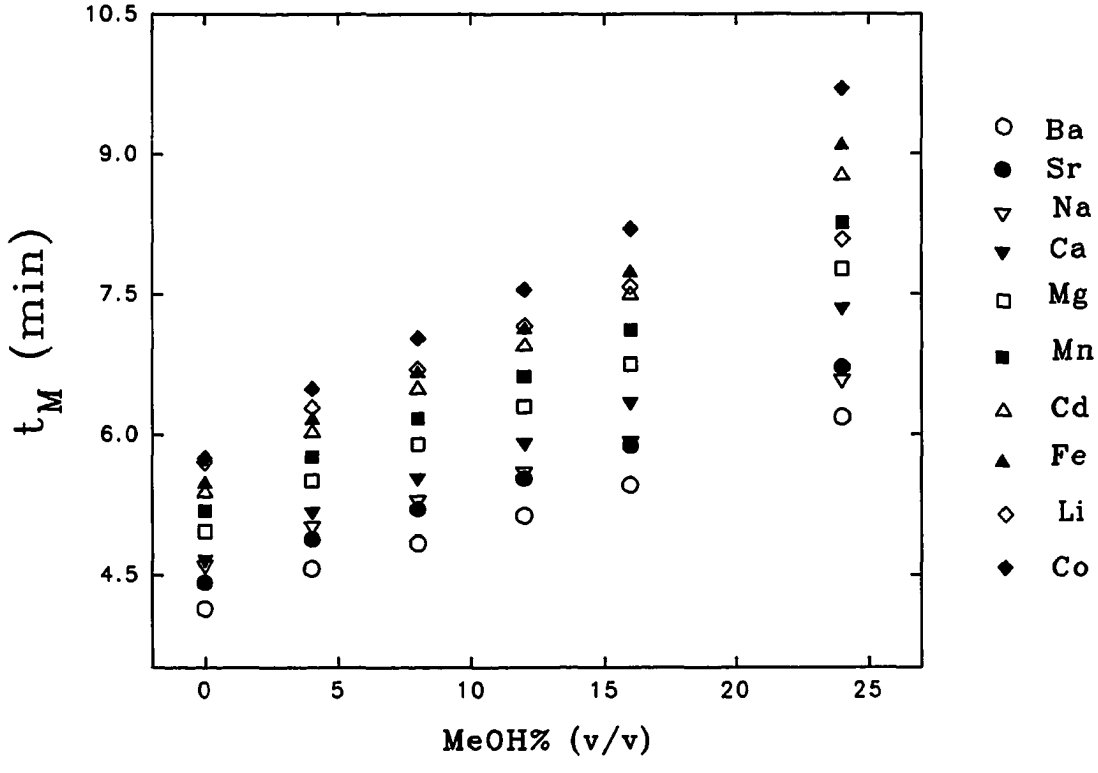

Figure 4. Change of migration time with methanol as a buffer modifier. Same conditions as described in Figure 2.

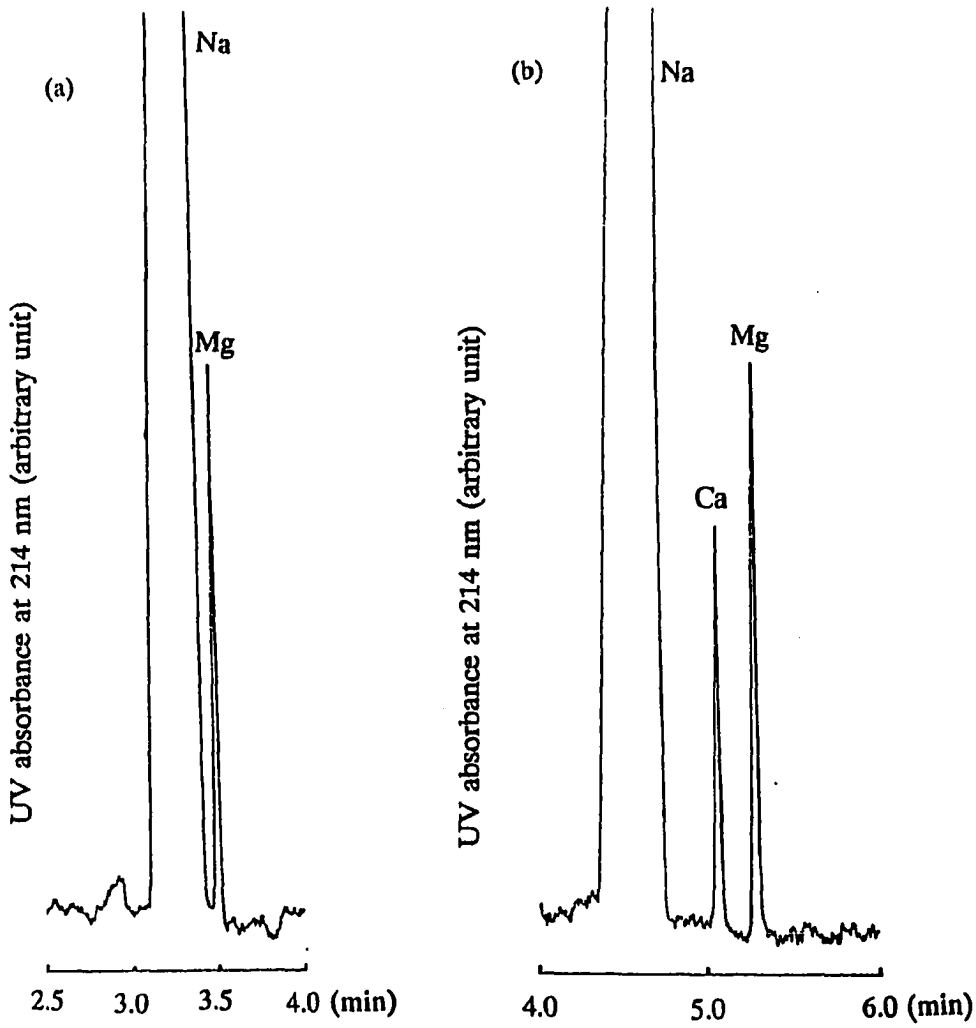

Figure 5. Effect of methanol in separation of 75 ppm Na^+ and 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}. Electrolyte, 15 mM lactic acid, 10 mM 4-methylbenzylamine, pH 4.3, (a) 8% (v/v) methanol; (b) 32% (v/v) methanol; applied voltage, 30 kV; injection time, 30 s.

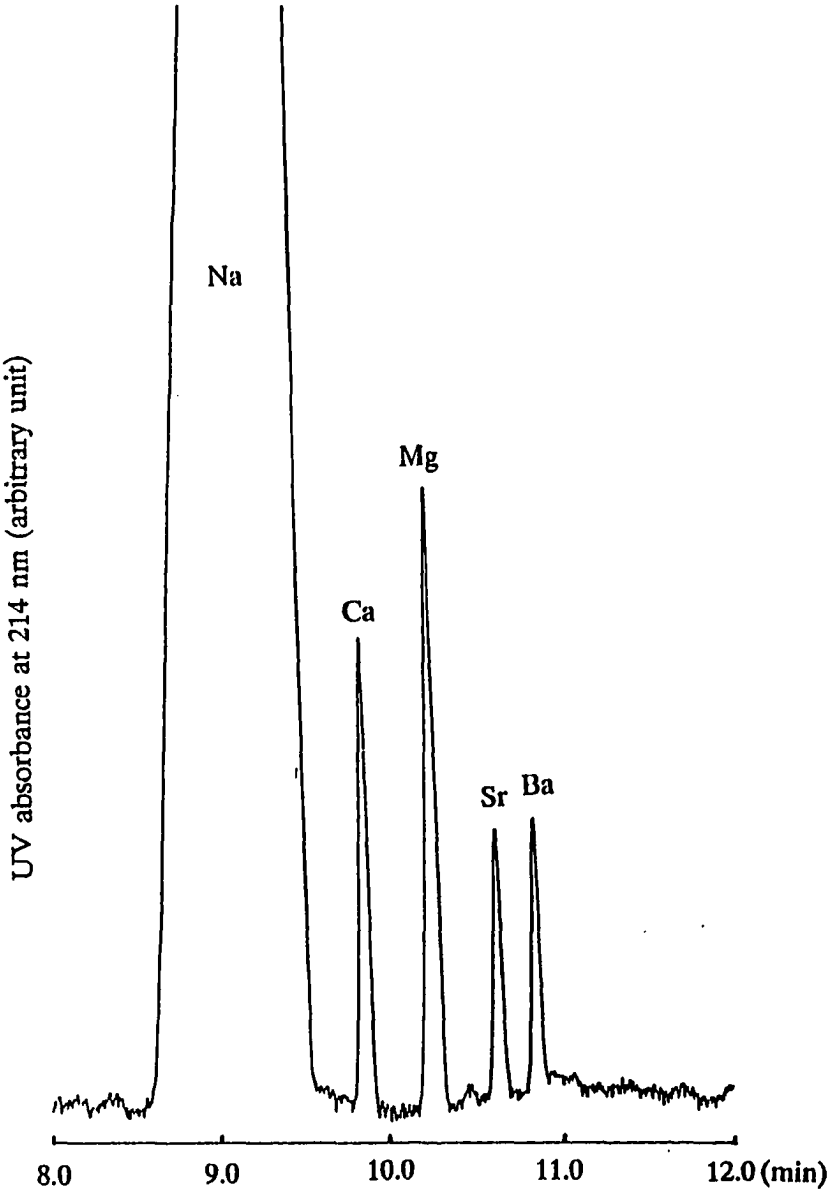

Figure 6. Effect of 18-crown-6 in separation of 75 ppm Na^+ and 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}. Electrolyte, 15 mM lactic acid, 10 mM 4-methylbenzylamine, pH 4.3, 32% (v/v) methanol; applied voltage, 15 kV; injection time, 30 s.

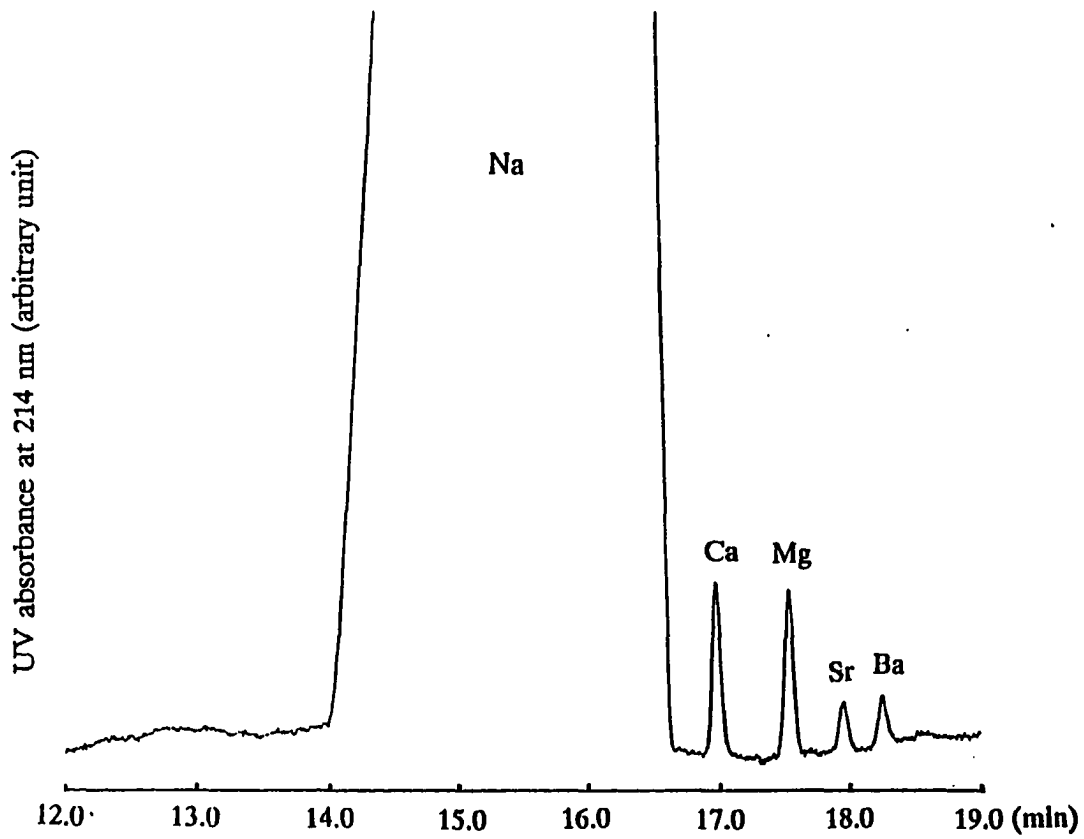

Figure 7. Separation of 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} from 1000 ppm Na^{+}. Electrolyte conditions same as Figure 6.; applied voltage 10 kV; injection time, 6 s.

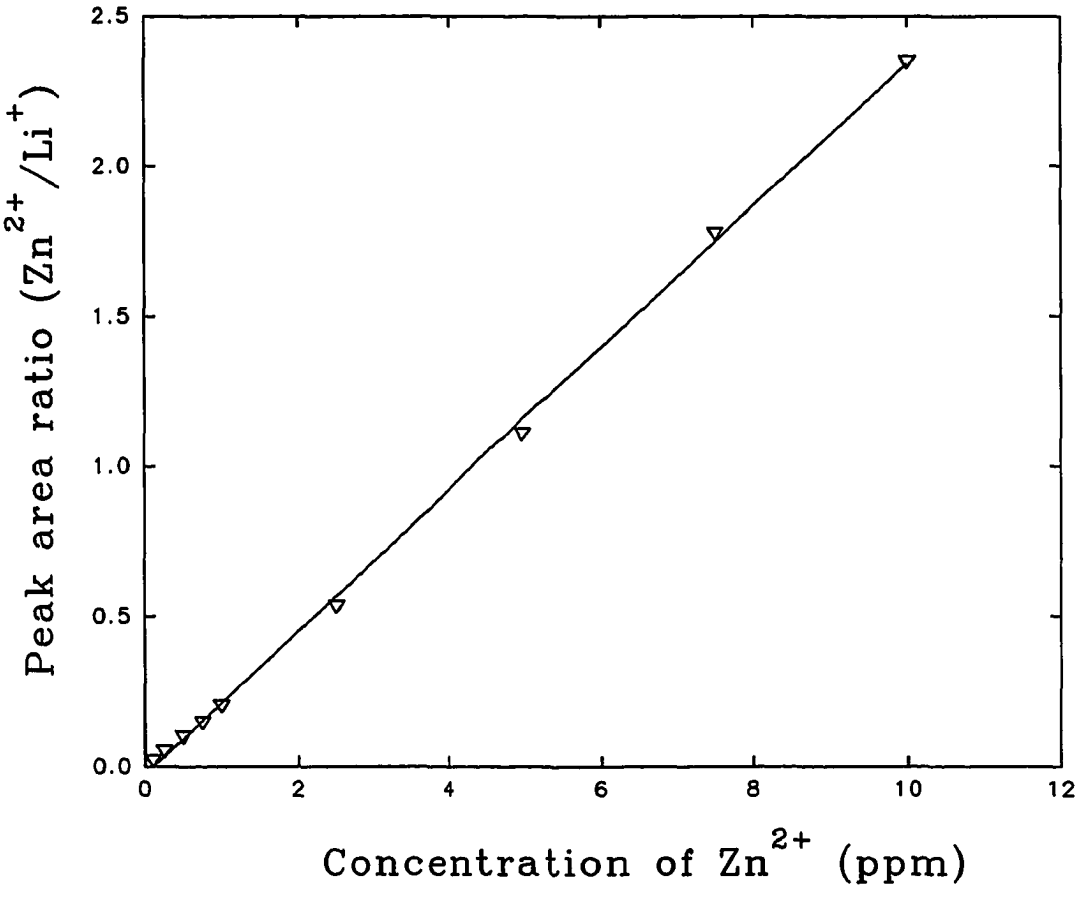

Figure 8. Calibration plot of Zn^{2+} using Li^{+} as an internal standard. Electrolyte, 15 mM lactic acid, 10 mM 4-methylbenzylamine, 0.6 mM 18-crown-6, 10%(v/v) methanol, pH 4.3; applied voltage, 20 kV; injection time changing with the concentration of Zn^{2+} standard solution, 60 s for 0.10 ppm and 0.25 ppm, 30 s for 0.50 to 2.5 ppm, 15 s for 5 ppm, 11s for 7.5 ppm, 8 s for 10.0 ppm; internal standard concentration, 0.2 ppm Li^{+} in 0.10 ppm or 0.25 ppm Zn^{2+} solution, 1.0 ppm Li^{+} in 0.5 or higher concentration of Zn^{2+} solution.

Table II. Quantitative aspects of some metal ions. Electrolyte, 15 mM lactic acid, 10 mM 4-methylbenzylamine, 0.6 mM 18-crown-6, 10% MeOH, pH 4.3; applied voltage, 20 kV. The concentration of all metal ion standards is from 0.1 ppm to 10.0 ppm.

Ion	Slope (1/ppm)	Correlation coefficient
Sr^{2+}	0.159	0.9983
Mg^{2+}	0.560	0.9992
Mn^{2+}	0.241	0.9989
Co^{2+}	0.277	0.9995
Zn^{2+}	0.236	0.9995

standard. A plot of peak height (relative to that of Li^+) against concentration was unsatisfactory over the range 1 to 10 ppm. This is probably because peak width tends to increase with concentration. However, a plot of peak area vs. concentration gave a linear plot over a 100-fold change in concentration (0.1 to 10 ppm). A typical plot (Figure 8) shows linearity over the entire range. Slopes and correlation coefficients are listed in Table II.

Peak efficiency

The electrophoretic peaks were examined by using a very fast chart speed on the recorder so that the various peaks would be quite spread out. The plate number (N) widely used in chromatography was calculated for Zn^{++} , which was one of the better shaped peaks. At 1 ppm, the calculated value of N was approx. 365,000, which would indicate very efficient electrophoretic behavior. However, the calculated plate number dropped drastically as the amount of zinc(II) injected was increased Figure 9. A somewhat similar decrease in plate number with increasing amounts of sample ions was noted in a recent paper (1).

The use of a plate number derived for chromatography to express the separating power of CE seems to be a poor practice. This is because peak broadening in chromatography and CE is fundamentally different. In chromatography plate numbers are relatively constant over a reasonable range of concentrations. This obviously is not true for CE where peaks become significantly broader as the concentration is increased. In chromatography the later peaks are always broader than the early-eluting peaks. In

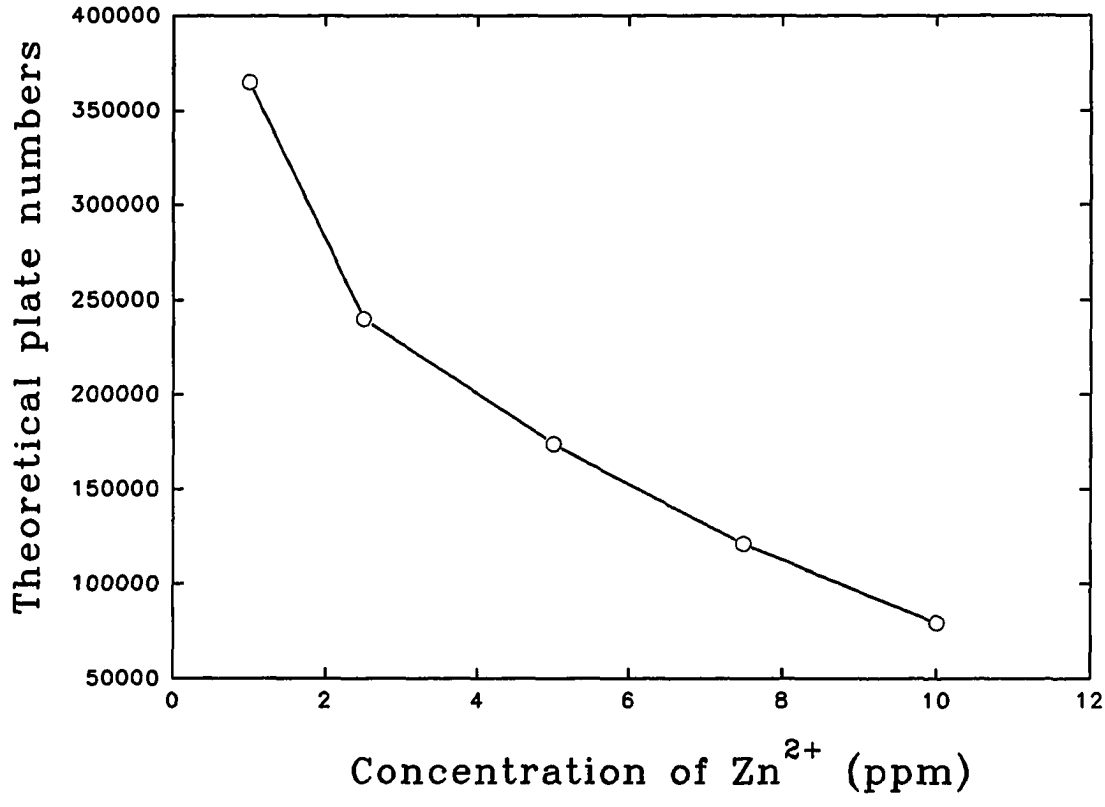

Figure 9. Change of theoretical plate number with analyte concentration. Same electrolyte conditions and applied voltage as described in Figure 8; injection time, 15 s. Theoretical number calculated using $(t_M/\sigma)^2$, where t_M=the migration time and $\sigma=2.35W_{1/2}$ which is the half width of a peak.

CE each ion zone tends to reach an equilibrium broadness which does not increase very much as the zone moves further along the column. This can result in some very high calculated plate numbers for later eluting peaks. Even in chromatography some highly inflated plate numbers are sometimes reported when a separation takes a long time and the value of t_M is quite high. In such cases the more accurate plate number (N_{eff}) would be only a small fraction of the inflated classical plate number (N).

Separation of uncomplexed metal cations.

Hydroxyisobutyric acid (HIBA) and lactic acid have been used extensively to separate groups of metal ions that have almost identical mobilities, such as the divalent transition metal ions and the trivalent lanthanides. Metal ions are complexed to varying degrees, resulting in differences in the overall rates of movement of the various metal ions. The zone of each element contains the free metal cation in equilibrium with one or more metal-ligand species. In the separation of the lanthanides with lactic acid as the ligand, the migration time has been shown to be a linear function of $\overline{n}$, the average number of ligands attached to the lanthanide (11). It will be seen that the equilibria between the free metal ion and the several complexed species must be quite fast in order to maintain a tight, compact zone. If the equilibria are not rapid, the different species would move at different rates and the zone for an element would become very diffuse.

In the lactic acid system no peak could be obtained for ions such as Al(III) which are known to have rather slow rates of complex formation. We therefore decided to study the separation of metal ions by CE under conditions where only free, uncomplexed ions

would be present. To avoid complications from hydrolysis of metal ions, a very acidic pH was chosen.

Attempts were made to separate metal ions such as copper(II) and cobalt(II), which have a reasonable absorbance in the UV spectral range. Using protonated ß-analine (pK_a=3.6) or formate (pK_a=3.75) as the buffer, Cu^{2+}, Co^{2+}, UO_2^{2+} and VO^{2+} were separated and directly detected at 214 nm. However, the detection sensitivity was rather poor. The absorbance of many other metal ions is so low that direct detection is not feasible. The situation becomes even worse if only a few detection wavelengths are available on the CE instrument.

For these reasons indirect detection is the better choice for multi-element detection with high sensitivity. In practice, not all buffer systems are suitable for the indirect detection. The baseline was very noisy and no metal ion peaks could be discerned with ß-alanine or formate as the buffer and 4-methylbenzylamine or phenylethylamine as the UV-visualizing agent. However, good peaks were obtained at pH 3.2 with nicotinamide serving both as the buffer (pK_a=3.3) and the UV-visualizing agent, and formate as the counterion.

Figure 10a shows a separation of several metal ions at pH 3.2 using 8.0 mM nicotinamide and indirect detection. It is now possible to obtain good peaks for Al^{3+} and VO^{2+}. In other experiments good peaks were obtained for UO_2^{++}, Cr^{3+} and Ag^{+}.

In Figure 10a Ca^{2+} and Sr^{2+} co-elute (peak 3) and Mg^{2+} and Na^{+} co-elute in peak 4. The separation was repeated under the same conditions but with 0.6 mM 18-crown-6 also added to the electrolyte (Figure 10b). The crown ether lengthened the migration times of

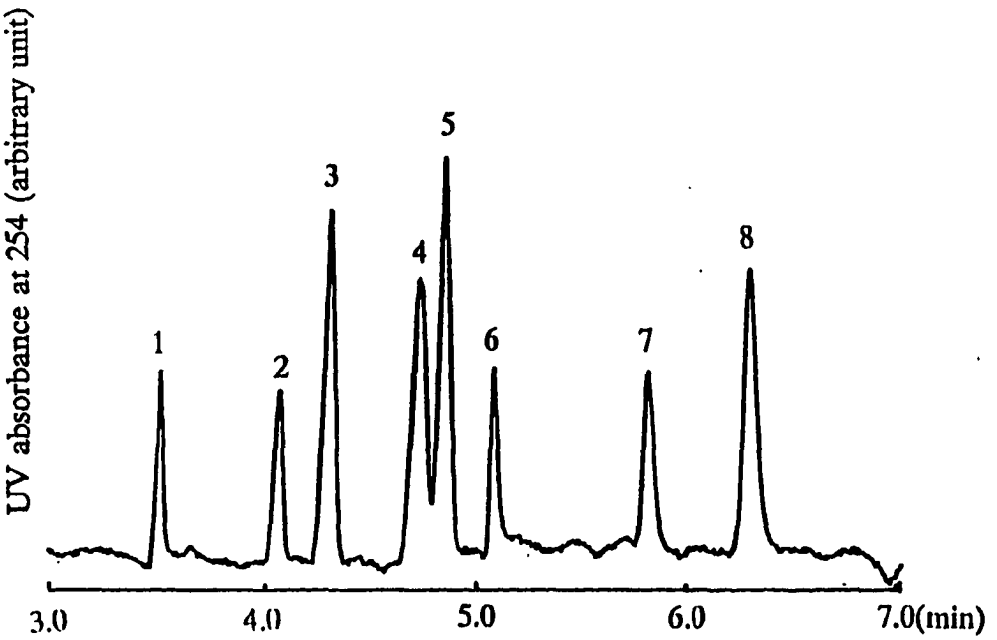

Figure 10(a) Separation of samplestandard mixture using an uncomplexing electrolyte. Electrolyte, 8 mM nicotinamide, pH 4.3 adjected with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1=K^+(1.5ppm), 2=Ba^{2+}(1.5ppm), 3=Sr^{2+}(1.5ppm) and Ca^{2+}(0.8ppm), 4=Mg^{2+}(0.5ppm) and Na^+(0.8ppm), 5=Al^{3+}(0.8ppm), 6=Cu^{2+}(ppm), 7=Li^+ (0.2ppm), 8=VO^{2+}(2ppm).

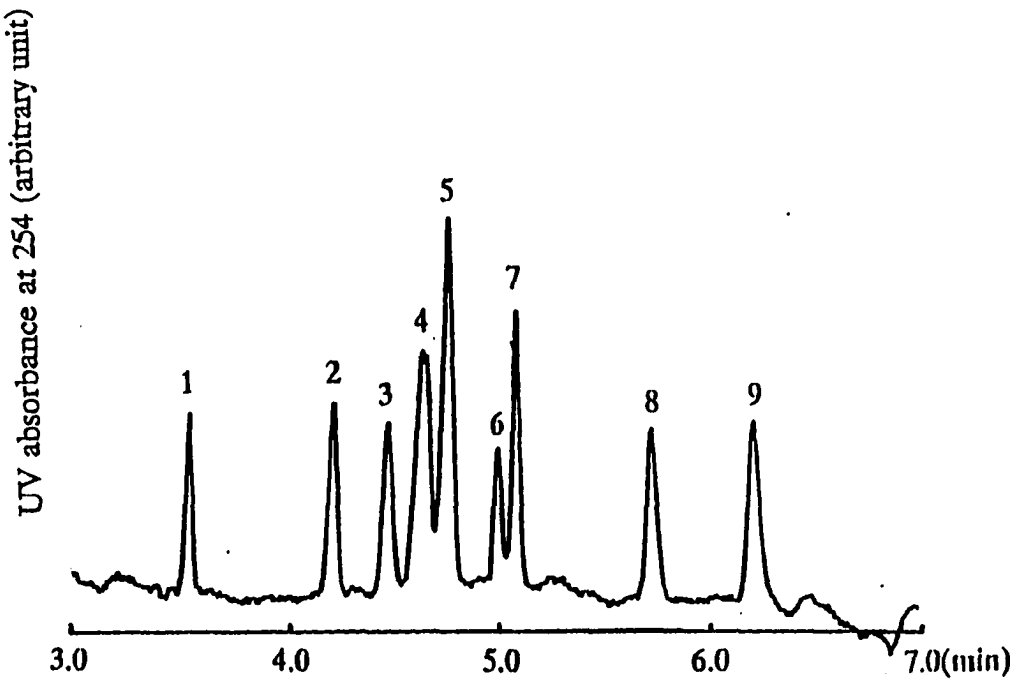

Figure 10.(b) Separation of the same sample standard mixture using an uncomplexing electrolyte with 18-crown-6. Electrolyte, 8 mM nicotinamide, 0.6 mM 18-crown-6, pH 4.3 adjested with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1=K^+(1.5ppm), 2=Ca^{2+}(0.8ppm), 3=Sr^{2+}(1.5ppm), 4=Mg^{2+}(0.5ppm) and Na^+(0.8ppm), 5=Al^{3+}(0.8ppm), 6=Cu^{2+}(0.8ppm), 7=Ba^{2+}(0.8ppm), 8=Li^+(ppm), 9=VO^{2+}(2ppm).

Sr^{2+} and Ba^{2+} so that separation of Ca^{2+} and Sr^{2+} was obtained.

The separation of Al^{3+} was tried under the same conditions as Figure 10b but with lactic acid added to the electrolyte. No aluminum peak was found, thus supporting the hypothesis that rapid equilibrium is essential in complexing systems. The crown ether in the electrolyte probably does not complex aluminum(III). By adjusting slightly the concentrations of nicotinamide and crown ether, and by adding methanol to the electrolyte, it was possible to completely resolve a mixture containing Mg^{++}, Al^{+++}, the alkaline earths and the first three alkali metal ions (Figure 11).

Conclusions

As found in previous chapter, lactate electrolyte is an excellent electrolyte system for separating metal cations if methanol is added as a electrolyte modifier. The effects of methanol on both electroosmotic mobility and electrophoretic mobility were investigated in this chapter. Methanol reduces both electroosmotic and electrophoretic mobilities, and thus increases migration time. The study shows resolution was increased greatly after methanol was added.

Generally, NH_4^+ and K^+ ions are difficult to be separated due to the same electrophoretic mobility. Adding 18-crown-ether to the lactate electrolyte changed mobility of K^+ more greatly than that of NH_4^+. It was also shown that 18-crown-6 changed migration times of Ba^{2+}, Sr^{2+} and Pb^{2+} because they have large size and fit in the crown ether ring very well. With using 18-crown-6 and methanol in lactate electrolyte, 1 ppm Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} could be separated from 1000 ppm Na^+.

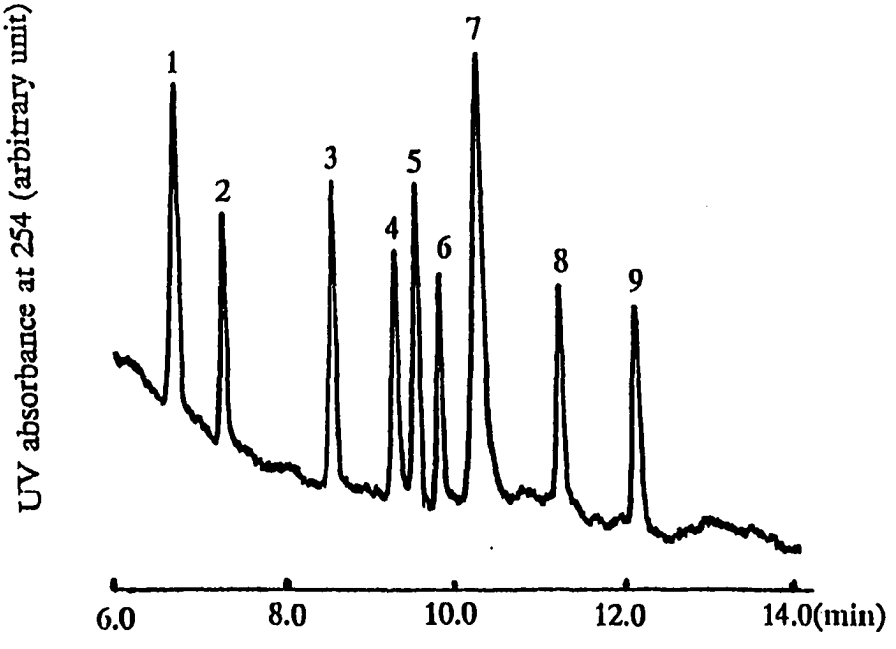

Figure 11. Electropherogram of a standard miture with 9 common cations using a nicotinamide electrolyte. Electrolyte, 8 mM nicotinamide, 12% methanol, 0.95 mM 18-crown-6, pH 3.2 adjusted with formic acid; applied voltage, 25 kV; injection time, 40 s. Peaks: 1=NH_4^+(1.5ppm), 2=K^+(1.5ppm), 3=Ca^{2+}(1.0ppm), 4=Na^+(1.0ppm), 5=Mg^{2+} (0.5ppm), 6=Sr^{2+}(1.0ppm), 7=Al^{3+}(1.0ppm), 8=Ba^{2+}(1.0ppm), 9=Li^+(ppm).

Some of the metal cations could not be separated in complexing electrolyte, probably because of their slow complexing kinetics. Uncomplexing buffer systems may be useful. Nicotinamide electrolyte showed that it was very good for separation of Al^{3+}, VO^{2+}, Cr^{3+}, UO_2^{2+} and Ag^{+}.

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We wish to thank Waters Division of Millipore for their gift of the Waters Quanta 4000 CE system and associated chemicals. We also thank Andrea Weston and Peter Jandik for valuable technical assistance in the initial stages of this research.

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CAPILLARY ZONE ELECTROPHORESIS OF NEUTRAL ORGANIC MOLECULES IN ORGANIC-AQUEOUS SOLUTION

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Key words:

Electrophoretic capillary chromatography
Organic modifiers
Hydrophobic association
Capacity factors

Summary

Very good separations of non-ionic organic compounds were achieved by capillary zone electrophoresis (CZE) using a tetraheptylammonium salt as an additive in aqueous-acetonitrile solvent. A systematic study was undertaken to determine the effect of experimental parameters on electroosmotic mobility and electrophoretic mobility. It was found that pH and acetonitrile concentration, as well as the type and concentration of quaternary ammonium salt, are important experimental variables. Under appropriate conditions, the separation window was enlarged and a broad range of electrically neutral organics, including both very hydrophobic compounds (e.g., polycyclic aromatic hydrocarbons) and fairly hydrophilic compounds were separated during a relatively short

running time. By adjusting separation conditions, high resolution CZE of specific group of neutral organics could be achieved. A method for calculation of capacity factor was proposed and capacity factors for a variety of non-ionic organic compounds were calculated.

1. Introduction

Micellar electrokinetic capillary chromatography (MECC) is a highly efficient technique for separating neutral organic compounds that cannot form ions. The technique was first reported by Terabe (1,2) and continues to receive considerable attention (3). Separations are based on differences in the ability of the various analytes to enter a charged micelle and thereby undergo electrophoretic migration. The direction of micelle flow is almost always counter to a larger rate of electroosmotic migration. However, the use of MECC is restricted by a limited elution range (4). Hydrophobic analytes partition so strongly into the micelle that they elute in a fairly narrow window close to the micelle itself. In addition, it is often difficult to dissolve sufficient amounts of hydrophobic compounds in the aqueous micellar solution to permit detection by standard on-column UV (4). Baluchunas and Sepaniak have also commented on the limited elution range of MECC (5). They used silanized capillaries to adjust the electroosmotic flow and 10% propanol in the electrolyte to increase the kinetic rate of analytes in and out of the micelle. The effects of organic mobile phase modifiers were discussed in a later paper (6). Gareil (7) discussed factors affecting migration time and resolution in MECC.

It is generally acknowledged that micelles are not stable in water-organic solvents

above 20 to 30% organic (4). Despite this, separation of testosterone esters has been reported in SDS solutions containing up to 50% acetonitrile (8). Sepaniak used bile salt micelles for MECC, which are more stable than conventional SDS micelles in the presence of higher percentages of organic solvents (9). Hydrophobic analytes show less affinity for these micelles and are eluted earlier. Palmer, Khaled, and McNair used an oligomer of sodium 10-undecylenate for separations of neutral organics in 20 to 40% methanol (4). Excellent separations of aromatic hydrocarbons were reported but some difficulties were encountered in reproducing retention times and in occasional precipitation of the oligomer.

In 1986 Walbroehl and Jorgenson used a tetrahexylammonium salt as an additive to facilitate separation of neutral organic compounds in aqueous-organic solvents containing 50% or more acetonitrile (10). They felt that no micelle existed but that separation of the analytes took place because of differences in solvophobic association in solution between the quaternary ammonium salt (Q^+) and the organic analytes. Conditions were such that the electroosmotic flow and electrophoretic migration of the Q^+ were in the same direction. The window available for separation again appeared to be rather small. Very little use seems to have been made of the approach (11).

In the present work it is shown that a wide variety of neutral organic compounds can be separated by CZE in solutions containing a fairly high percentage of acetonitrile when a suitable quaternary ammonium is added to the electrolyte. A systematic study was made of the effect of type and concentration of Q^+, pH, and percentage of acetonitrile on both the electroosmotic- and electrophoretic migration of the analytes. These experimental parameters were then adjusted to give a good window for separation and to optimize

conditions for separation of various types of analytes.

2. Experimental

Capillary electrophoresis was performed by using Waters Quanta 4000 Capillary Electrophoresis System (Millipore Waters, Milford, MA, USA) and fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA), 50 μm i.d. with length of 60 cm. The on-column detection window was 52.5 cm apart from the injection end of capillaries. UV detection was employed at 254 nm. Injection time was 30 seconds with hydrodynamic mode. Positive power supply of 12 kV was used for electrophoresis. Electropherograms were plotted using a Servogor 120 flatbed recorder (Goerz Instruments, Austria) with chart speed of 1.0 cm/min.

All standards and electrolytes were prepared with analytical-reagent grade chemicals and 18 MΩ deionized water by a Barnstead Nanopure II System (Sybron Barnstead, Boston, MA, USA).

Buffer solutions were prepared by mixing tetraalkylammonium halide (Aldrich, Milwaukee, WI, USA), acetonitrile (Fisher Scientific, Fair Lawn, NJ, USA) and sodium borate (Fisher Scientific, Fair Lawn, NJ, USA) solution. Adjustment of pH was done by adding 100 mN phosphoric acid solution (Millipore Waters, Milford, MA, USA). Sample standards contained about 40% acetonitrile.

At the beginning of each experimental day, the capillary was flushed with 0.1 M KOH solution for 10 min and followed with deionized water for 15 min. Before each run, the capillary was purged with running buffer for 5 min. Between two runs, 10 min

careful rinse of capillary with deionized water solution containing 50% (v/v) acetonitrile was followed by 5 min rinse with pure methanol (Mallinckrodt, Paris, KY).

3. Results and Discussion

3.1 Principles

In CZE neutral organic molecules undergo only electroosmotic flow and therefore migrate at the same rate through the capillary. Separation of neutral compounds becomes possible when a large ionic substance is added to the electrolyte that can associate to varying degrees with the analytes. Sample compounds that associate strongly with the ionic additive undergo greater electrophoretic migration than those that are more weakly associated.

Our early experiments confirmed an earlier report that neutral organic compounds can be separated in acetonitrile-water using a large quaternary ammonium salt (Q^+) such as tetrahexylammonium bromide as the ionic additive (10). However, the difference in migration times of the most polar and the least polar compounds tested was rather small. It was apparent that a broader window for separations would be needed for this technique to be capable of broad practical application. With this in mind a systematic study was undertaken to determine the effect of experimental parameters on electroosmotic and electrophoretic mobility.

Investigation of the effects of varying experimental parameters was carried out using several organic compounds: acetophenone, nitrobenzene, 1-bromo-4-nitrobenzene, benzophenone, and anthraldehyde. A positive power supply was used so that both

electroosmotic flow and electrophoretic flow were towards the detector. Preliminary experiments showed that a good separation of the test mixture could be obtained using an aqueous-organic electrolyte containing 40% acetonitrile, buffered at an alkaline pH, and containing 50 mM of a suitable quaternary ammonium salt (Q^+). Injection of a small amount of pure water provided a suitable marker for measuring electroosmotic flow.

3.2 Type of ionic additive

No separation was possible when Q^+ was a tetrabutylammonium- or a tetrapentylammonium salt. Very good separations were obtained when Q^+ was a tetrahexylammonium- or tetraheptylammonium salt. Migration times of the neutral analytes were shorter with the tetraheptylammonium salt, indicating a greater degree of association. A few experiments were attempted with Q^+= tetraoctylammonium, but its use was abandoned owing to low solubility in the solvent mixture.

A partial separation was obtained of another test mixture using SDS as a negative-ion additive in ~40% acetonitrile. However, the results were not nearly as good as those using Q^+.

3.3 Migration mode

In MECC a negatively charged micelle is generally used in conjunction with a positive power supply. This gives a counter migration in which the electroosmotic flow is toward the detector and the smaller electrophoretic flow is in the opposite direction. With Q^+ as the ionic additive, a positive power supply results in co-migration (both electroosmotic- and electrophoretic migration towards the detector). This means that the order of elution of neutral analytes is exactly the opposite as in MECC. A negative power

supply in conjunction with Q^+ failed to give any analyte peaks in a reasonable time.

3.4 Effect of pH

At pH 9.2 the presence of a tetraheptylammonium- or tetrahexylammonium salt in 40% acetonitrile lowers the electroosmotic mobility significantly (Figure 1). This could be a result of a coating of Q^+ on the capillary surface. The electroosmotic mobility rises with increasing pH, leveling off around pH 10.5. The reason for this is not clear, but a practical effect is that pH control is a useful way to adjust the electroosmotic mobility. The electrophoretic mobilities of the six analytes remained virtually constant with respect to pH (Figure 2).

3.5 Effect of acetonitrile concentration

At a fixed pH (9.85) the electrophoretic mobility of the analytes investigated decreases linearly with an increasing concentration of acetonitrile in the electrolyte. The rate of decrease is much larger for the tetraheptylammonium salt (Figure 3) than it is for the tetrahexylammonium salt (Figure 4). These linear plots tend to fan out so that there is a greater difference in electrophoretic mobilities at lower concentrations of acetonitrile.

This behavior is consistent with a mechanism in which solvation of analytes by acetonitrile competes with solvation of analytes by the hydrocarbon chains on the quaternary ammonium salts. The latter solvation is apt to occur to different degrees with the various analytes, thus contributing to differences in their migration rates.

Electroosmotic mobility at fixed pH (9.85) shows a sharp increase as a higher percentage of acetonitrile is incorporated into the electrolyte (Figure 5). The increase occurs at a somewhat lower concentration of acetonitrile for the tetrahexylammonium salt.

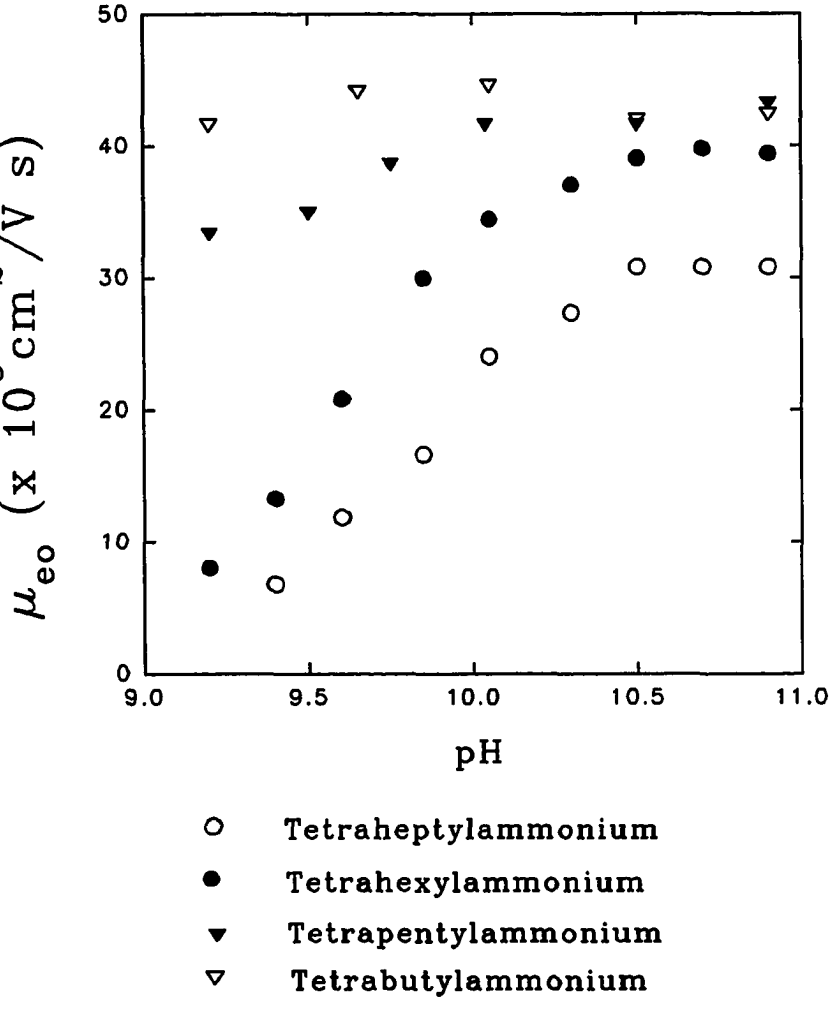

Figure 1. Effect of buffer pH on electroosmotic mobility. Electrolyte, 50 mM tetraalkylammonium bromide, 8 mM borate, 40% (v/v) CH_3CN; applied voltage, 12 kV.

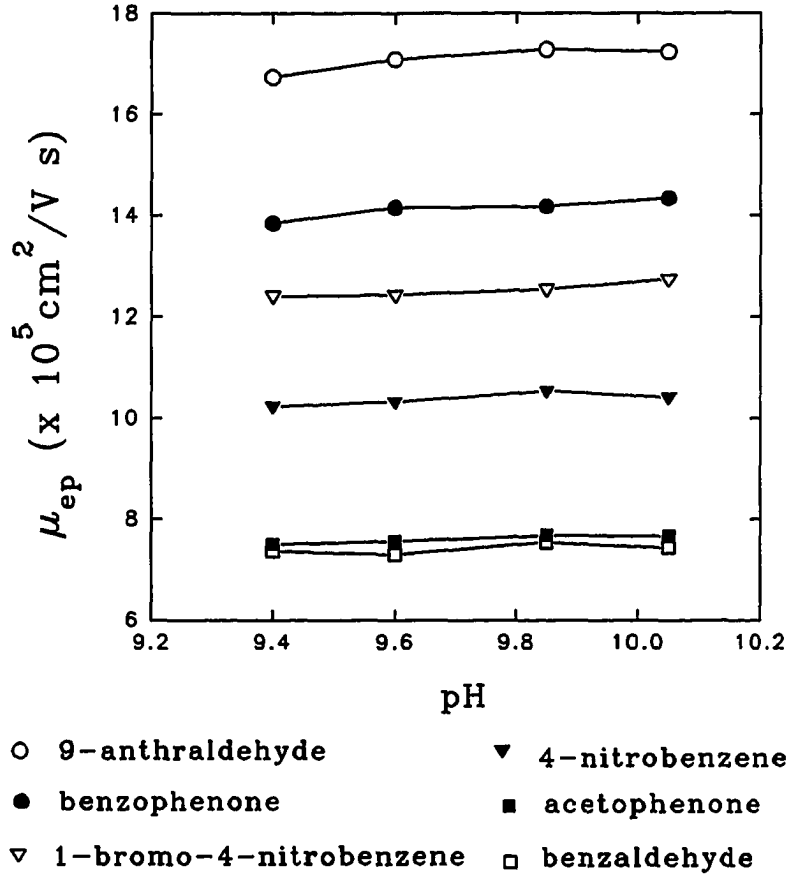

Figure 2. Effect of buffer pH on electrophoretic mobility. Electrolyte, 50 mM tetraheptylammonium bromide, 8 mM borate, 40% (v/v) CH_3CN; applied voltage, 12 kV; injection, 30 s.

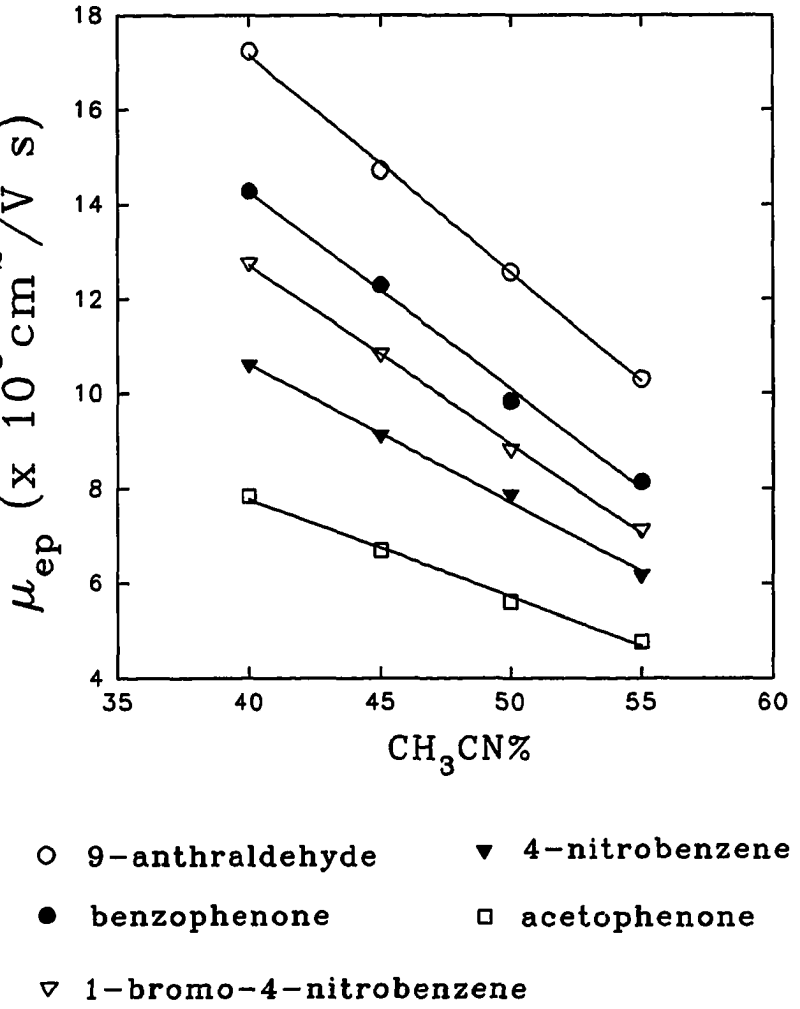

Figure 3. Electrophoretic mobility change with acetonitrile concentration in a buffer containing tetraheptylammonium. Electrolyte, 50 mM tetraheptylammonium bromide, 8 mM borate, pH 9.85; applied voltage, 12 kV; injection, 30 s.

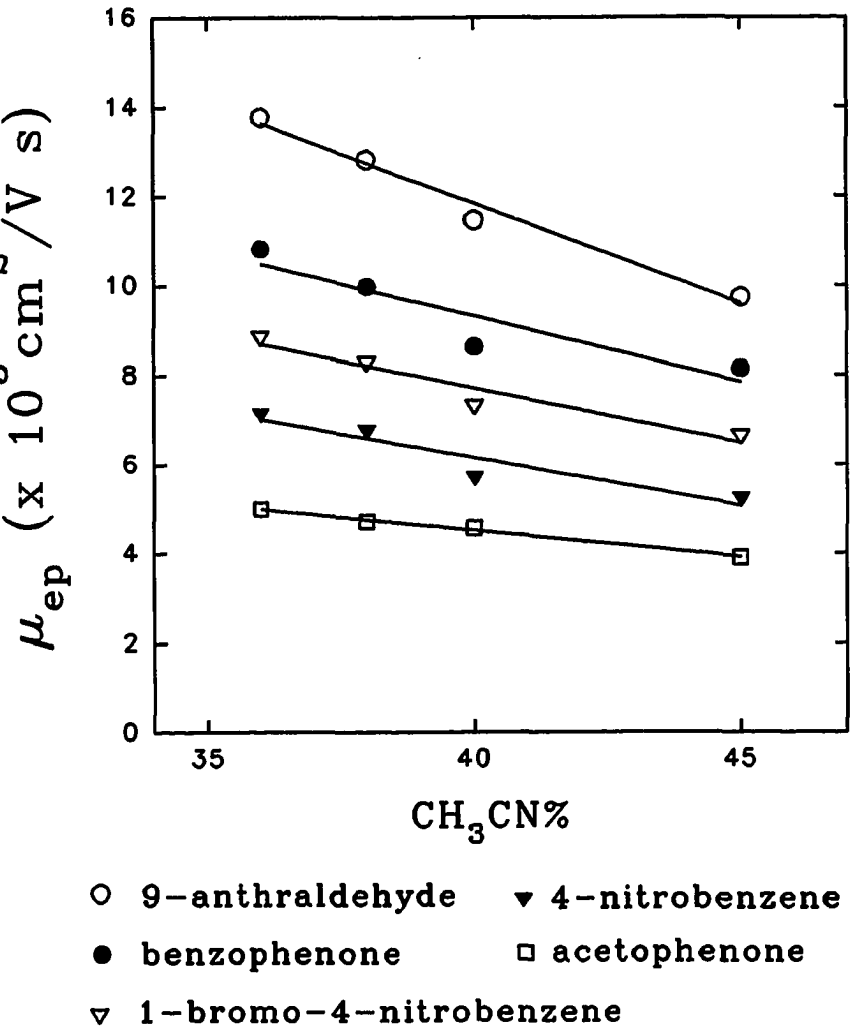

Figure 4. Electrophoretic mobility change with acetonitrile concentration in a buffer containing tetrahexylammonium. Electrolyte, 50 mM tetrahexylammonium bromide, 8 mM borate, pH 9.85; applied voltage, 12 kV; injection, 30 s.

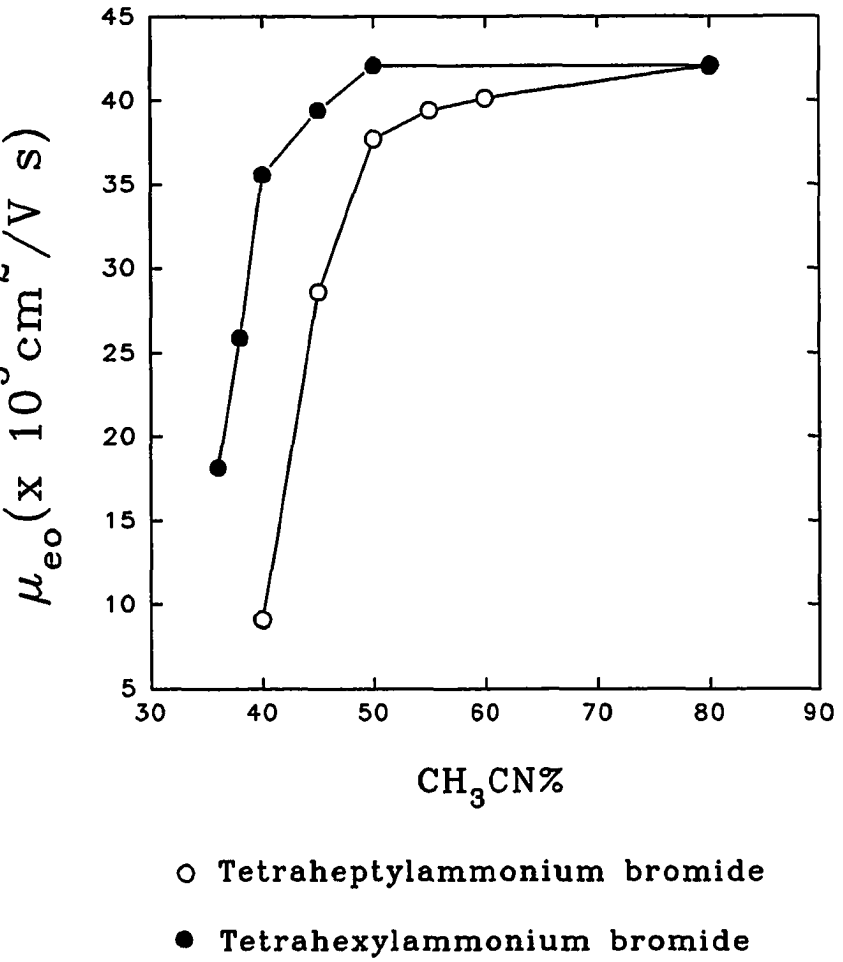

Figure 5. Electroosmotic mobility change with acetonitrile concentration. Electrolyte condition and applied voltage are same as described in Figure 3 and Figure 4.

This behavior is most likely due to a shift in the following equilibrium:

$$Q^+ \text{ (surface)} \rightleftharpoons Q^+ \text{ (solution)}$$

At a higher percentage of acetonitrile, better solvation of Q^+ would be expected to shift this equilibrium more to the right, thus restoring the negative surface charge from ionized silanol groups and increasing the electroosmotic mobility. A higher concentration of acetonitrile would be needed to desorb the tetraheptylammonium salt than would be required for the lower molecular weight tetrahexylammonium salt.

It should be noted that when this very large increase of electoosmotic mobility with respect to ACN concentration is combined with the linear decrease in electrophoretic mobility (Figs. 3 and 4), the net mobilities of the analytes increase with respect to acetonitrile concentration over much of the range studied.

3.6 Effect of Q+ concentration

The effect of tetraheptylammonium concentration on electroosmotic and electrophoretic mobility was studied at fixed pH (9.85) and fixed concentration of acetonitrile in the electrolyte. Notice that the ACN concentration selected (42%) was near the lower end of electroosmotic mobilities in Figure 5. The electroosmotic mobility shows a significant (but fairly small) increase as the tetraheptylammonium salt concentration is increased in steps from 30- to 55 mM (Figure 6). Electrophoretic mobilities of the several analytes increase in linear fashion over the same concentration range (Figure 7). This is indicative of a greater degree of association between the analytes and the tetraheptylammonium ion as the concentration of the latter is increased.

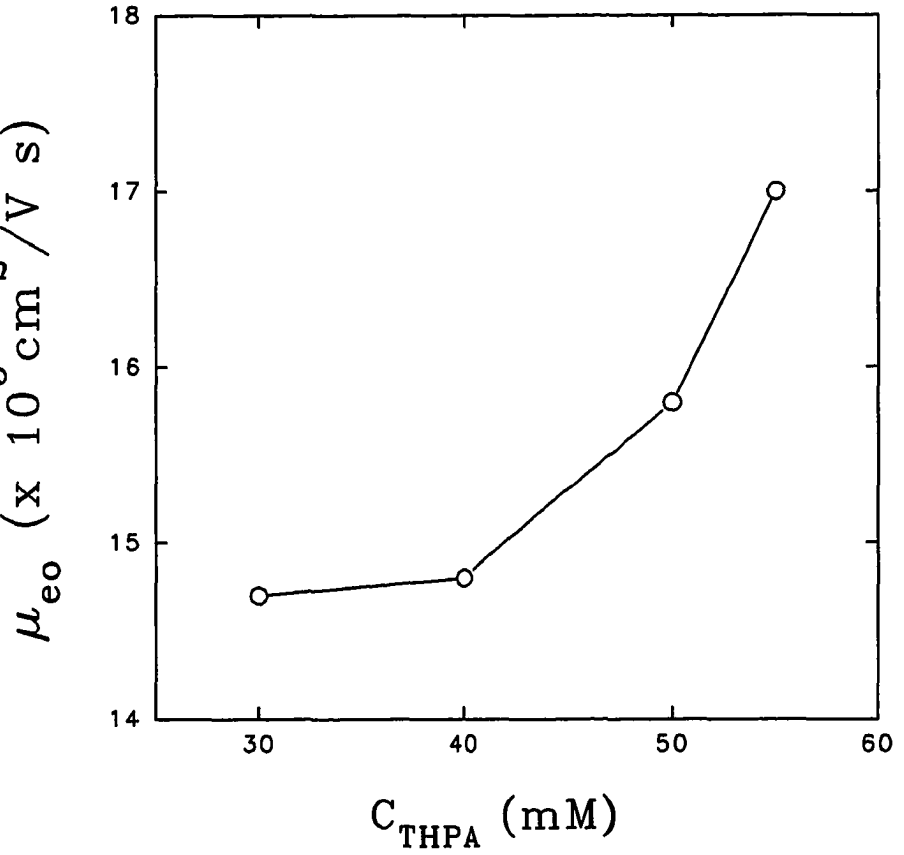

Figure 6. Effect of tetraheptylammonium concentration in buffer on electroosmotic mobility. Electrolyte, 30 - 50 mM tetraheptylammonium bromide, 8 mM borate, 42% (v/v) CH_3CN, pH 9.85; applied voltage, 12 kV

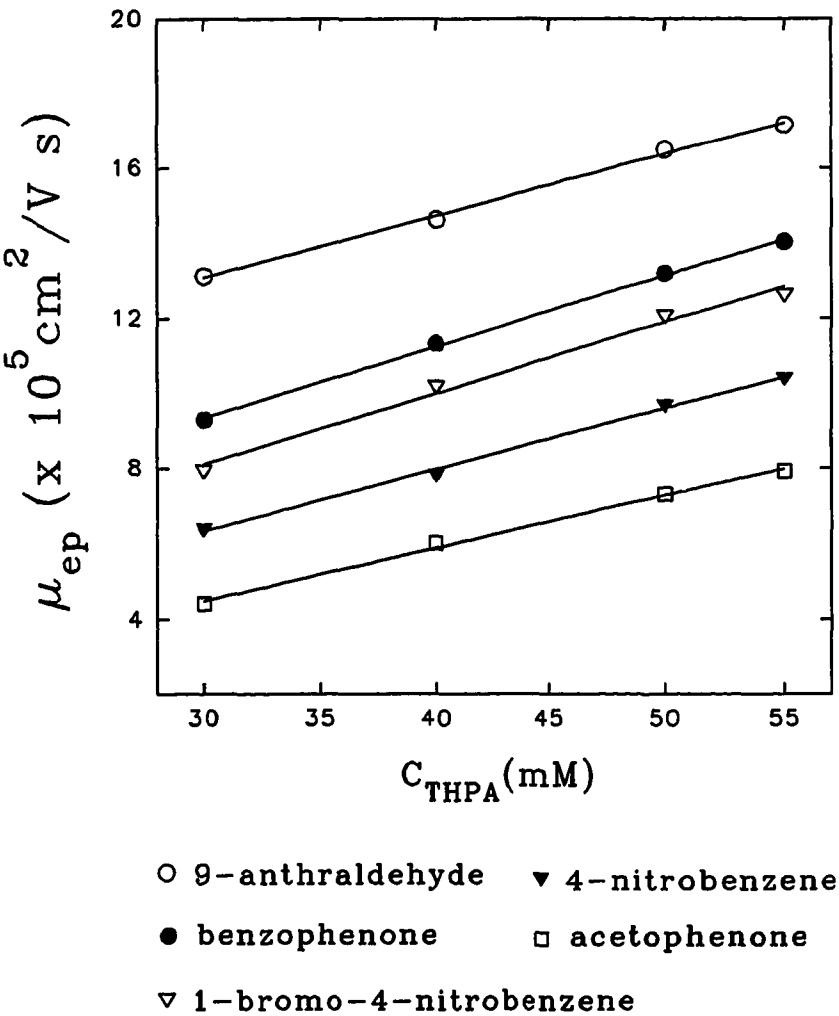

Figure 7. Effect of tetraheptylammonium in buffer on electrophoretic mobility. Electrolyte condition and applied voltage are same as described in Figure 6; injection time, 30 s.

3.7 Separation of Organic Analytes

Rather small changes in pH and in the percentage of acetonitrile have a profound effect on the speed of a separation and on the window available for separation of neutral organic compounds. The window can be expanded by selecting conditions where the electroosmotic flow is not too large compared with the electrophoretic flow. Figure 8 shows a separation with 15 peaks. By reducing the pH from 9.4 to 9.1, resolution of the peaks has improved (Figure 9) but at a cost of a somewhat longer time for the separation.

Figure 3 shows greater differences in electrophoretic mobility at lower concentrations of acetonitrile in the electrolyte. However, the electroosmotic flow drops precipitously below about 48% acetonitrile (Figure 5). Separation of the sample mixture at a lower percentage of acetonitrile than that used in Figures 8 and 9 provides better resolution but the separation time is longer owing to the very low electroosmotic flow (Figure 10).

In these separations the largest, most hydrophobic molecules appear first because they are the most strongly associated with the Q^+. This order of elution is the opposite to that in reversed-phase HPLC. The early elution of large PAH analytes by CZE may prove to be advantageous in determining these compounds in the presence of much larger amounts of more polar organic compounds.

3.8 Mechanism of separations

The existence of a quaternaty ammonium micelle seems doubtful in aqueous-organic solutions containing a high percentage of an organic solvent (4,10). In particular, short-chain alcohols prevent micellization when their concentrations are higher than ~15 to

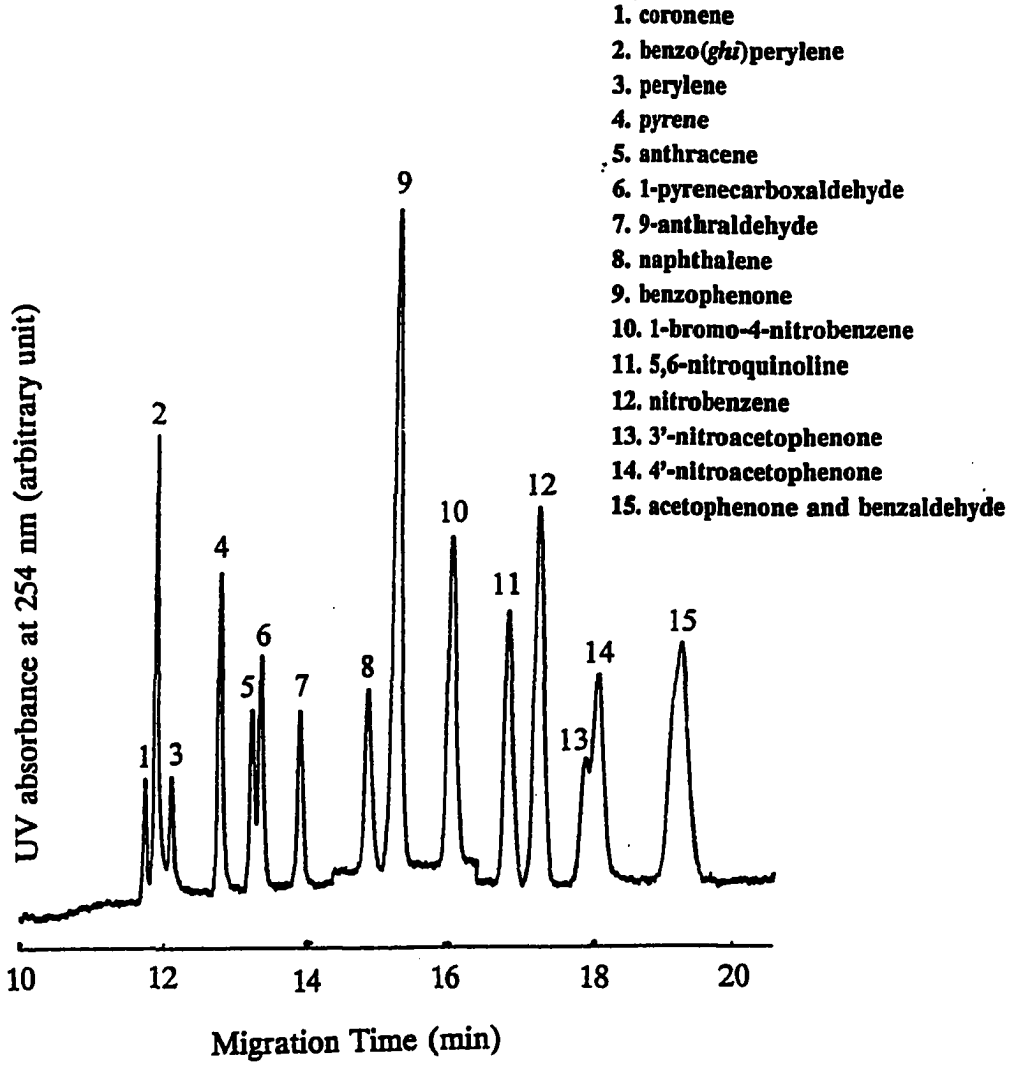

Figure 8. Electropherogram of 16 non-ionic aromatic organic compounds. Electrolyte, 50 mM tetraheptylammonium bromide, 8 mM borate, 42% (v/v) CH_3CN, pH 9.4; applied voltage, 12 kV; injection time, 30 s.

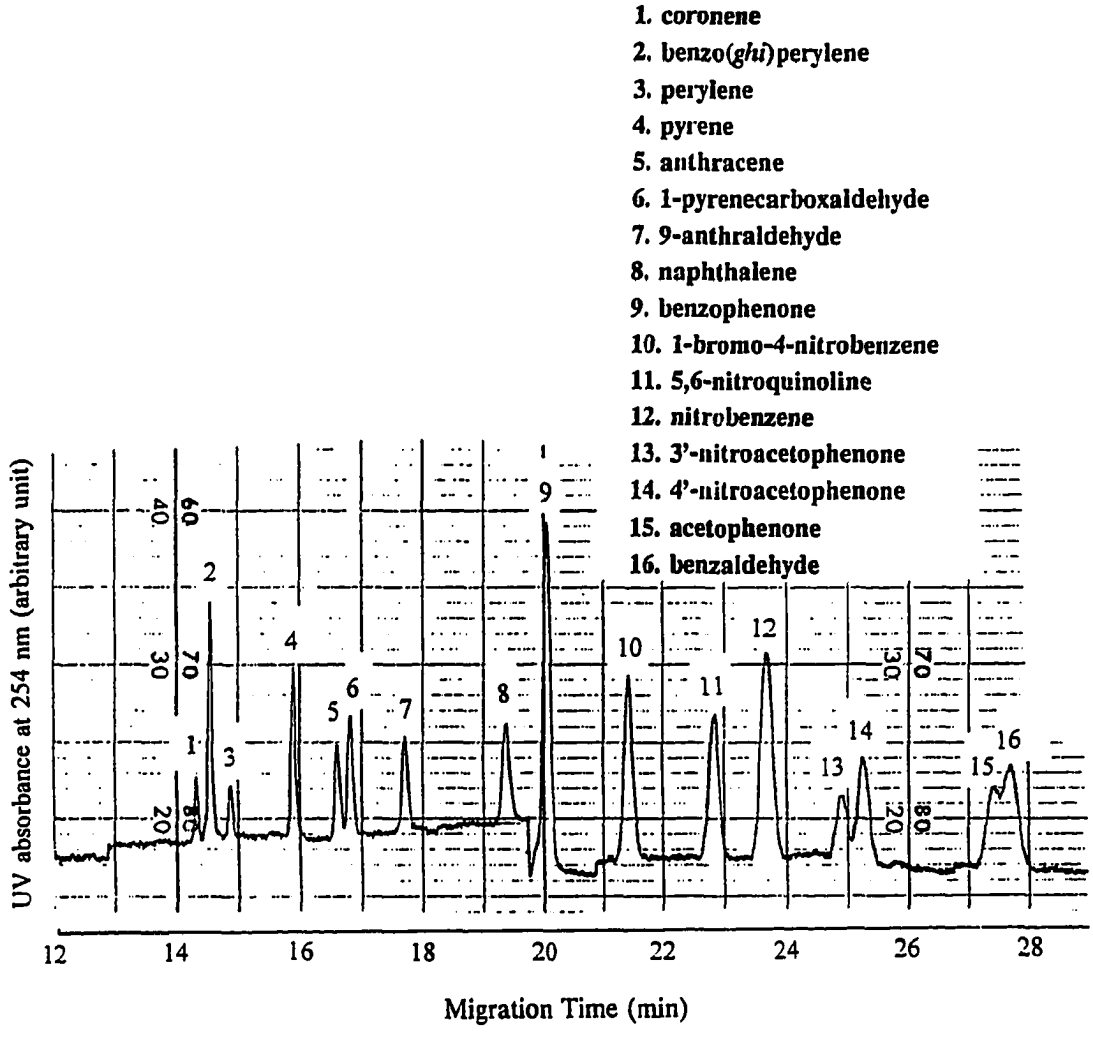

Figure 9. Electropherogram of 16 non-ionic aromatic organic compounds under same conditions as described in Figure 8 except pH 9.1.

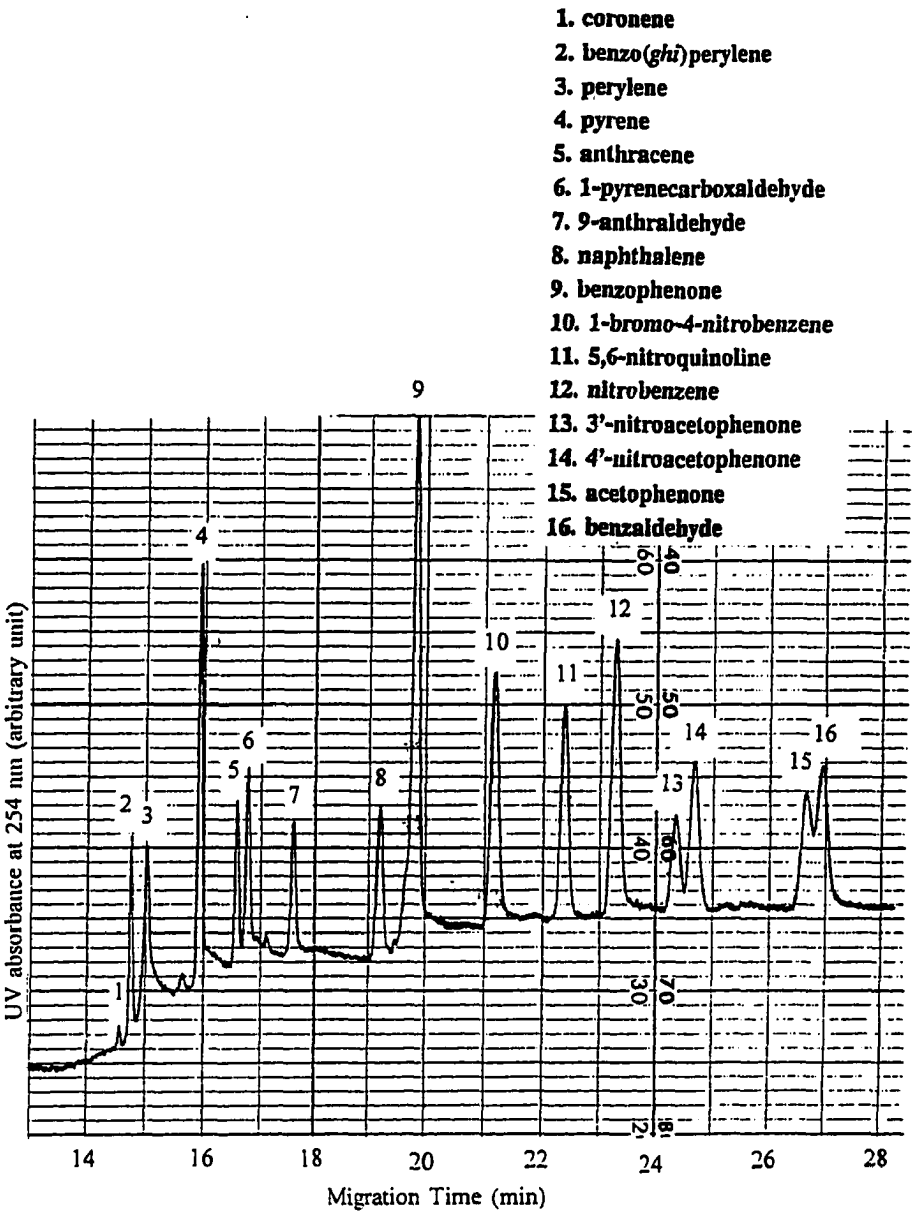

Figure 10. Electropherogram of 16 non-ionic aromatic organic compounds under sameconditions as described in Figure 8 except 40% (v/v) of CH_3CH.

20% by volume (12,13). Geometric considerations would also be expected to preclude micelle formation when the Q^+ additive has four chains of several carbon atoms each (14,15). This does not mean that the Q^+ molecules are evenly disbursed throughout the water-acetonitrile solution. Water is known to form hydrogen-bonded aggregates. In solution these might well be micro domains containing Q^+ molecules in some kind of aggregation with one another. Hydrophobic analytes could then form solvophobic "complexes" with the hydrocarbon chains of the quaternary ammonium ions. This is essentially the type of interaction proposed by Walbroehl and Jorgenson (10).

The electrophoretic mobility of neutral organic analytes was found in Figure 1 to be higher with tetraheptylammonium salts than with tetrahexylammonium salts. This is consistent with more complete complexation of the neutral analytes when the hydrocarbon chains of the Q^+ are longer.

Solvophobic association between Q^+ and the organic analytes would be in competition with solvation of the analytes by acetonitrile. It is therefore no surprise that the electrophoretic mobility of the analytes decreases with increasing percentages of acetonitrile in the solvent (Figures 3 and 4). The linear increases in electrophoretic mobility with increasing Q^+ concentrations noted in Figure 7 can be explained by more complete "complexation" of the neutral analytes.

3.9 Capacity factors

In chromatography the capacity factor (k) of a sample compound is defined as the time spent in the stationary phase divided by the time spent in the mobile phase in the column. In our co-migration system electroosmotic migration has a function equivalent to

the stationary phase in liquid chromatography. Of course electroosmotic migration involves movement, but all of the analytes move at the same rate. Separation is due to the electrophoretic migration that occurs when the analytes are associated with the positively charged quaternary ammonium ion. Thus, if we subtract the rate of electroosmotic migration from the total rate of migration, the capacity factor (k) can be defined as the time spent by an analyte uncomplexed by Q^+ (no electrophoretic migration), divided by the time spent associated (complexed) with Q^+ (electrophoretic migration). The fraction of an analyte "associated" with Q^+ can be written:

$$f_Q = \frac{V_s - V_{eo}}{V_Q - V_{eo}} \qquad (1)$$

where V_Q is the linear velocity of the Q^+, V_{eo} is the linear velocity of electroosmotic flow, and V_s is the linear velocity of a sample analyte. Similarly the fraction not associated with Q^+ can be written:

$$f_{aq} = \frac{V_Q - V_s}{V_Q - V_{eo}} \qquad (2)$$

$$k = \frac{f_{aq}}{f_Q} = \frac{V_Q - V_s}{V_s - V_{eo}} = \frac{t_{eo}\,(t_s - t_Q)}{t_Q\,(t_{eo} - t_s)} \qquad (3)$$

where t_{eo}, t_Q and t_s are the migration times of the osmotic flow marker, Q^+ and the sample analyte respectively.

The values for t_{eo} and t_s are easily measured from an electropherogram. t_Q is the migration time of an analyte that is completely associated with the Q^+ additive. This was

estimated to be the migration time of a large hydrophobic molecule, coronene. Capacity factors of 16 neutral organic compounds, calculated by equation 3, are given in Table I.

4. Conclusions

The highly efficient separations of CZE can be extended to neutral organic solutes simply by adding a suitable quaternary ammonium salt to the electrolyte and operating in a solvent containing an appreciable proportion of acetonitrile. The mechanism of the separation involves association in solution between the analytes and the added Q^+. In contrast to HPLC and most MECC, the largest most hydrophobic molecules have the shortest migration times.

A systematic study revealed that pH and acetonitrile concentration, as well as the type and concentration of Q^+, are important experimental variables. A pH between approximately 9 and 10 is necessary to ensure a reasonable electroosmotic flow. If the acetonitrile concentration is too low, electroosmotic flow is very low and separations are unreasonably slow. However, increasing concentrations of acetonitrile reduce the electrophoretic flow resulting from association of the analytes with the Q^+. The best separations were obtained with approximately 40 to 50% acetonitrile. When appropriate experimental conditions are used, a broad window for separation is possible. This method can be used for separation of a wide variety of neutral organic compounds.

Acknowledgements

We wish to thank Waters Division of Millipore for their gift of the Waters Quanta

Table I. Capacity factors for 16 aromatic compounds

t_M(min)	k'	Compounds
11.7	0.00	coronene
		3,4,9,10-dibenzpyrene
11.8	0.02	benzo(*ghi)*perylene
12.0	0.05	perylene
		benzo(*a*)pyrene
12.7	0.20	pyrene
13.2	0.31	anthracene
		benzo(*a*)anthracene
13.3	0.33	1-pyrenecarboxaldehyde
13.9	0.49	9-anthraldehyde
14.8	0.77	naphthalene
15.2	0.92	benzophenone
16.0	1.27	1-bromo-4-nitrobenzene
18.8	1.72	5,6-nitroquinoline
17.2	2.00	nitrobenzene
17.9	2.59	3'-nitroacetophenone
18.8	2.69	4'-nitroacetophenone
19.1	4.19	acetophenone
19.2	4.37	benzaldehyde

4000 CE system and associated chemicals and supplies. We are also grateful to Professor Edmondo Pramauro, University of Torino for information on micelles.

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HPCZE OF NON-IONIC COMPOUNDS USING A NOVEL ANIONIC SURFACTANT ADDITIVE

A paper submitted to *Analytical Chemistry*

Youchun Shi and James S. Fritz

Abstract

Excellent separations of non-ionic organic compounds were obtained by adding dioctyl sulfosuccinate (DOSS) to an acetonitrile(~40% v/v)-water electrolyte. Separation is based on differences in the strength of analyte-DOSS association "complexes" , or solvophobic interaction, in solution which results in differences in electrophoretic mobility. Micelle formation is not believed to occur. The effect of varying apparent pH, applied voltage, acetonitrile concentration and DOSS concentration was studied with regard to electroosmotic mobility and electrophoretic mobility. Under optimum conditions, excellent separations of 23 organic compounds were obtained. By varying the concentration of DOSS, it was possible to calculate formation constants for both 1:1 and 2:1 DOSS-analyte complexes.

Introduction

Capillary electrophoresis (CE) of non-ionic analytes cannot be performed in a free

solution due to lack of electric charges of analytes. This problem can be solved by using an additive to form a pseudophase. In micellar electrokinetic capillary chromatography (MECC), which was introduced by Terabe (1,2), micelles are used as a pseudophase. MECC separations are based on different partitioning of analytes between solvent phase and micellar phase. Sodium dodecyl sulphate (SDS) has been extensively used for electrolyte additive in MECC and proven very useful for separation of water-soluble analytes (3,4). However, SDS micelles are likely to disintegrate in electrolyte solution containing more than 20% (v/v) of many common organic modifiers, and SDS MECC has a limited elution range. Hydrophobic analytes are difficult to elute owing to their very small solubility in water and high partition coefficients into the pseudophase. Thus, SDS MECC is not a good choice for separation of hydrophobic compounds (5), although separation of testosterone ester has been reported in SDS solution containing up to 50% acetonitrile (6).

Bile salts have been used in MECC for separations of hydrophobic compounds (7,8) because the solubilizing power of micelles is lower than that of SDS micelles. Palmer *et al* used a monomolecular pseudophase for separation of hydrophobic analytes such as alkyl phthalate and polycyclic aromatic hydrocarbons (PAHs) (5) which are difficult to analyze by SDS MECC.

Several years ago, Walbroehl and Jorgenson reported separation of several non-ionic compounds by capillary zone electrophoresis (CZE), using tetrahexylammonium ion as a pseudophase additive in a aqueous medium containing 50% acetonitrile (v/v) (9). Due to the existence of four long carbon chains and high concentration of acetonitrile,

tetrahexylammonium salts will not form micelles (10). The interaction between tetrahexylammonium and analytes was called solvophobic association. However, the separation had a small separation window and could not resolve enough compounds. Little attention had been paid to this method until recently Shi and Fritz performed a systematic study of CZE of hydrophobic compounds using four long chain quaternary ammonium electrolyte systems (11). It was found that pH, concentration of acetonitrile and type and concentration of quaternary ammonium salts were important variables. Under appropriate conditions, a broad range of non-ionic organic compounds including many PAHs were separated using tetraheptylammonium ion as the additive.

Sodium dioctyl sulfosuccinate (DOSS) is used as a liquid-phase additive in the present work in order to separate non-ionic organic compounds by CZE. The more accurate chemical name for DOSS is di-2-ethylhexyl sulfosuccinate.

In the pure water, DOSS has lower critical micelle concentration (CMC), 2.5 mM at room temperature (12), than SDS, 8.1 mM at 25 °C (13). In aqueous solution containing 50%(v/v) acetonitrile, DOSS still has strong enough hydrophobic interaction with analytes, which makes it suitable for separation of hydrophobic analytes. It is shown that DOSS might be even more suitable than four long chain quaternary ammonium ions for two reasons. It gives even larger separation windows and has less interaction with capillary wall. Systematic studies of the DOSS electrolyte buffer were undertaken to determined the effects of experimental parameters on electroosmotic mobility and electrophoretic mobility. Separation of more than 20 hydrophobic analytes was achieved in 12 minutes using direct UV detection at 254 nm.

Experimental Section

A Waters Quanta 4000 Electrophoresis system (Waters, Milford, MA) was employed for capillary electrophoresis. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ) used for CE had 50 μm i.d. and 52.5 long between injection end and detection window. Direct UV absorbance detection was performed at 254 nm. Thirty seconds were set for hydrodynamic injection. Electropherograms were collected at speed of 15 points per second and plotted by a Chromperfect data acquisition system (Justice Innovations, Mountain View, CA).

All standards and electrolyte solutions were prepared with 18 MΩ deionized water from a Barnstead Nanopure II system (Syboron Barnstead, Boston, MA). Analyte standards were dissolved in water-*ca.* 30% (v/v) acetonitrile containing about 30 mM DOSS. Preparation of electrolyte buffer solutions included mixing DOSS (Aldrich, Milwaukee, WI), acetonitrile (Fisher Scientific, Fair Lawn, NJ) and sodium borate (Fisher Scientific) and adjusting pH of the solution by adding phosphoric acid (Fisher Scientific).

Between each run, capillaries were rinsed with 0.5 M NaOH for 15 minutes, deionized water for 15 minutes and running buffer for 12 minutes.

Results and Discussion

Principles

In CZE non-ionic organic molecules undergo only electroosmotic flow and therefore migrate at the same rate through the capillary. Separation of non-ionic

compounds becomes possible only when a large ionic substance is added to the electrolyte which can associate to varying degrees with non-ionic analytes and cause them to undergo electrophoretic migration. Sample compounds that associate strongly with the ionic additive have a larger electrophoretic mobility magnitude than those that are more weakly associated. DOSS was found to be an effective anionic additive for forming association "complexes" with neutral organic analytes.

A positive power supply was employed so that electroosmotic flow was toward the detection end of the capillary (toward the negative electrode) and the anionic additive migrate in the opposite direction. Since the electroosmotic mobility (μ_{eo}) of any analyte was always greater in absolute magnitude than the electrophoretic mobility (μ_{ep}) in the opposite direction, the net mobility (μ) was always positive (toward the detector). The stronger the interaction between an analyte and the negatively charged DOSS, the longer the migration time of the analyte.

In this counter-migration separation mode migration times become longer and longer as the negative electrophoretic vector and the positive electroosmotic vector become closer in their absolute magnitude. However, resolution of chemically similar molecules becomes better at longer migration times.

A negative power supply was tried briefly to obtain a separation based on co-migration. However, this would necessitate the use of a positively charged chemical to coat the capillary surface and reverse the direction of electroosmotic flow. This mode was not successful, probably because of interaction of the positively charged chemical with negatively charged DOSS.

Effect of pH

Preliminary experiments showed that good separations of several neutral aromatic compounds could be obtained under the following conditions: an alkaline apparent pH, 40% acetonitrile (v/v in water), 50 mM DOSS, 8 mM sodium borate, and 15-30 kV applied voltage. Each of the experimental variables was then studied to determine its effect on separation system.

The pH-dependence of electroosmotic mobility in fused-silica capillaries has been determined by Lukacs and Jorgenson (14). Their study shows that electroosmotic mobility increases with pH over the range 3 to 8 and reaches a plateau around pH 8. This was explained by dissociation of surface silanol groups at higher pH to leave a negatively-charged surface.

Electroosmotic mobility in the DOSS electrolyte in 40% (v/v) acetonitrile showed different and unexpected behavior at higher pH. The μ_{eo} increased up an apparent pH of 9.0 and then decreased above 9.0. The term "apparent pH" is used here because the DOSS electrolyte contained 40% acetonitrile, which made the measured pH different from what it would be in water alone. Figure 1 shows the change of μ_{eo} with apparent pH in 50 mM DOSS electrolyte containing 40% acetonitrile. The reason for this abnormal behavior is not clear, but from a practical view separation around apparent pH 9.0 has two advantages. First, the fastest separation will be obtained at this pH. Second, and more importantly, results will be more reproducible around apparent pH 9.0 where the change of μ_{eo} with pH is much smaller than at other pH values.

The electrophoretic mobility of analytes was hardly affected by pH change in the

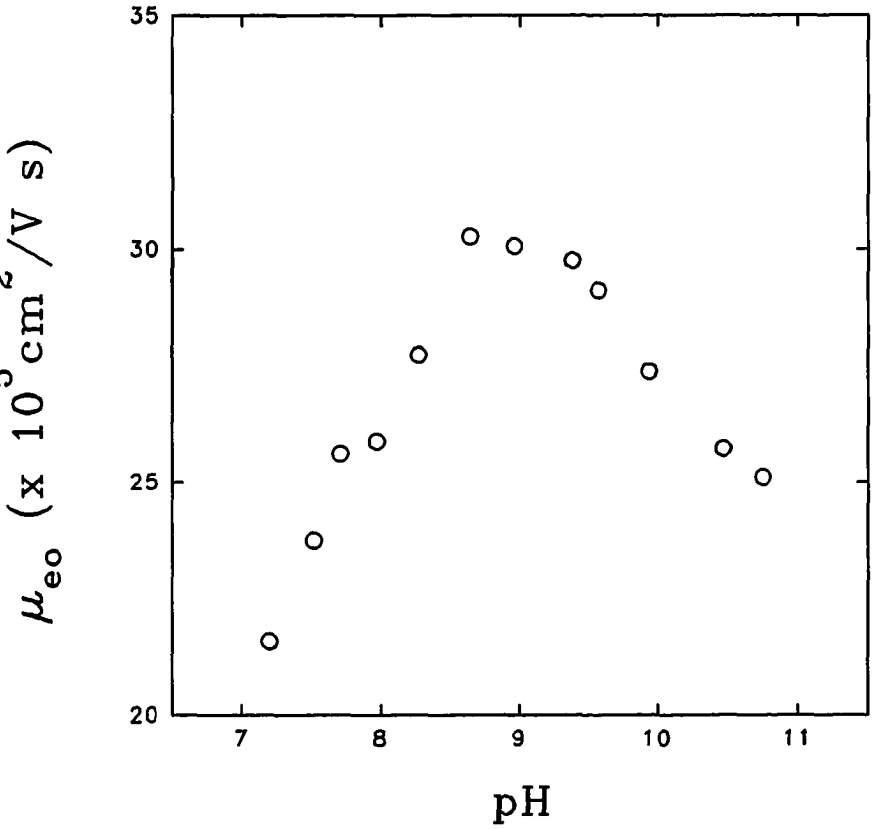

Figure 1. Influence of apparent pH on electroosmotic mobility. Electrolyte, 50 mM sodium dioctyl sulfosuccinate, 8mM sodium borate, 40% (v/v) acetonitrile; applied voltage, 30 kV; current, 45 - 49 μA.

region between approximately apparent pH 8 to 10.5 (Figure 2).

Effect of applied voltage

According to theory (15, 16), use of a higher voltage will yield more theoretical plates (thus giving sharper peaks) and shorter migration times of sample components. However, higher voltage can cause greater joule heating (15, 17) and thus cause peak broadening. Migration times can become too short to permit good resolution of sample analytes.

Figure 3 compares separation of 23 non-ionic compounds under identical conditions except for the applied voltage. As expected, the migration times become longer as the applied voltage is decreased, and resolution of the analytes becomes better. Resolution of most of the analytes in this complex sample is really quite good at 20 kV. However, a reasonable separation is still obtained at 30 kV and total migration time required is less than 11 minutes.

Effect of acetonitrile concentration

At apparent pH 9.0, the electroosmotic mobility decreased linearly as the content of acetonitrile in the electrolyte was increased (Figure 4). The relationship between electroosmotic mobility, μ_{eo} and ζ potential, which was derived by von Smoluchowski (18) is

$$\mu_{eo} = -\epsilon_0 \epsilon \zeta / \eta \qquad (1)$$

where ϵ_0 is the permittivity of vacuum, ϵ dielectric constant of the medium and η the

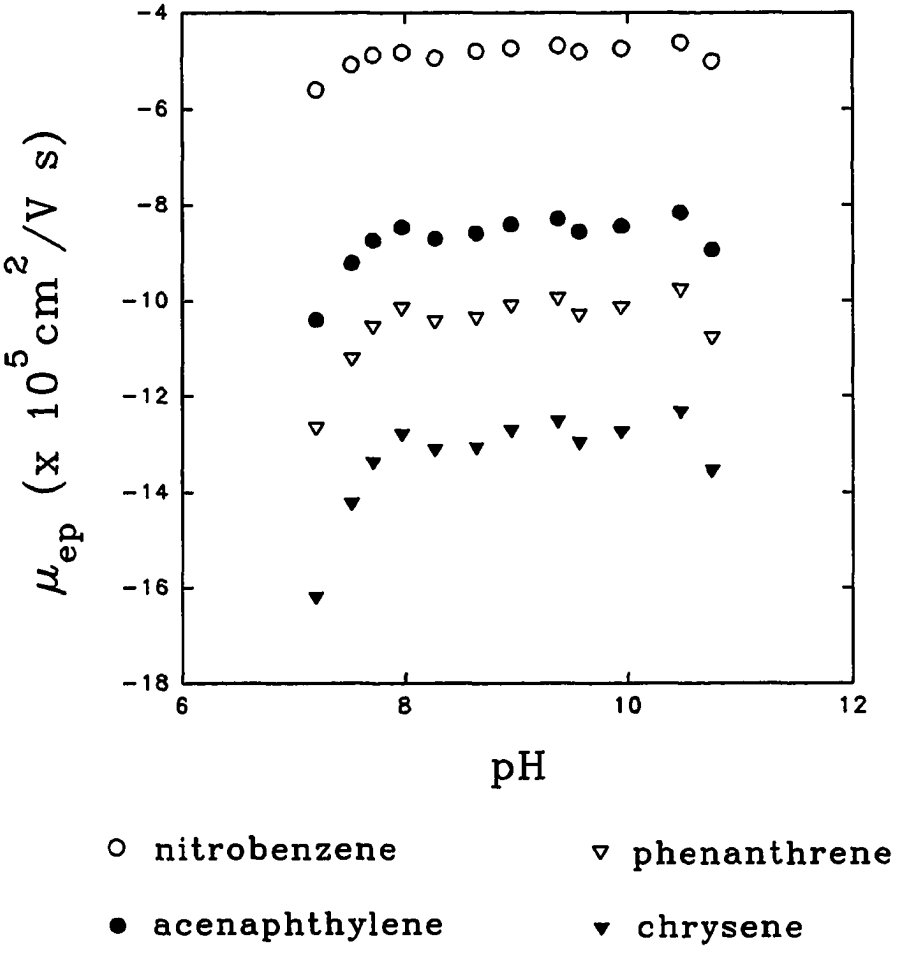

Figure 2. Effect of apparent pH on electrophoretic mobilities of several non-ionic compounds. Same conditions in Figure 1.

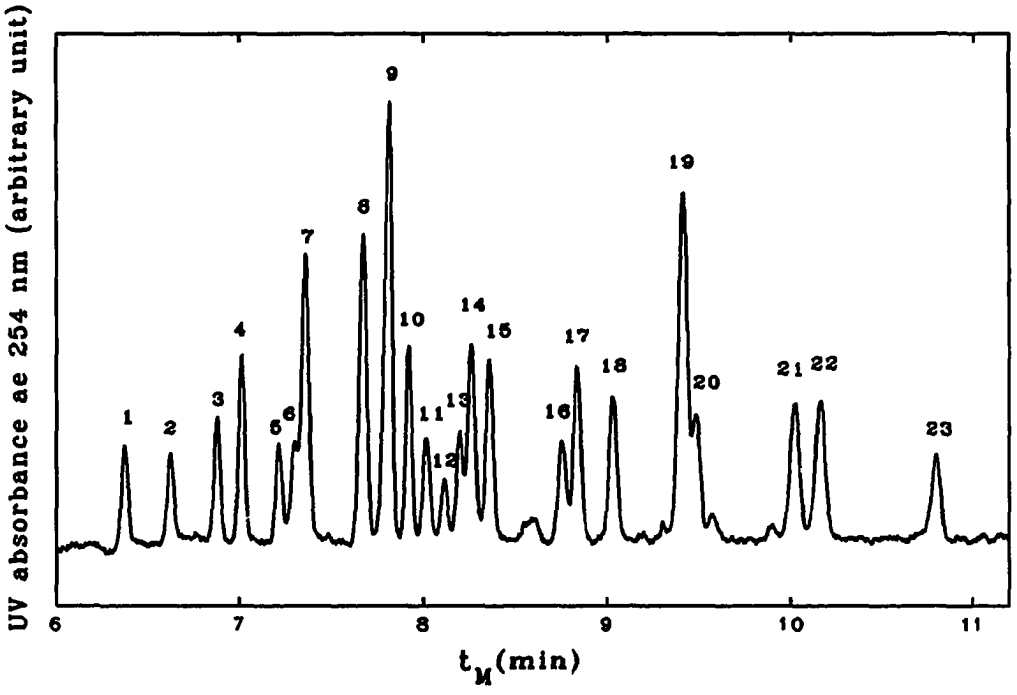

Figure 3A. Separation of non-ionic aromatic compounds using different applied voltage: (A) 30 kV; current, 49 μA; (B) 25 kV; current, 38 μA; (C) 20 kV; current, 29 μA. Electrolyte, 50 mM sodium dioctyl sulfosuccinate, 8 mM sodium borate, 40% (v/v) acetonitrile, pH 9.0. Peaks, 1=acetophenone; 2=nitrobenzene; 3=unidentified; 4=5,6-benzoquinoline; 5=benzophenone; 6=azulene; 7=naphthalene; 8=acenaphthylene; 9=acenaphthene; 10=fluorene; 11=3-aminofluoranthene; 12=9,10-dimethylbenz(a)anthracene; 13=benz(a)anthracene; 14=phenanthrene; 15=anthracene; 16=fluoranthene; 17=pyrene; 18=2,3-benzofluorene; 19=chrysene; 20=2,3-benzphenathrene; 21=perylene; 22=benzo(a)pyrene; 23=benzo(ghi)perylene.

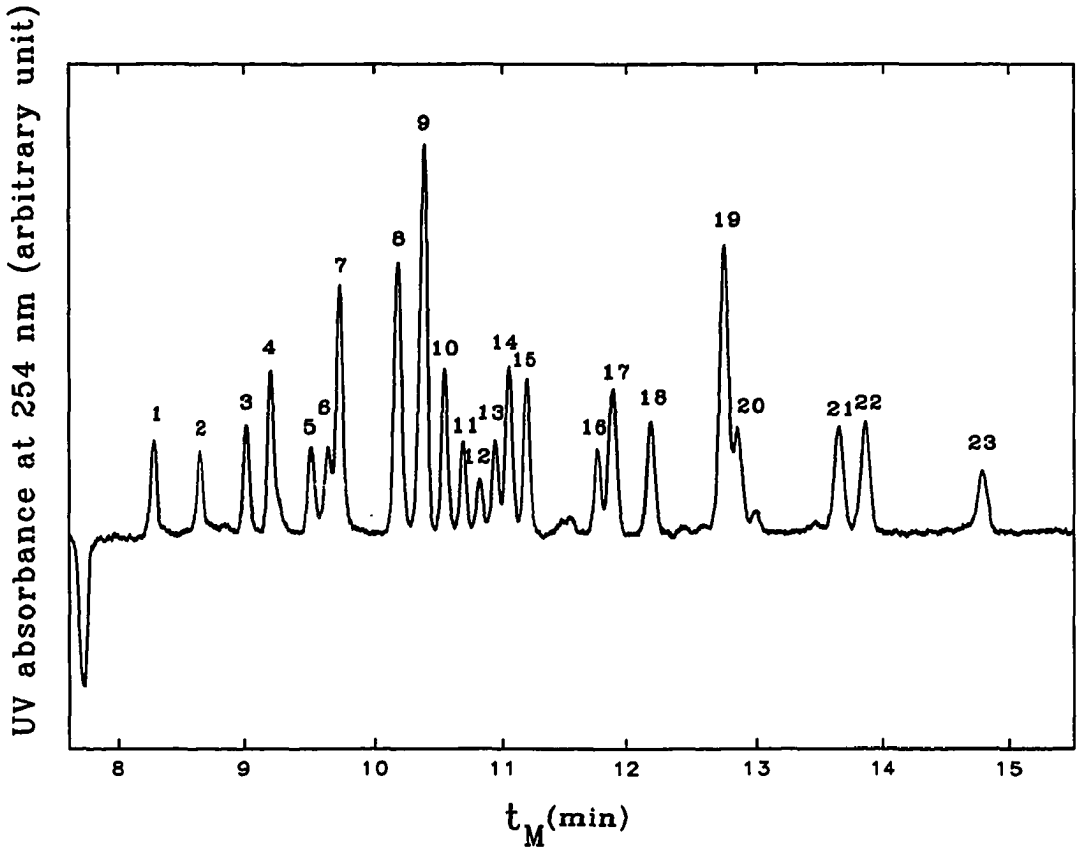

Figure 3B. Applied voltage, 25 kV; current, 38 μA. For all other conditions and peak identifications, see Figure 3A.

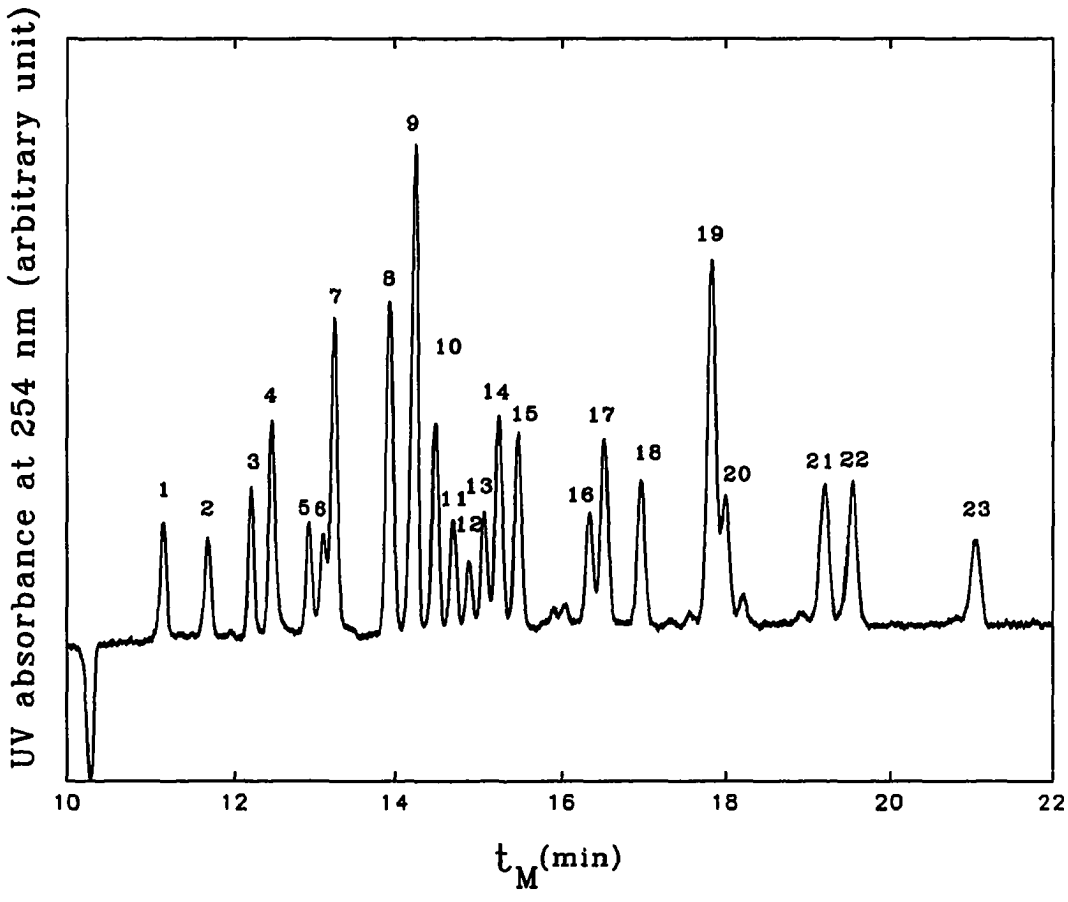

Figure 3C. Applied voltage, 20 kV; current, 29 μA. For all other conditions and peak identifications, see Figure 3A.

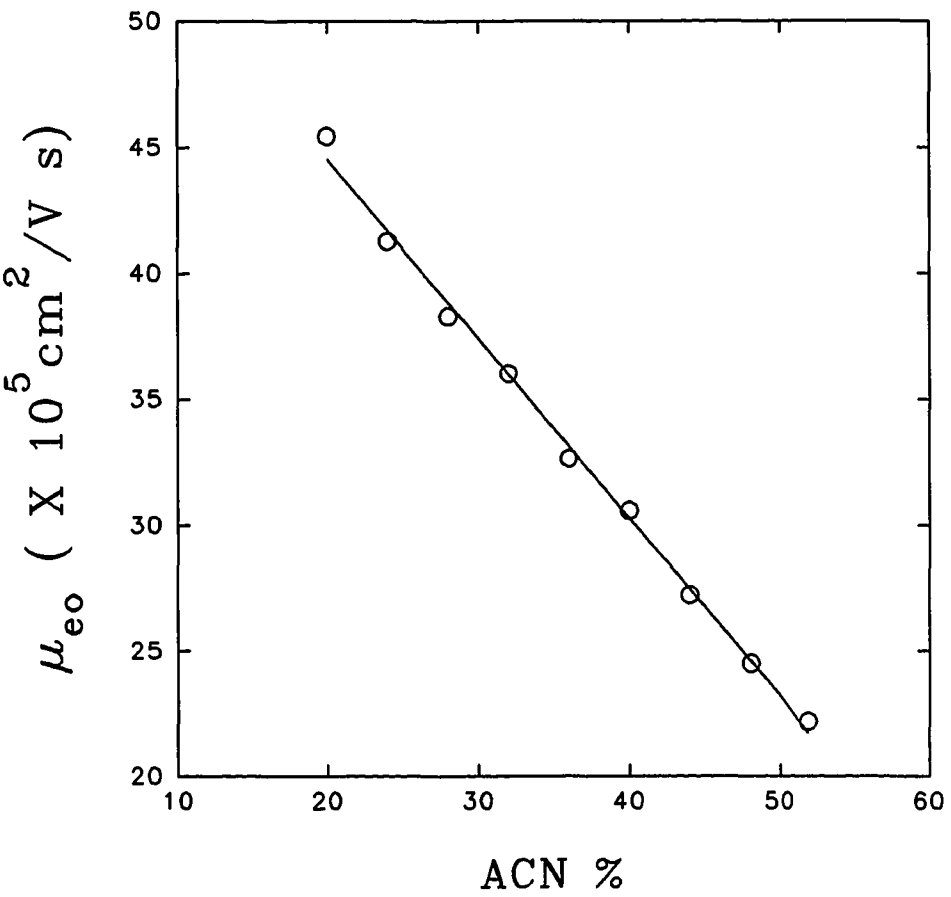

Figure 4. Dependence of electroosmotic mobility on acetonitrile content. Electrolyte, 50 mM sodium dioctyl sulfosuccinate, 8 mM sodium borate, apparent pH 9.0; applied voltage, 30 kV.

viscosity of the medium. Schwer and Kenndler (19) studied the influence of organic solvents on electroosmotic mobility and concluded that the decrease of electroosmotic flow with increasing content of acetonitrile is caused mainly by low dielectric constant, which leads to a low value for the ζ.

Figure 5 shows that the magnitudes of electrophoretic mobilities of non-ionic compounds decreased as the content of acetonitrile in the electrolyte increased (notice the negative sign of the electrophoretic mobility should be omitted when the only magnitudes are compared). The reason is easy to understand. The electrophoretic mobility of non-ionic compounds is a result of their association with DOSS. A higher concentration of acetonitrile (ACN) solvates the analytes (A) more strongly and reduces the strength of analyte-DOSS complexes. The solvophobic interaction between the analytes and DOSS can be expressed using the following equilibrium equation:

$$A(ACN) + DOSS^- \rightleftharpoons A(DOSS)^- + ACN \qquad (2)$$

Figure 5 shows that large differences in electrophoretic mobilities occurred at lower concentrations of acetonitrile, a fact that could improve the resolution of sample analytes. However, an acetonitrile concentration that is too low reduces the solubility of large analytes and would require very long separation times. Therefore, a modest reduction in acetonitrile concentration is very beneficial. Figure 6 shows a separation of 23 compounds in 36% (v/v) acetonitrile. Although the separation time is somewhat longer, resolution is appreciably better than obtained in 40% (v/v) acetonitrile (Figure 3).

Effect of DOSS concentration

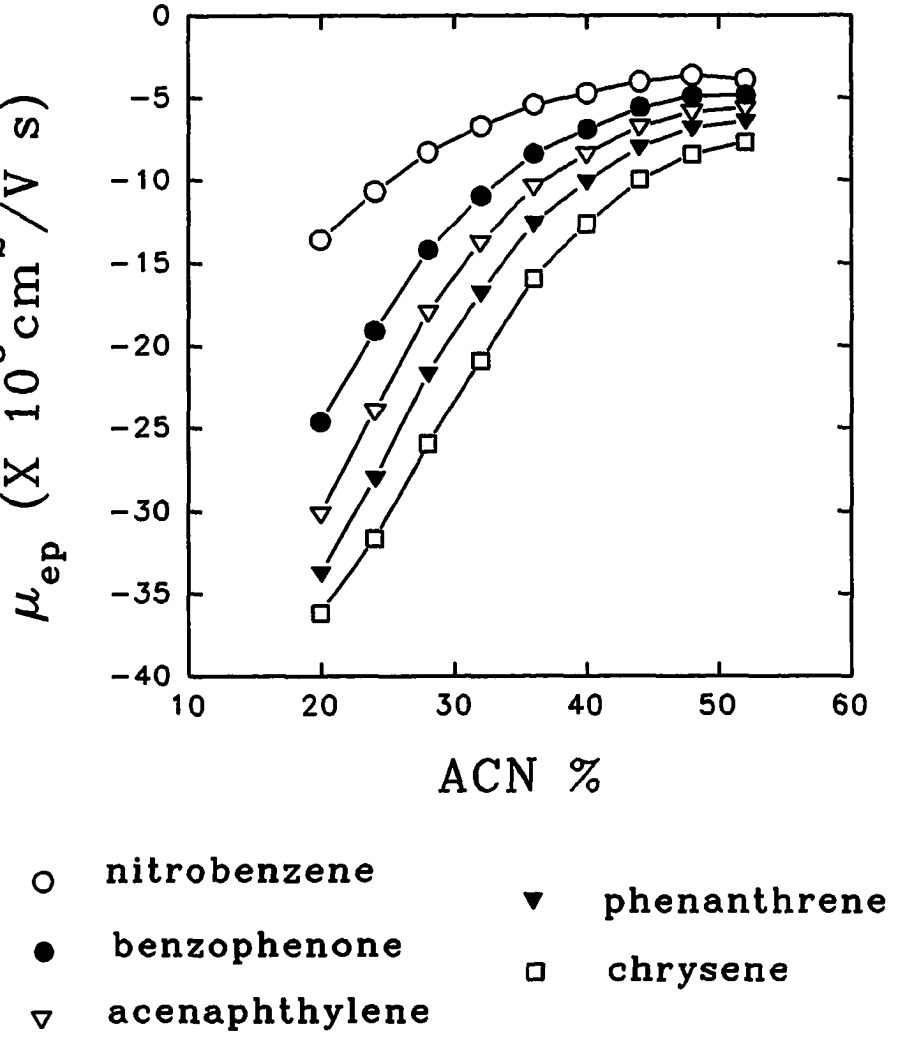

Figure 5. Change of electrophoretic mobilities with acetonitrile content. For conditions, see Figure 4.

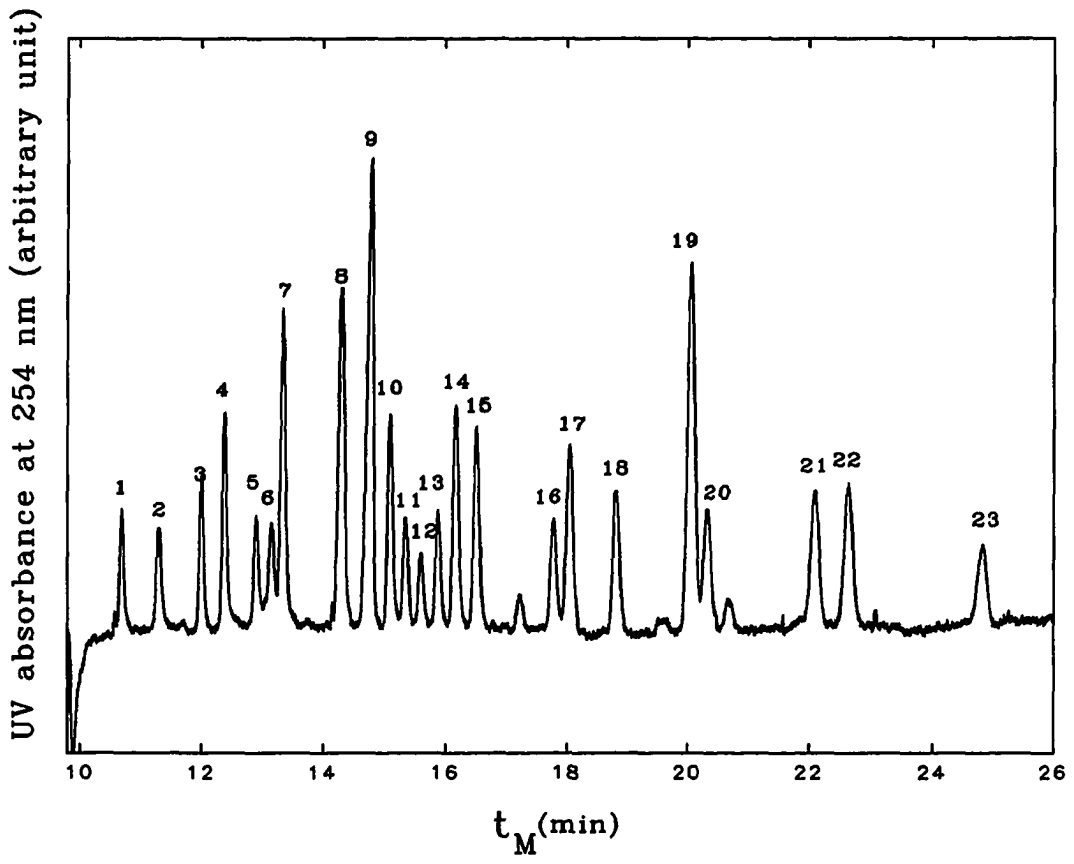

Figure 6. Separation of non-ionic aromatic compounds in electrolyte containing 36% (v/v) acetonitrile. Electrolyte, 50 mM sodium dioctyl sulfosuccinate, 8 mM sodium borate, apparent pH 9.0; applied voltage, 20 kV; current, 30 μA. For peak identifications, see Figure 3.

The effect of DOSS concentration on μ_{eo} was studied at apparent pH 9.0 in 40% (v/v) acetonitrile (Figure 7). This decrease can be explained by the increased ionic strength and viscosity of the electrolyte. Electrophoretic mobilities of analytes became increasingly negative with increasing DOSS concentration (Figure 8) owing to more complete association with DOSS.

For practical separations the window for separation is very narrow (~0.7 minutes) at 10 mM DOSS (40% acetonitrile and 25 kV) and resolution is very poor (Figure 9A). The separation window increased to ~1.8 minutes at 20 mM DOSS, ~3.8 minutes at 30 mM (Figure 9B), ~5.6 minutes at 40 mM, and ~8.3 minutes at 50 mM. The best separation was obtained at 60 mM DOSS (Figure 9C) where the separation window was ~12.3 minutes. At 70 mM DOSS the separation window was ~18 minutes but the baseline had become noisy.

Estimation of association constants

Estimation of solvophobic association constants was undertaken in DOSS electrolyte buffers containing 40% (v/v) acetonitrile. The concentration of acetonitrile was so high that the formation of micelles was unlikely (20, 21). The major interaction between the surfactant and the organic analyte was solvophobic association, which can be expressed using the following equations:

$$A + D \rightleftharpoons AD$$

$$AD + D \rightleftharpoons AD_2$$

$$\ldots \qquad (3)$$

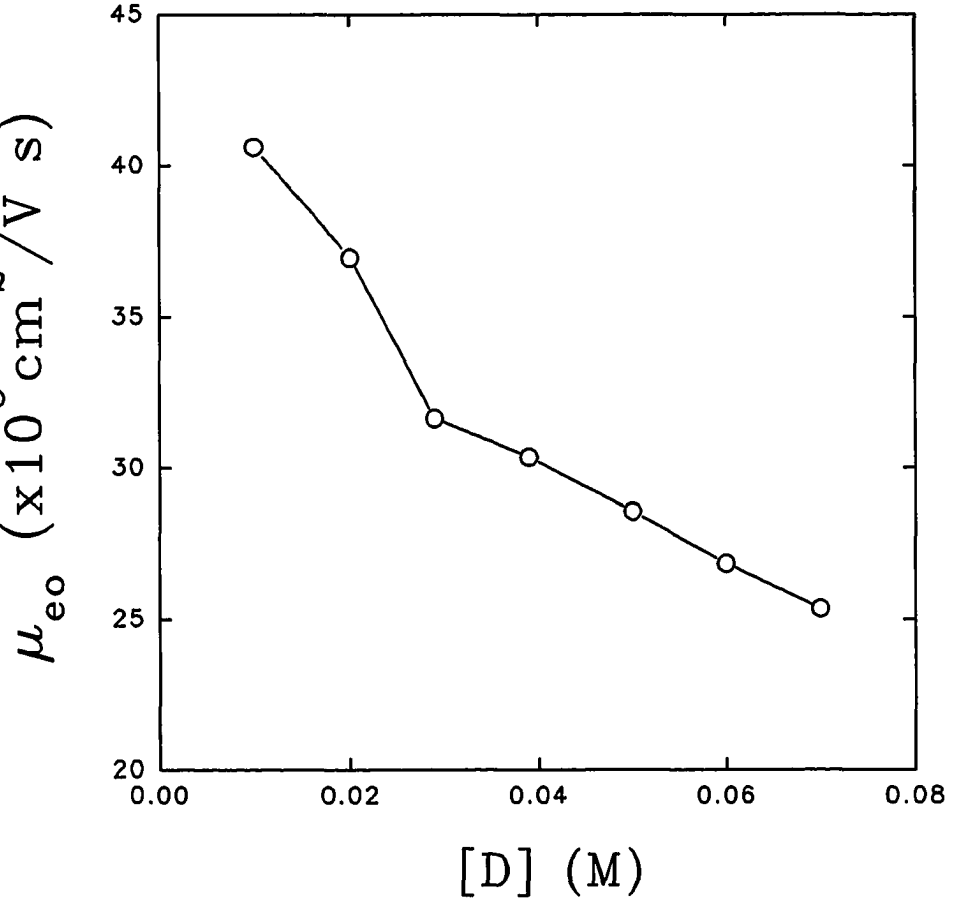

Figure 7. Electroosmotic mobility vs. concentration of sodium dioctyl sulfosuccinate. Electrolyte, 6 mM sodium borate, apparent pH 9.0; applied voltage, 25 kV.

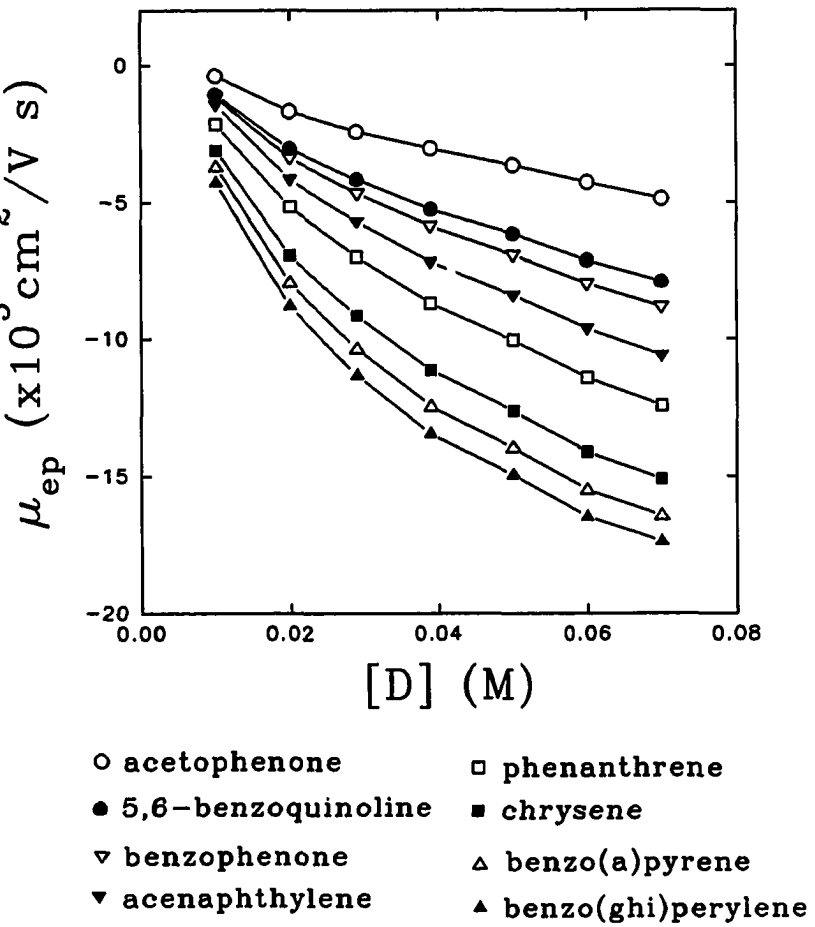

Figure 8. Effect of concentration of sodium dioctyl sulfosuccinate on electrophoretic mobilities. Same conditions in Figure 7.

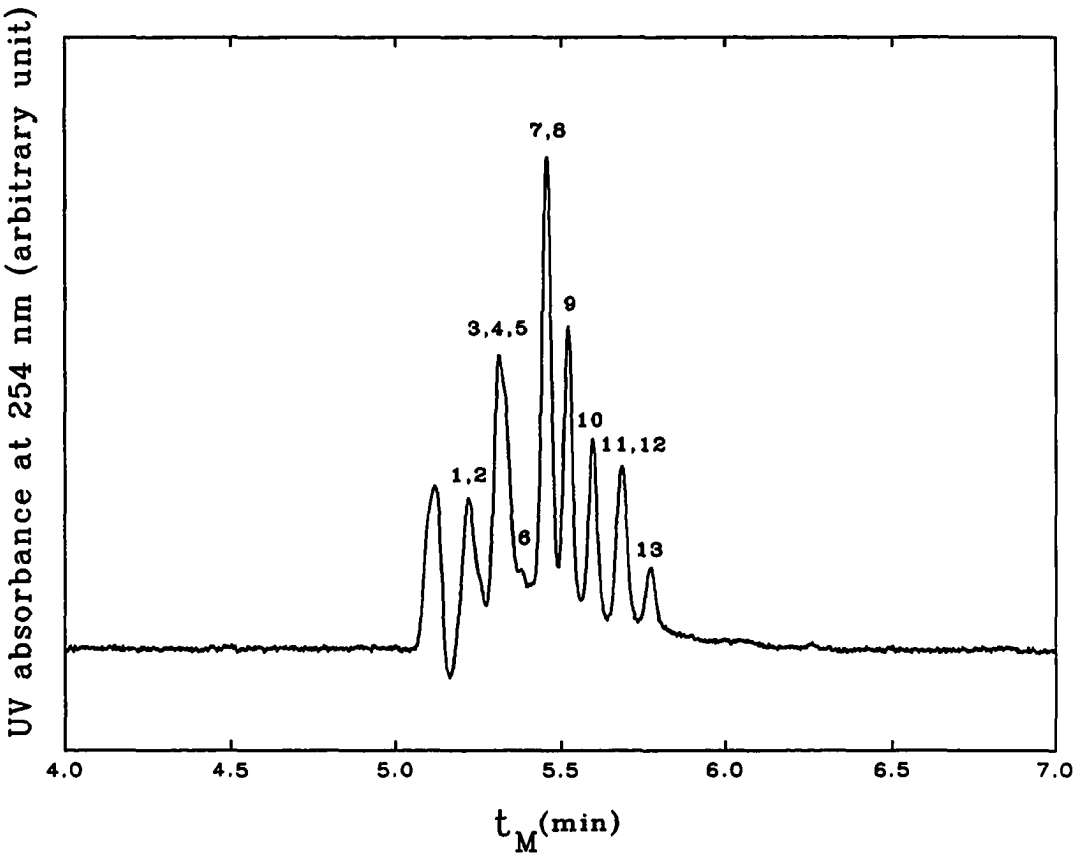

Figure 9A. Influence of DOSS concentration on resolution. [DOSS] = 10 mM. Other conditions are same as described in Figure 7. Peaks, 1=acetophenone; 2=nitrobenzene; 3=5,6-benzoquinoline; 4=benzophenone; 5=azulene; 6=acenaphthylene; 7=phenanthrene; 8=anthracene; 9=pyrene; 10=chrysene; 11=perylene; 12=benzo(a)pyrene; 13=benzo(ghi)perylene.

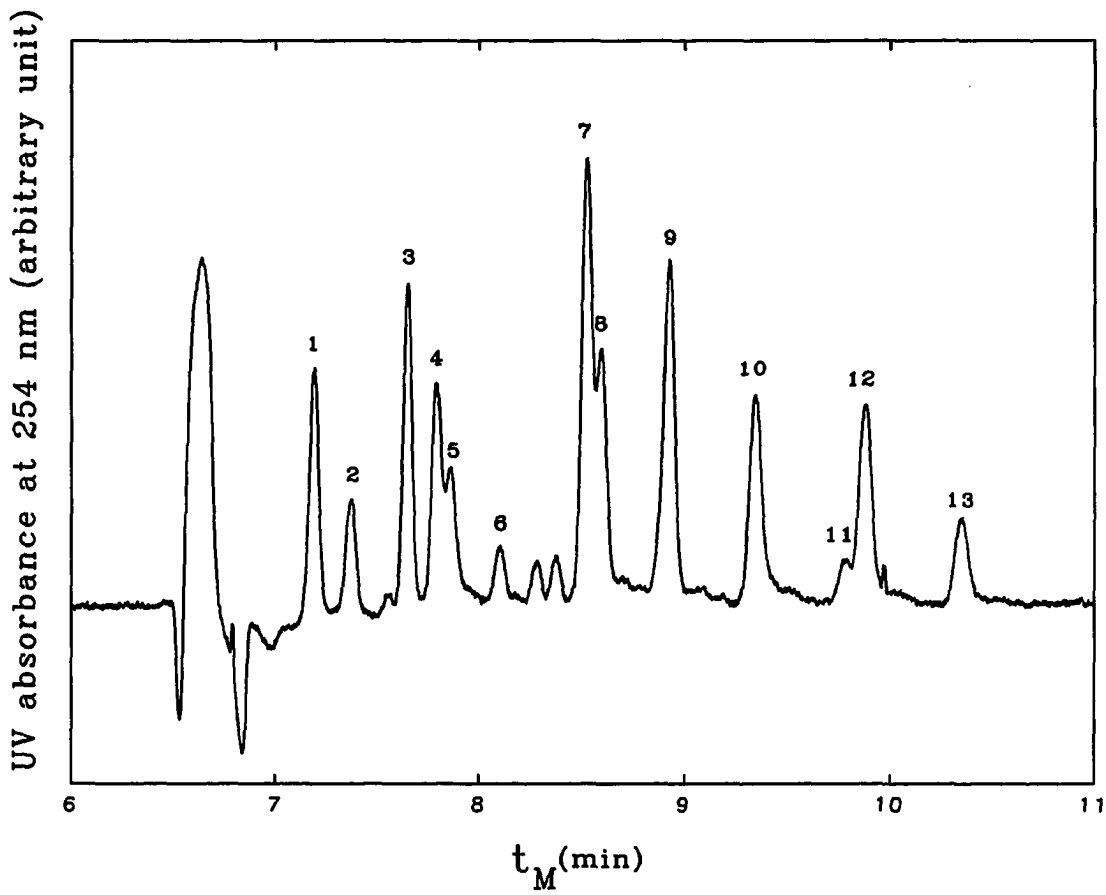

Figure 9B. [DOSS]=20mM. All other conditions and peak identifications are same as described in Figure 9A.

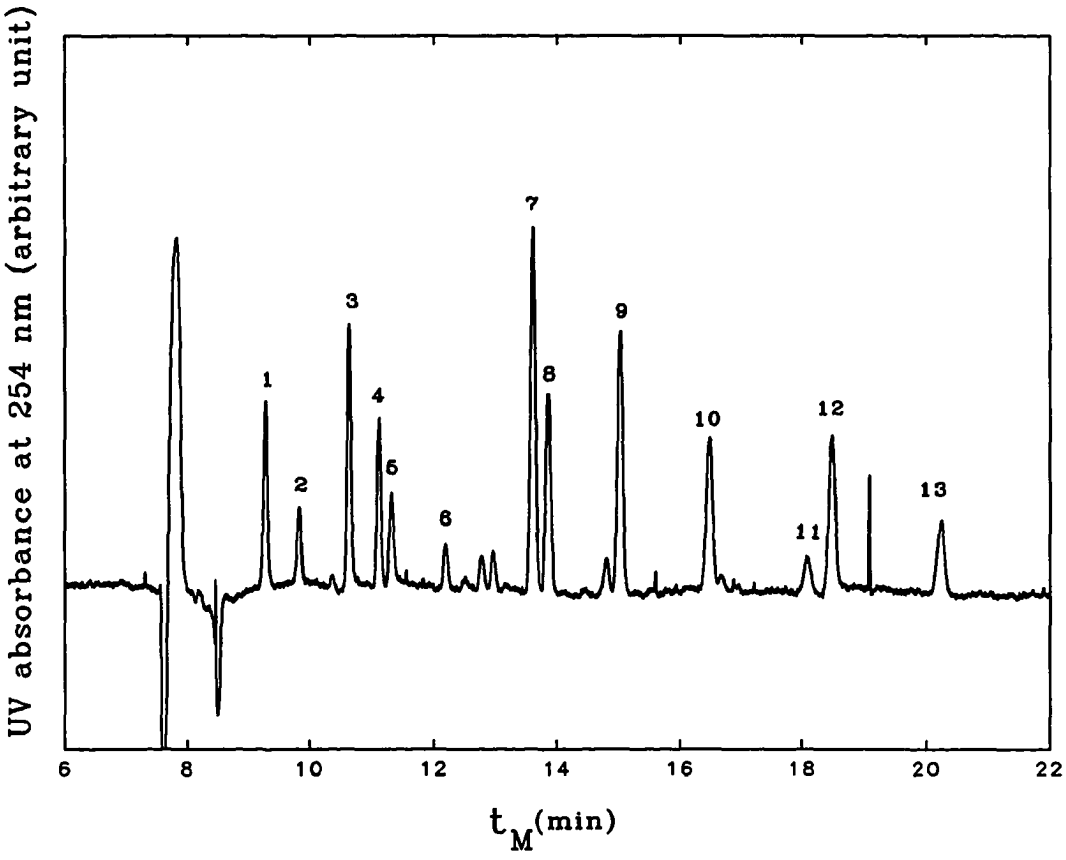

Figure 9C. [DOSS]=30mM. All other conditions and peak identifications are same as described in Figure 9A.

where A is an analyte and D is the dioctyl sulfosuccinate. The stepwise association constants are

$$K_1 = [AD]/[A][D]$$

$$K_2 = [AD_2]/[AD][D]$$

... (4)

If cumulative association constants are used, then

$$\beta_1 = [AD]/[A][D]$$

$$\beta_2 = K_1K_2 = [AD_2]/[A][D]^2$$

... (5)

The fraction of free analyte, α_0, is (1, 2):

$$\alpha_0 = [A]/([A]+[AD]+[AD_2]+...]) \quad (6)$$

Its reciprocal is

$$1/\alpha_0 = ([A]+[AD]+[AD_2]+...])/[A] = 1 + [AD]/[A] + [AD_2]/[A] +...$$

$$= 1 + \beta_1[D] + \beta_2[D]^2 \quad (7)$$

Equation (8) gives the relationship of α_0 and mobilities (1,2):

$$\alpha_0 = (\mu^D - \mu)/(\mu^D - \mu_{eo}) \quad (8)$$

where μ is the apparent mobility of the analyte, μ_{eo} the electroosmotic mobility and μ^D the apparent mobility of an analyte completely complexed with dioctyl sulfosuccinate.

Both μ and μ_{eo} can be calculated directly using experimental data. If the value of μ^D were also known, it would not have been difficult to calculate α_0 using equation (8) and then calculate the association constants in equation (7) using 2nd order regression. Unfortunately, the mobility of dioctyl sulfosuccinate, μ^D, is unknown, and unlike MECC

with SDS, it couldn't be obtained using Sudan III (1) or quinine hydrochloride (22) as a marker. Actually, Sudan III and quinine hydrochloride were eluted earlier than many analytes because their solvophobic interaction with DOSS in 40% (v/v) acetonitrile solution was not very strong. It was difficult to choose an appropriate DOSS marker in the electrolyte solution.

However, it could be found that the distances between separation peaks were very large between last few large PAH molecules after careful examination of the peak distribution fashion in electropherograms, while large PAH molecule peaks should be close together if complete complexation occurs(11). It can be assumed that the association between the largest PAH molecules and DOSS is far from completed. Thus, compared with μ and μ_{eo}, the apparent mobility of DOSS, μ^D, is quite small.

Rearranging equation (8) gives

$$\alpha_0 = [(\mu^D - \mu_{eo}) - (\mu - \mu_{eo})]/(\mu^D - \mu_{eo})$$

$$= 1 - (\mu - \mu_{eo})/(\mu^D - \mu_{eo}) = 1 - \mu_{ep}/\mu^D_{ep} \qquad (9)$$

where $\mu_{ep} = \mu - \mu_{eo}$ is the electrophoretic mobility of the analyte and $\mu^D_{ep} = \mu^D - \mu_{eo}$ is the electrophoretic mobility of the surfactant molecules. Combining equations (7) and (9), one can obtain

$$\mu^D_{ep}/(\mu^D_{ep} - \mu_{ep}) = 1 + \beta_1[D] + \beta_2[D]^2 + \ldots \qquad (10)$$

Using the above assumption, $\mu^D \approx 0$, the first estimation of μ^D_{ep} is

$$\mu^D_{ep} = \mu^D - \mu_{eo} \approx -\mu_{eo} \qquad (11)$$

With replacing μ^D_{ep} in equation (10) with equation (11) and making a 2nd-order regression using SigmaPlot software, cumulative association constants were obtained

(Table I).

When 3rd-order regression was made, some negative values were obtained. Actually, the possibility for an analyte combining with more than two DOSS molecules is very small. Therefore, it is assumed that only three kinds of species, or A, AD and AD_2 existed in the solution.

In order to obtain more reliable β values, the 2nd-order estimation of μ^D_{ep} was done. Using the β_1 and β_2 values of different analytes at certain concentration of DOSS and the following equation

$$\mu_{ep} = (1 - \alpha_0) \times \mu^D_{ep} \qquad (12)$$

which can be directly derived from equation (9), the value μ^D_{ep} at this DOSS concentration was calculated by a 1st-order regression (Table II). Because the assumption of $\mu^D \approx 0$ is more suitable for early eluted analytes, only the β values of the first seven peaks were used for μ^D_{ep} estimation.

Using the second estimation values of μ^D_{ep} and repeating the step of calculation of β_1 and β_2, new β values were obtained using 2nd-order regression (Table III). At low concentration of DOSS, separation peaks were merged and relative reading error of migration times was large. Therefore, sometimes the datum at [D]=0.01 M was not used in order to get a regression with a intercept closer to 1.0000.

Conclusions

Use of dioctyl sulfosuccinate as a solvophobic additive is quite efficient to achieve high performance capillary zone electrophoresis of a broad range of non-ionic aromatic

Table I. The cumulative solvophobic association constants calculated using a 2nd-order regression. The values of electrophoretic mobility, μ^{D}_{ep}, used for regression were $-\mu_{eo}$ values. The ideal intercept is equal to 1.0000. Electrolyte, 40% acetonitrile, 6 mM sodium borate, pH 9.0; applied voltage, 25 kV.

Analyte	β_1	β_2	intercept	r
acetophenone	2.33	15.5	0.9962	0.9990
nitrobenzene	3.30	20.7	0.9888	0.9986
5,6-benzoquinoline	4.06	34.3	0.9962	0.9991
benzophenone	4.52	44.6	0.9932	0.9992
azulene	5.07	43.3	0.9985	0.9992
acenaphthylene	5.06	73.7	0.9952	0.9990
phenanthrene	5.42	115	1.0088	0.9990
anthrathene	5.56	122	1.0078	0.9989
pyrene	5.56	161	1.0190	0.9987
chrysene	5.42	211	1.0353	0.9985
perylene	4.24	281	1.0641	0.9983
benzo(a)pyrene	4.14	297	1.0656	0.9981
benzo(ghi)perylene	2.54	397	1.0997	0.9977

Table II. Estimation of μ^{D}_{ep} values using estimated β values. The ideal intercept is 0.00.

[D] (M)	$-\mu_{eo}$ x 10^5 cm^2/V s	μ^{D}_{ep} x 10^5 cm^2/V s	intercept x 10^5 cm^2/V s	r
0.01	-41.2	-44.6	0.51	0.9336
0.02	-37.1	-40.7	0.50	0.9917
0.03	-31.7	-35.5	0.38	0.9960
0.04	-30.5	-32.2	0.38	0.9983
0.05	-28.7	-28.8	0.30	0.9993
0.06	-27.0	-27.5	0.28	0.9997
0.07	-25.5	-26.0	0.18	0.9998

Table III. The second estimatimation of cumulative solvophobic association constants using the μ^{D}_{ep} values estimated from first estimation of β_1 and β_2.

Analyte	β_1	β_2	intercept	r
acetophenone	2.14	17.7	0.9947	0.9999
nitrobenzene	2.55	28.6	0.9977	0.9999
5,6-benzoquinoline	3.72	37.8	0.9929	1.0000
benzophenone	4.14	48.3	0.9897	1.0000
azulene	4.56	48.3	0.9870	1.0000
acenaphthylene	4.26	80.8	0.9984	1.0000
phenanthrene	4.94	117	1.0008	1.0000
anthrathene	5.07	124	0.9996	1.0000
pyrene	5.08	161	1.0079	0.9999
chrysene	5.00	209	1.0200	0.9999
perylene	4.03	272	1.0426	0.9999
benzo(a)pyrene	3.96	287	1.0432	0.9998
benzo(ghi)perylene	2.69	360	1.0693	0.9997

compounds, including PAHs and fairly hydrophilic compounds. Compared with cationic additives (9, 11), DOSS has a negative charge and less interaction between the surfactant and the uncoated capillary wall due to the repulsion, which results in more reproducible separations. Counter-migration separation mode enables larger analytes to stay longer in capillaries and thus gives excellent separation for large molecules. The systematic study indicates that apparent pH, applied voltage, acetonitrile concentration and DOSS concentration are key experimental parameters of high resolution separations. Solvophobic association constants between analytes and DOSS can be estimated using 2nd order regression. The excellent separation of PAHs using DOSS in ~40% (v/v) acetonitrile proves again that CZE using solvophobic association is method of separation of hydrophobic neutral compounds, where MECC has its difficulty.

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GENERAL CONCLUSIONS

Capillary zone electrophoresis of some analytes can be difficult if the analytes do not have large enough differences in electrophoretic mobilities. However, high resolution CZE of transition metal cations and non-ionic organics were achieved using appropriate electrolyte modifiers.

Fairly weakly complexing reagents were used for separation of transition metal ions and lanthanides. If a metal ion is more strongly complexed, it will have smaller electrophoretic mobility than those less strongly complexed ions. Methanol in the electrolyte reduced both electroosmotic and electrophoretic mobilities so that migration times of ions were increased to allow the formation of completely separated analyte zones. By adding a certain amount of methanol in lactate electrolyte buffer, 27 metal cations were baseline separated in six minutes. Mobility of large metal cations was increased by 18-crown-6 while that of small size metal ions was not, where size matching between metal cations and the crown ether plays an important role. Mobilities of potassium and ammonium became different mobilities when 18-crown-6 existed in the electrolyte. By utilizing effects of methanol and 18-crown-6, resolution was enhanced so that 1 ppm magnesium, calcium, strontium and barium could be separated from matrix containing 1000 ppm sodium. Lactate buffer also offered a good quantitative analysis result of metal cations when an internal standard was used. Some of metal cations could not be separated using complexing electrolyte because of the slow complexation kinetics

which diffuses the analyte zone. However, using a non-complexing electrolyte system containing nicotinamide Al^{3+}, Ag^{+}, Cr^{3+}, VO^{2+} and UO_2^{2+} were able to be separated.

Micellar electrokinetic capillary chromatography is not very useful for hydrophobic non-ionic compounds because micelles are not stable in the solution containing more than 20% common organic solvent, and also hydrophobic compounds have little solubility in water solution. However, CZE of hydrophobic neutral organics could be achieved using certain quaternary ammoniums as an electrolyte additive through a "solvophobic association" mechanism, in which association occurs between neutral analytes and an ionic quaternary ammonium and makes the analytes go through electrophoretic flow. Tetrahepylammonium (THPA) was found to be a very good solvophobic additive for separation of hydrophobic non-ionic organic compounds. Concentration of organic solvent, concentration of THPA and pH affected electroosmotic and electrophoretic mobilities. By changing electrolyte pH and acetonitrile concentration, separation window and migration times could be controlled. At pH range 9.1-9.5, acetonitrile concentration of 40-42%, high efficient separations of 16 aromatic compounds, including many PAH, were obtained. High concentrations of THPA were used to achieve high resolution, but it caused a wall effect in capillary which changed migration time and peak shapes. Rinsing capillary with methanol after each run could reduce the wall effect.

Dioctylsulfosuccinate (DOSS) is another excellent solvophobic additive. It has a negative charge and it does not adsorb on capillary wall. Counterelectroosmotic CZE of hydrophobic neutral organic compounds was performed. The fastest and the most

reproducible separation was obtained at pH 9.0 because of the highest electroosmotic mobility. The more water in electrolyte, the stronger is the solvophobic interaction. Therefore, higher resolution occurred at relatively lower acetonitrile concentration range but the solubility problem of hydrophobic analytes may appear at too low acetonitrile concentration range. Performing the CZE of neutral molecules at 30 kV caused problems with Joule heating and gave analysis time too short to allow the analyte to form completely separated zones. With 36% (v/v) acetonitrile in the electrolyte solution and applying 20 kV high performance capillary electrophoresis of 23 hydrophobic non-ionic aromatic compounds was achieved. Using the mobility data at electrolytes containing different concentrations of DOSS and a 2nd-order regression program, estimation of association constant between the analytes and DOSS was possible.