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Methods development for separation of inorganic anions, organic acids and bases, and neutral organic compounds by ion chromatography and capillary electrophoresis

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Jie Li

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ABSTRACT

A novel anion-exchange resin containing three amine groups was prepared by reaction of a chloromethylated polystyrene-divinylbenzene (PS-DVB) resin with diethylenetriamine. After being protonated by contact with an aqueous acid, this resin can be used for ion-chromatographic separation of anions. The charge on the resins can be varied from +1 to +3 by changing the mobile phase pH. The selectivity of the new ion exchangers for various inorganic anions was quite different from that of conventional anion exchangers. The performance of this new anion exchanger was studied by changing the pH and the concentration of the eluent, and several different eluents were used with some common anions as testing analytes. Conductivity detection and UV-visible detection were applied to detect the anions after separation. The new resin can also be used for HPLC separation of neutral organic compounds. Alkylphenols and alkylbenzenes were separated with this new polymeric resin, and excellent separations were obtained under simple conditions.

For the separation of neutral compounds by electrokinetic chromatography, separations are usually carried out in predominantly aqueous solution in order to preserve the charged micelle necessary for the separation. We now show that PAH compounds can be separated efficiently by capillary electrophoresis in pure methanol or in aqueous-organic mixtures containing a high percentage of methanol. Sodium tetradecyl sulfate was the preferred surfactant. The effects of pH, solvent composition, surfactant structure and surfactant concentration on the separations were studied. Reproducible migration times and linear calibration plots were obtained.

Addition of either ethanesulfonic acid or protonated triethylamine to the background electrolyte was found to markedly improve the separation of protonated anilines by capillary electrophoresis. These additives appear to form a thin coating on the capillary surface via a dynamic equilibrium. This results in a change in electroosmotic flow and reduces interactions of the sample cations with the silica surface. A mixture of ten substituted anilines could be separated, including several positional isomers. Migration times of the sample cations were reproducible with a RSD less than 1.0%.

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

CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with a general introduction containing a review of pertinent literature. This is followed by two research papers that have been published. The third and fourth papers have been submitted for publication. Permission from the publisher extending reproduction and distribution rights has been obtained. A general conclusion section follows these four papers. Each paper is similar to the published version, although additional figures and tables have been added. Figures and tables are contained in the text of the paper at the appropriate location. References cited within each paper are listed after the conclusions of each paper.

Anion-Exchange Chromatography

Ion exchange is one of the oldest separation processes described in the literature [1]. Modern ion-exchange chromatography was pioneered by Small et al. [2]. They developed ion-exchange resins of low capacity and high chromatographic efficiencies, and achieved automatic detection by introducing conductivity detection for ionic species. For a sensitive detection of ions via their electrical conductance, the effluent from separation column was passed through a "suppressor" column to reduce the background conductance of the eluent. In 1979, Fritz et al. [3] described an alternative separation and detection scheme for ion-exchange chromatography, where the separation column was directly coupled to the conductivity cell. For this chromatographic setup, ion-exchange resins with low capacities

have to be employed so that eluents with low ionic strengths can be used. In addition, the eluent ions should exhibit low equivalent conductances to ensure sensitive detection of sample components. Since then, many other important improvements, including the developments of stationary phases with high efficiency [4-14], employment of various eluent species [15-26], and introduction of different detection methods [21, 27-36], have make ion-exchange chromatography a versatile technique for both inorganic and organic analyses. Different retention models were also proposed for better understanding of retention behavior of ions on the ion-exchangers [37-42].

Anion-exchange chromatography is based on an anion-exchange process occurring between the mobile phase and anion-exchange groups on the stationary phase. Separation of anions is accomplished with quaternary ammonium groups of the stationary phases. Usually, when sample mixtures are loaded onto the separation column which has been equilibrated with the mobile phase, sample anions will replace the mobile phase anions that are attracted to the ion-exchange sites, so they are retained by the fixed charges on the stationary phase. Various sample ions remain a different length of time within the column due to their different affinity toward the stationary phase, therefore, separation is possible. Sample anions interact with the stationary phases through ion-exchange processes as well as other non-ionic interactions. The most important non-ionic interaction is adsorption via hydrophobic interaction or water-structure induced ion-pairing [43]. Highly polarizable inorganic anions, such as iodide, thiocyanate and oxygen-containing metal anions, and organic anions usually have much stronger retention on the stationary phases because of this adsorption phenomenon [44] than anions without adsorptive interactions.

Stationary Phases

Stationary phases used in anion-exchange chromatography can be characterized both by the nature of the ion-exchange groups and by the nature of the supporting materials. Most IC separations of anions are performed on strong base anion-exchangers containing quaternary amine functional groups, although less substituted amines can form weak base exchangers. The supporting materials can be classified as inorganic and organic (polymeric) materials. Organic polymers are predominant as supporting material because they show very high stability toward extreme pH conditions. Anion-exchangers with different supporting materials can provide different selectivities, so the development of new stationary phases for anion separation have been of great interest.

Polymer-based anion-exchangers

Polystyrene-divinylbenzene (PS-DVB) copolymers, polymethacrylate, and polyvinyl resins are several organic materials that are tested for their suitability as support materials for polymer-based anion exchangers. Polystyrene-divinylbenzene copolymers are the most widely used substrate materials [45-48]. Their stability over pH range between 0 and 14 allows the employment of eluents with extreme pH values. The copolymerization of styrene with divinylbenzene (DVB) is necessary to impart the mechanical stability to the resin. The degree of crosslinking is determined by the percentage of divinylbenzene in the reaction mixture. Reaction of PS-DVB copolymer to produce a strong-base anion-exchanger resin generally proceeds via chloromethylation using chloromethylmethylether in the presence of a suitable catalyst, such as zinc chloride. After chloromethylation, a second reaction with an amine produces the required anion-exchanger. Because of the extreme toxicity of

chloromethylmethylether and the difficulty in controlling the degree of chloromethylation, an alternative chloromethylation procedure, which used paraformaldehyde and concentrated hydrochloric acid in the absence of catalyst, was reported by Barron and Fritz [49]. This method allowed a good control of the ion-exchange capacity of the final resin.

In addition to PS-DVB copolymers, several other polymeric anion-exchangers have also been studied as potential stationary phases for IC. An anion-exchanger based on methacrylate polymer was introduced in 1983 for separation of inorganic anions [50,51]. This type of resin provides very high chromatographic efficiency, but is more sensitive to eluent pH than PS-DVB resin. Polyvinyl-based anion-exchange resins have been available since 1984 [52,53]. These resins are stable at pH values between 0 and 14, allowing the use of many eluents; however, they exhibit low efficiency.

There are advantages and disadvantages associated with these polymer-based anion-exchangers. Polymer resins can tolerate eluents and samples with extreme pH values. This makes it possible that anions from very weak acids, such as borate and cyanide, can be analyzed by ion chromatography. Poor efficiency was a problem at the early stage of IC using polymer resin [54]. With the advanced technology in making surface-functionalized resins, modern, small diameter anion-exchanger resins can provide efficiencies equivalent or superior to those obtained on silica exchangers of similar characteristics [53]. One significant drawback of polymeric anion-exchanger resins is that they are subject to pressure limitations, especially for polymethacrylate. The softness of this type of material restricts the column length and the eluent flowrates that can be used. Another limitation about polymer resins is the permissible percentage of organic modifier in the mobile phase cannot go high

because of the crosslinking in the polymer structure. This restriction can often limit the approaches taken to regenerate the columns fouled with organic materials.

Silica-based anion-exchangers

Parallel to the development of organic polymers as anion-exchanger substrate, a number of silica-based anion-exchangers have been introduced over the past years [55-58]. Generally, silica substrates are grouped according to their particle size, and microparticulate beads with particle sizes in the range of 3 to 10 μm are preferred to pellicular ones. Two groups of silica materials can be recognized. For polymer-coated materials, silica particles are first coated with a layer of polymer, and then the polymer layer is derivatized to introduce the desired functional groups for separations. For functionalized silica materials, functional groups are chemically bonded directly to silica particles.

The prime advantage of silica-based materials is the favorable chromatographic efficiency [53]. Silica can be obtained as small particles with a narrow size distribution; being non-swelling and rigid, these materials can be packed at high pressure to produce a uniform and stable chromatographic bed that is not subject to stringent pressure or flowrate limitations during usage. Moreover, organic modifiers can be used freely with functionalized silica materials to manipulate ion-exchange selectivities or to reduce column fouling by organic sample components. Another advantage is that the retention mechanism is frequently simpler than that with other materials because of low probability of secondary interactions between solute ions and silica substrate [59].

A number of drawbacks exist with the use of silica-based anion-exchangers. One of these is the restricted pH range over which the columns can be operated, usually between

pH 2-8. The pH values below 2.0 can cause the cleavage of the functional groups from the silica substrate and result in the loss of ion-exchange capacity. On the other hand, eluents or samples of alkaline pH could lead to the dissolution of silica matrix. Also, metal ions like Cu^{2+}, Pb^{2+} and Zn^{2+} can be retained on the silica-based anion-exchangers and cause inference with anion analyses [60]. This happens because silica itself can act as both anion- and cation-exchangers [61]; and metal ions can be retained also by adsorption of their anionic complexes formed with eluent species [62].

Other types of anion-exchangers

Except the two most popular support materials described above, anion-exchangers based on other materials have also been developed, including latex-agglomerated anion-exchangers [63,64], crown ether phases [65-67], silica and alumina phases [61,68,69]. A strong anion-exchange stationary phase of quaternized polyethylenimine-coated zirconia was also described [70]. Hollow fibers were used as anion-exchangers as well [71]. Although less popular than silica- and polymer-based anion-exchangers, these IC packing materials have different properties, and often offer distinguished selectivities.

Mobile phases

The range of mobile phase species used in anion-exchange chromatography is enormous. Several important eluent characteristics include the compatibility with the detection mode, nature and concentration of the competing ions, eluent pH, buffering capacity and organic modifier content. In general, the kind of eluent applied for anion separations depends mainly on the detection system employed as well as the solute anions being separated. For anion separation with chemical suppressors, salts of weak acids are

usually employed as eluents because they exhibit a low background conductivity after suppression. Carbonate, bicarbonate and their mixture [72, 73], borate [74], hydroxide [75] and some amino acid anions [76,77] with sodium as a suitable cation can fit into this category to separate a variety of anions. Non-suppressed anion-exchange chromatography requires the eluent species with low background conductivity to enable a sensitive conductivity detection of anions to be analyzed. Salts of aromatic carboxylic acids, such as benzoates, phthalates and pyromellitic acid, are the most widely used eluent species for the separation of anions by non-suppressed IC [20,22,78,79], although others are also used, including aliphatic carboxylic acids [80-83], sulfonic acids [84-86] and inorganic eluents [87-89].

In many cases, additives can be included in the mobile phases to dynamically modify the stationary phases and bring different selectivities. Jun et al. modified a polymeric PRP-1 reversed-phase column by coating it with hexadecyltrimethylammonium bromide and used it for the separation of inorganic anions and monocarboxylic acids [90]. Knox and Wan adsorbed polyethyleneimine onto porous graphitic carbon and obtained chromatographic performance similar to that of bonded ion-exchange silica gels [91]. Three sulfobetaine surfactants were adsorbed onto a C18 column for separating inorganic anions with water as eluent [92].

Detection

Conductivity detection is the most common detection mode for anion-exchange chromatography. Sample anions can be detected based on their conductance with or without chemical suppression of eluents. Amperometric and potentiometric detection are also

applicable in anion-exchange chromatography [93-95], and they offer much higher sensitivity than conductivity. Spectroscopic detection methods that have been used in anion-exchange chromatography include UV-visible [96], fluorescence[97] and refractive index [98] detections. Detection of sample anions with all of the detection modes mentioned here can be performed either directly or indirectly.

In Chapter 2 of this dissertation, a novel polymeric anion-exchanger based on polystyrene-divinylbenzene was prepared by modifying the PS-DVB resin particles with diethylenetriamine. Its capacity can be gradually changed by varying eluent pH. This new anion-exchanger was compatible with different mobile phase species for both direct UV and conductivity detection. It was applied for separating common inorganic anions and organic compounds.

Capillary Electrophoresis of Basic Compounds

Capillary electrophoresis (CE) has proven to be a rapid and versatile analytical technique that combines simplicity with high efficiency. The narrow diameter (normally between 20 and 100 μm) of the silica capillaries allows the application of high voltages and ensures rapid heat dissipation, and complex mixtures of analytes can be resolved and recorded as sharp signals due to lower risk of zone broadening. Jorgenson and Lukacs were the first to produce highly efficient CE separations [99,100]. Their publications drew the attention of a number of scientists from various disciplines (analysts, physical chemists, and biochemists) and marked the beginning of the process for CE development. The introduction of commercial CE instrumentation from late 1988 also enhanced the speed of development

and application of this technique. Variations in capillary design and the discovery of a number of modes of CE operation have enabled the continued success and application of this separation technique over the last 20 years.

CE is a technique for separating charged molecules based on their movement through a medium under the influence of an applied electric field. The separation efficiencies can reach as high as 10^5-10^6 theoretical plates. In its diverse modes of operation, including capillary zone electrophoresis (CZE) [101-103], micellar electrokinetic chromatography (MEKC) [104-108], capillary gel electrophoresis (CGE) [109-112], capillary isotacophoresis (CITP) [113-115], capillary isoelectric focusing (CIEF) [116,117], and capillary electrochromatography (CEC) [118-121], CE can be applied to analyze a wide variety of analytes ranging from low molecular weight analytes such as inorganic anions [122-126], metal cations [127-130], drugs [131-133] to larger molecules such as carbohydrates [134-138], peptides [139-141], proteins [142-144], DNA [145-148], bacteria [149,150], and single cells [151-153].

Separation by CE is based on different electrophoretic mobilities of ions (μ_{ep}, $cm^2/V{\cdot}s$), which are governed by their charge/size ratio [116],

$$\mu_{ep} = \frac{q}{6\pi\eta r} \qquad (1)$$

where q is the net charge, η is the viscosity of the buffer, and r is the hydrated radius. According to Eq. 1, electrophoretic mobilities are independent of electric field (E) and capillary length (L). However, both mobilities (μ) and velocities (v) can be measured experimentally:

$$v = \frac{L_d}{t_m} \tag{2}$$

$$\mu = \frac{v}{E} = \frac{L_d \cdot L_t}{t_m \cdot V} \tag{3}$$

where L_d is the length of the capillary to the detector, L_t is the total length of the capillary, t_m is the migration time, and V is the applied voltage.

A prominent phenomenon in CE is electroosmosis (EO). Electroosmosis occurs due to the surface charge on the wall of the capillary. An anionic charge on the capillary surface presumably owing to the ionization of silanol groups at most pH conditions results in the formation of an electrical double layer. When an electric field is applied, the layer of positive charge migrates toward the negative electrode. Since ions are solvated by water, the fluid in the buffer is mobilized as well and dragged along by the migrating cations, resulting in the bulk flow of liquid in the direction of the cathode, known as electroosmotic flow (EOF). The electroosmotic mobility (μ_{eo}) as defined by Smoluchowski in 1903 is given by

$$\mu_{eo} = \frac{\varepsilon_0 \xi}{4\pi\eta} \tag{4}$$

where ϵ_0 is the dielectric constant, η is the viscosity of the buffer, and ξ is the zeta potential on the surface. The magnitude of the EOF is largely affected by the pH of the solution. This is because the degree of dissociation of the silanol groups (which has a pK_a of 6-7) on the capillary wall is dependent upon the pH of the solution, and so is the zeta potential. Other experimental conditions, such as temperature, the buffer concentration, organic solvent

concentration, and chemical additives, can also be manipulated to vary both magnitude and direction of the EOF. The measured mobilities according to Eq. 2 are truly the sum of the electrophoretic (μ_{ep}) and electroosmotic mobilities (μ_{eo}):

$$\mu = \mu_{ep} + \mu_{eo} \tag{5}$$

CE separation of basic compounds are usually achieved through different approaches. Organic bases can be separated as protonated cations by operating at acidic pH [154-159]. A popular method of choice for separation of basic compounds is micellar electrokinetic chromatography (MEKC). Various analytes have been successfully resolved by MEKC, including pharmaceuticals [160,161], amino acids [156,162-164], proteins and peptides [165-167], and nucleosides and bases [168,169]. Most commonly used surfactants in MEKC are sodium dodecyl sulfate (SDS) and cetyltrimethylammonium chloride (CTAC). Nonionic and zwitterionic surfactants have also been employed for MEKC [170,171]. For MEKC separation of chiral compounds, synthetic or naturally occuring chiral surfactants are needed for the chiral resolution [172-174]. These surfactants can form micelles in the background electrolytes under certain conditions, thus allowing the partitioning of the analytes between micelles and bulk solution. Basic drugs were also separated by CE in nonaqueous media [175].

Another way to achieve the separation of basic compounds, especially for basic proteins, is to coat the silica capillary surface. Proteins are polyelectrolytes, and adsorption usually occurs because of columbic attractions between the negatively charged capillary surface and the positive charges on the protein molecules, resulting in either tailing peaks

or even complete adsorption of the protein to the capillary surface, i.e., no peaks. Both permanent and dynamic coating has proven to be successful in overcoming this problem. Capillaries can be coated by various cellulose derivatives [176-178], poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) [179,180]. Cationic amines have been applied for this purpose as well [167,181].

Chapter 4 of this dissertation deals with the CE separation of some alkyl-substituted anilines using ethanesulfonic acid or triethylamine as the BGE additives. These additives can decrease, eliminate or reverse the EOF, preventing the adsorption of the basic analytes onto the capillary surface. Because these additives are quite small, they usually form very thin coating on the surface, thus are easy to remove by simply rinsing capillary with organic solvents and water.

Nonaqueous Capillary Electrophoresis

Nonaqueous capillary electrophoresis (NACE) has been gaining popularity over the last several years [182-186]. Compared with CE separations performed in aqueous solution, NACE utilizes organic solvents as separation media to alter electroosmotic flow and electrophoretic mobilities of analytes and to achieve different selectivities [187]. Organic solvents provide various polarity, viscosity, dielectric constant and autoprotolytic properties, so analytes can be solvated and migrate differently in organic solvents. Highly hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAHs), are especially suitable for the NACE analysis because they have better solubility in many organic solvents than in aqueous phase.

To make an appropriate medium for NACE analysis, an organic solvent should be able to maintain a stable electric current between electrodes, requiring an adequate solubility for added ionic species; the solvent should allow a reasonable electroosmotic flow and electrophoretic mobility for the analyte, so analysis can be complete within a reasonable time; the solvent should provide enough selectivity for the separation, which is based on the differences in the effective charge-to-hydrodynamic radius ratio of the analytes. The advantages about NACE are that currents are lower in nonaqueous media than they are in aqueous buffers of the same ionic strength [184], so it is possible to achieve high separation efficiency; also, CE using nonaqueous media is more compatible with mass spectrometry detection. However, organic solvents generally absorb light in the UV region more than water does, which is a clear disadvantage. In this case, indirect UV detection [188,189] or alternative detection methods [190] can be applied. For example, improved detection limits were reported for N,N-dimethylformamide with electrochemical detection for inorganic anions when compared with the results in aqueous buffer with UV detection [188]. Various solvents have been tested for NACE separations, among which methanol [182,183,186, 191-194] and acetonitrile [194-198] are most commonly used because of their popular use as organic modifier in CE applications and their low toxicity compared with many other solvents. Formamide, N-methylformamide, N, N-dimethylformamide and dimethyl sulfoxide have also been the choice for many NACE analyses [188,194,199-202] because they can often provide unique selectivities and allow fast analysis.

Highly hydrophobic compounds such as PAHs have been the subject of many reports [194,195,203-210] due to their abundance in environment and the adverse health effects to

which they are linked. Micellar electrokinetic chromatography (MEKC) with various surfactants [207,209,210] has been most successful for this type of separations because charged micelles allow the partition of these analytes between bulk solution and micelles, and nonionic analytes can migrate under the electric field by interacting with micelles. Separations of PAH compounds with cyclodextrans (CD) and CD-modified MEKC [205,206,208] have been possible as well. CD additives improve the separation by forming inclusion complexes with analytes. Because of the very high hydrophobicity of PAH compounds, organic modifiers [211-213] are frequently used to increase their solubility in aqueous electrolytes, which could influence the micelle properties and thereby the separation mechanism.

Several groups described the separation of PAH compounds by NACE. Six PAHs were separated by Walbroehl and Jorgenson [214] with electrolyte solution containing tetraalkylammonium ions and 50 - 100% acetonitrile. Miller et al [195] obtained PAH separations in acetonitrile with planar organic cations, such as tropylium ion and 2,4,6-triphenylpyrylium ion, and they found that charge-transfer interactions as well as electrostatic and dispersive forces play important roles in PAH-cation binding. Nonaqueous media containing 65% acetonitrile without supporting electrolyte was used for resolution of 11 PAH compounds by capillary electrochromatography (CEC) [194].

Chapter 3 of this dissertation described the NACE separation of PAH compounds in pure methanol or methanol-water mixture containing a high percentage of methanol. Several anionic surfactants as well as their concentrations were compared about their effect on the separation, and other important parameters were also studied, including apparent pH of the

electrolyte and the effect of methanol content on analyte migrations.

Ion Chromatography - Capillary Electrophoresis

Ion chromatography and capillary electrophoresis are two major techniques for doing ion analyses. The different separation mechanisms make them complementary to each other. Generally, ion chromatography suffers from the poorer separation efficiency and lower resolving power than CE. On the other hand, some isomeric ions are not easily separated by CE purely based on the differences in electrophoretic mobilities. The technique of combining ion chromatography with CE sounds very promising for the separation of closely related ionic compounds that cannot be separated by CZE itself.

Ionic polymers have been frequently applied to improve electrophoretic separations. For example, polyethyleneimine [215,216], polyamide [217], polybrene [216,218] and other polycationic polymers [216] have been examined to see their potential in improving protein separations by CE. Capillary columns coated with glycoside-bearing polymer were also characterized for separating basic proteins [219]. However, these polymers work by coating the silica capillary surface, so the surface becomes positive and prevents the adsorption of positively charged proteins. They are not really involved in modifying the selectivity.

Terabe et al [220-222] were the first to employ ion-exchange interactions for CE separation. They proposed a simple theory for this combined separation mechanism, and isomeric organic acids were easily resolved by adding an ion-exchange polymer to CE electrolytes. Cassidy and coworkers also reported some of their work in this area [223-225]. Okada separated several aromatic disulfonates by CE based on their ion-pair formation with

polyammonium ions [226]. Polyammonium ions with various chain lengths were expected to act as a molecular ruler and recognize the structures of aromatic disulfonates.

Ion-exchange capillary electrochromatography (IE-CEC) has been reported by Smith and Evans [227] for the efficient analysis of highly polar pharmaceutical compounds, such as antidepressants imipramine and nortriptyline, etc. Using capillary packed with strong acid cation-exchangers, plate numbers in excess of eight million per meter were observed. Wei et al [228] also demonstrated the potential of IE-CEC for separation of basic pharmaceutical compounds.

In chapter 5 of this dissertation, electrophoretic separation of both inorganic and organic anions was obtained with an anion-exchange polymer added to the background electrolytes. Unlike previous work in this area where the effect of added salt on the separation was neglected, a detailed study about the type and concentration of added salt, as well as other important variables including electrolyte pH, and type and concentration of the ion-exchange polymer was carried out. Excellent separations for anions with similar mobilities were obtained rapidly and efficiently.

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CHAPTER 2. NOVEL POLYMERIC RESINS FOR ANION-EXCHANGE CHROMATOGRAPHY

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Abstract

A novel anion-exchange resin containing three amine groups was prepared by reaction of a chloromethylated polystyrene-divinylbenzene (PS-DVB) resin with diethylenetriamine. After being protonated by contact with an aqueous acid, this resin can be used for ion-chromatographic separation of anions. The charge on the resins can be varied from +1 to +3 by changing the pH at which the ion chromatographic separation was carried out. The selectivity of the new ion exchangers for various inorganic anions was quite different from that of conventional anion exchangers. The performance of this new anion exchanger was studied by changing the pH and the concentration of the eluent, and several different eluents were used with some common anions as testing analytes. Conductivity detection and UV-

visible detection were applied to detect the anions after separation. The new resin can also be used for HPLC separation of neutral organic compounds. Alkylphenols and alkylbenzenes were separated with this new polymeric resin, and excellent separations were obtained under simple conditions.

1. Introduction

Ion chromatography is one of the most common and most widely used techniques for the separation of various ionic compounds. Two types of ion chromatography are in practical use now, the suppressor-based system developed by Small et al.[1], and single-column ion chromatography introduced by Fritz et al.[2]. Since the introduction of ion chromatography, a lot of research has been conducted to understand and vary the ion-exchange selectivity and improve ion chromatographic separation of anions[3-13], especially in the development of stationary phases[8-13]. Several types of stationary phases for the separation of anions have been developed, including silica-based anion exchangers[13], poly(styrene-divinylbenzene) (PS-DVB) copolymer anion-exchangers[8-11], and macroporous hydroxyethyl methacrylate-based anion-exchanger[12]. There are also mixed-bed ion-exchange materials developed to resolve anions and cations simultaneously[14-16].

In anion-exchange chromatography it is known that a mobile phase containing a 2- anion is generally a more powerful eluent than a 1- anion at the same concentration. This idea has been carried a step further by using the 3- anion of 1,3,5-benzenetricarboxylic acid or the 4- anion of pyromellitic acid in the mobile phase[17, 18]. Since ionization of polycarboxylic acids occurs in a stepwise fashion, the average charge on these compounds

can be reduced simply by making the pH more acidic. In this way the eluting power of the mobile phase can be varied by changing pH.

Although polymer resins containing polyethylenimine have been used to separate proteins by anion-exchange chromatography[19, 20], most of the anion-chromatographic separations are performed on columns containing single quaternary amine functional groups. Such groups are ionized completely so that changes in pH have little effect on the resins affinity for sample anions. In this paper a new type of anion exchanger is described for the separation of anions by ion chromatography. The anion exchanger is a PS-DVB copolymer containing diethylenetriamine functional groups. Thus, such a group contains three amino groups in the same molecule. The charge on the triamine groups can be varied by changing the pH so that one, two or all three of the nitrogen atoms in each group are protonated. This constitutes a powerful tool to alter the selectivity of the resin.

2. Experimental

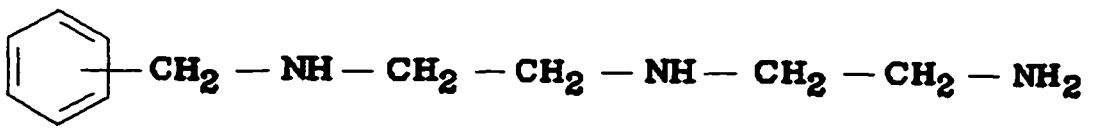
2.1. Chromatographic system

The chromatographic system consisted of several components. A Dionex DXP pump (Dionex, Sunnydale, CA, USA) was used to deliver a flow of 1 ml/min. A 7010 Rheodyne injector (Rheodyne, Berkeley, CA, USA) delivered 10-μl samples which were detected with either a Kratos Spectroflow 783 UV absorbance detector (Kratos Analytical Instrument, Ramsey, NJ, USA) or an Alltech (Deerfield, IL, USA) 320 conductivity detector. Separations were recorded by a Servogor 120 chart recorder (Abb Goerz Instruments, Vienna, Austria) and a Shimadzu C-R3A Chromatopac integrator (Shimadzu Corporation,

Kyoto, Japan). Columns were packed with a Shandon Southern (Sewickley, PA, USA) HPLC packing pump at 3000 p.s.i. (1 p.s.i. = 6895 Pa).

2.2. Preparation of anion-exchange resins

Figure 1 shows the chemical structures of the anion-exchange resin made in our laboratory. This resin was prepared from 5-μm macroporous polystyrene-divinylbenzene (Sarasep, Santa Clara, CA, USA). A 5-g amount of resin was wetted with glacial acetic acid (ca. 5 ml/g resin) then filtered and rinsed with concentrated hydrochloric acid. A 75-ml volume of concentrated hydrochloric acid with 2.2 M formaldehyde was added to the resin with stirring. The resin mixture was reacted for 2.5 minutes then poured into ice water to quench the reaction. After filtration and washing with deionized water and methanol, the chloromethylated PS-DVB resin was mixed with 25-40% diethylenetriamine in methanol.

a. 

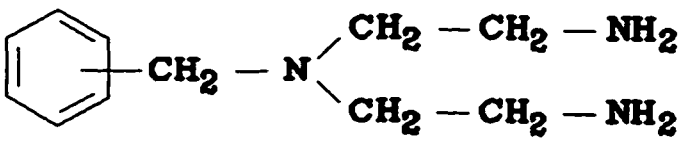
b. 

Fig. 1. Schematic description of the structure of the new anion-exchange resins.

This resin mixture was reacted for 24 hours at temperature 70 - 80°C, after which the reaction was quenched with ice water. The resultant resin was washed with deionized water, 2-propanol and methanol, and then dried in air for overnight. Four batches of resin were prepared by this procedure. The capacity of the protonated resin, measured by acid-base titration with sodium hydroxide, was 0.175 ± 0.013 meq per gram of dry resin. The average nitrogen content by elemental analysis was 0.26 meq/g. The lower exchange capacity could be due to incomplete protonation of the three nitrogens or caused by some nitrogens being located deep within the resin.

2.3. Reagents and chemicals

Methanol, 2-propanol and acetonitrile were of HPLC grade and used as obtained from Fisher Scientific (Pittsburgh, PA, USA). Diethylenetriamine was 99+% and obtained from Aldrich (Milwaukee, WI, USA). All salts and other reagents were of the best grade available and used as obtained from Fisher Scientific, Aldrich, J. T. Baker (Phillpsburg, NJ, USA) and Lancaster (Windham, NH, USA). All eluents were prepared daily. Stock solutions were used to prepare all sample solutions by diluting to desired concentrations with D.I.water or mobile phase. A Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA) was used to further deionize distilled water for all eluents and sample mixtures.

2.4. Chromatographic procedures

A flow of 1 ml/min was selected for all chromatographic separations. The column was protonated with 50 mM hydrochloric acid before the desired eluent was used. Sample

injections were made when the baseline was stable. The eluted species were detected by UV-vis detector at 200 nm or 254 nm with an output range of 0.010 AUFS, or by a conductivity detector with the output range of 0.5 S.

Capacity factors, k', were calculated according to expression: $k' = (t_r-t_0)/t_0$. The system dead time, t_0, used to calculate the capacity factor k', was measured by injecting D.I.water into the system. An average of at least three replicates was used to perform all calculations.

3. Results and Discussion

3.1. Conditions for separation

Several anions were separated chromatographically at pH 7.5-7.7 using different salts in the mobile phase with direct UV detection at 200 nm. The results in Table 1 show that perchlorate is a significantly better eluting anion than chloride. However, sulfate and hydrogen phosphate both give even shorter retention times by virtue of their 2- charge.

Not all anions absorb sufficiently to use direct UV detection. When conductivity detection was used, potassium hydrogen phthalate (KHP) or sodium benzoate was the preferred material to be used in the mobile phase. The relatively large phthalate or benzoate anions have lower conductivities than the anions to be separated and detected. Chromatograms for separation of inorganic anions are given in Figure 2 using KHP at pH 3.8 and in Figure 3 using sodium benzoate at pH 6.4. At pH 3.8, the triamine resin is more fully protonated than it is at pH 6.4. This pH effect is demonstrated particularly well for the divalent sulfate anion. The retention time for sulfate is ca. 21 min at pH 3.8, but is only ca.

Table 1. Retention times of several inorganic anions with different eluents[a].

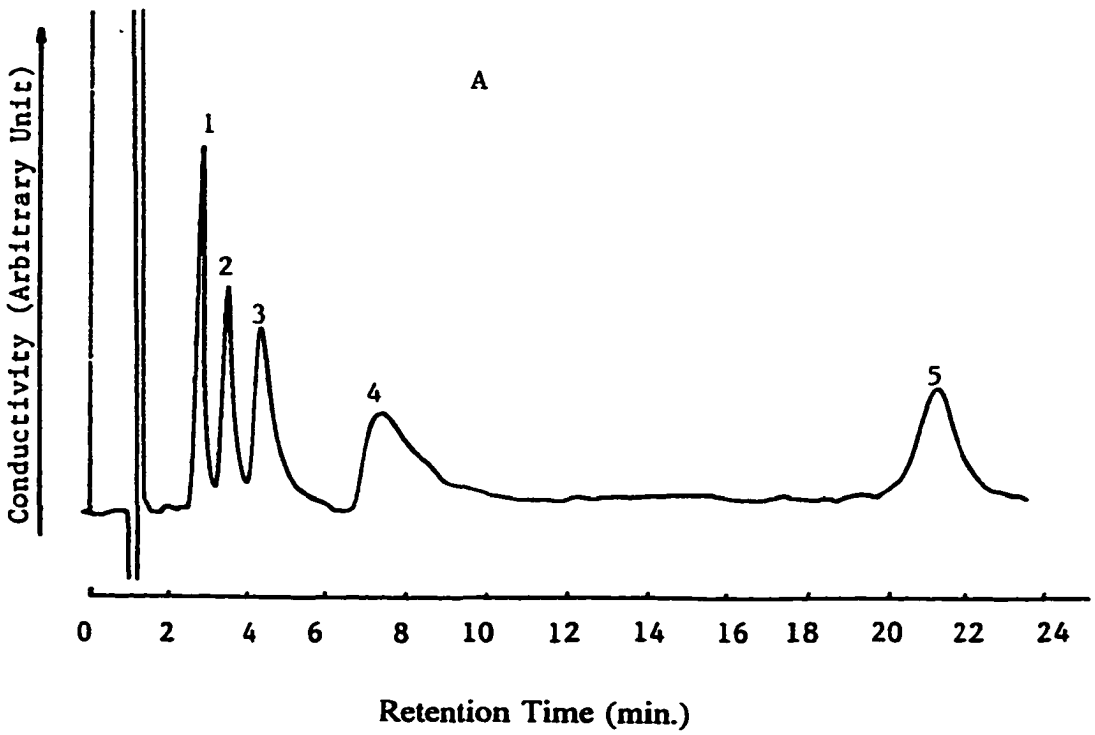
Anion	Retention Time, min.			
	$NaClO_4$[b]	Na_2SO_4[b]	NaCl[b]	Na_2HPO_4[b]
Bromide	3.45	2.15	4.51	1.98
Nitrate	3.98	2.75	5.55	2.54
Iodide	6.25	7.01	12.4	5.95
Thiocyanate	13.7	ND[c]	25.6	17.4

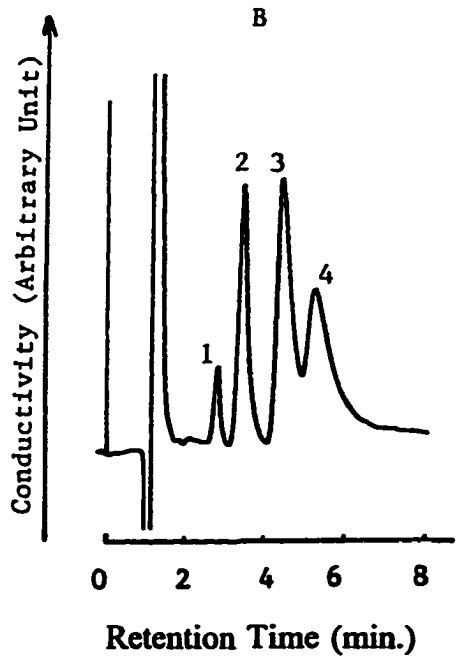
a. Column was 10 cm long packed with PS-DVB triamine resin.

b. Each eluent is 5.0 mM at pH 7.5-7.7.

c. ND = not detected.

Figure 2. Separations of inorganic anions on column packed with PS-DVB triamine resin. Column: 100 x 4.6 mm.; detection: conductivity. Eluent: 2.5 mM potassium hydrogen phthalate, pH 3.77. Peaks: (A) 1 = chloride (30 ppm); 2 = bromide (118 ppm); 3 = nitrate (85 ppm); 4 = iodide (300 ppm); 5 = sulfate (100 ppm). (B) 1 = iodate (200 ppm); 2 = bromate (200 ppm); 3 = nitrite (80 ppm); 4 = chlorate (150 ppm).


A
Conductivity (Arbitrary Unit)
1
2
3
4
5
0
2
4
6
8
10
12
14
16
18
20
22
24
Retention Time (min.)


B
Conductivity (Arbitrary Unit)
1
2
3
4
0
2
4
6
8
Retention Time (min.)

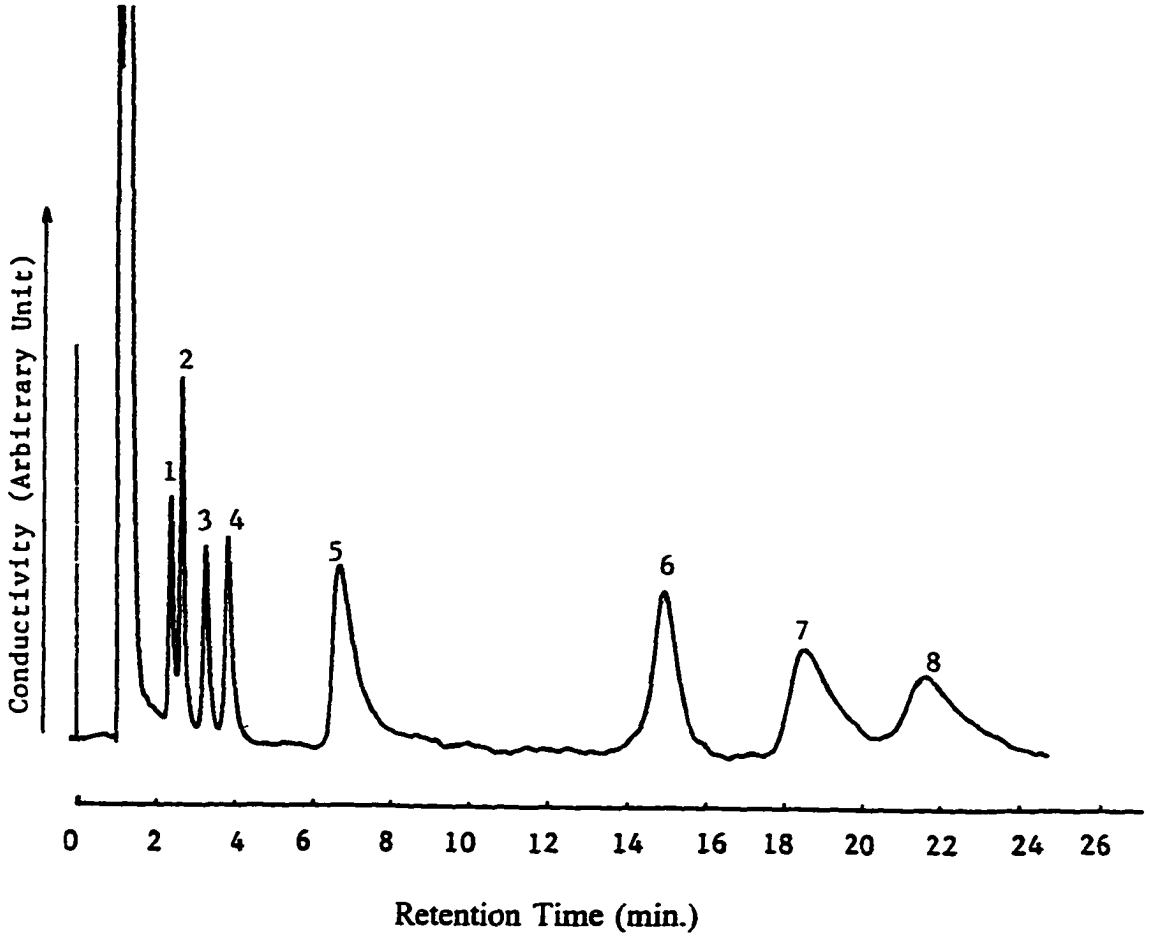


Figure 3. Separation of inorganic anions on triamine column. Column: same as Figure 2. Eluent: 5.0 mM sodium benzoate at pH 6.40. Peaks: 1 = fluoride (22 ppm); 2 = chloride (20 ppm); 3 = bromide (63 ppm); 4 = nitrate (45 ppm); 5 = iodide (200 ppm); 6 = sulfate (100 ppm); 7 = molybdate (200 ppm); 8 = chromate (200 ppm).

15 min at pH 6.4 despite the fact that benzoate is generally considered to be a weaker eluent than phthalate.

In Figure 3 it will be noted that a good separation was obtained for eight anions with the divalent anions sulfate, molybdate and chromate eluting much later than the monovalent anions. This unique selectivity with the new resin is quite different from that on the conventional anion exchangers, such as trimethylamine modified PS-DVB resin where sulfate and chromate ions were eluted earlier than iodide[21]. The classical plate numbers were calculated for the first six peaks, and the results were summarized in Table 2. The average plate number (N = 2,900 for a 10-cm column) compares well with ordinary commercial columns.

3.2. Effect of pH

Perhaps the most interesting aspect of the triamine resin is its ability to assume a 3+, 2+ or 1+ charge with varying degrees of protonation. The higher the charge on the protonated triamino functional groups, the more strongly a sample anion should be retained. The effect of pH was studied by using a fixed concentration (15 mM) of sodium perchlorate in the mobile phase and carefully equilibrating both the resin and the mobile phase at various pH values. Since perchlorate is the anion of a strong acid, sodium perchlorate should be completely ionized over the entire pH range that was used.

The results of the pH study for six anions are given in Figure 4. As expected, the capacity factors for bromide, nitrate and iodide decrease steadily as the pH is raised and the resin nitrogens become less fully protonated. Thiocyanate, which is more strongly retained

Table 2. Theoretical plate numbers (N) for anions separated on a triamine resin column. Conditions: 100 x 4.6 mm column, 5.0 mM sodium benzoate at pH 6.4, conductivity detection.

Peak No.	Anion	N
1	Fluoride	4,900
2	Chloride	3,800
3	Bromide	2,100
4	Nitrate	2,100
5	Iodide	2,000
6	Sulfate	2,500

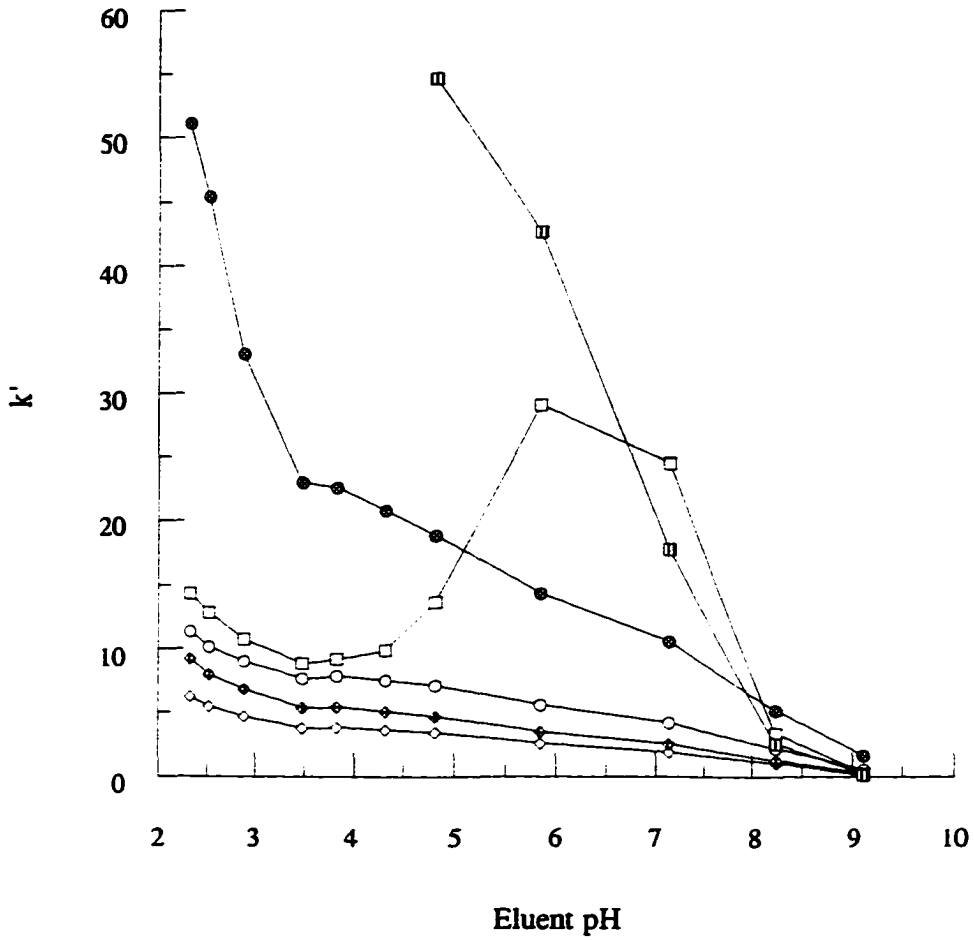


Figure 4. Eluent pH vs. anion capacity factor, k'. Conditions: 100 x 4.6 mm column; 15 mM sodium perchlorate at different pH value; UV detection at 200 nm. Symbols: ◇ Br^-; ◆ NO_3^-; ○ I^-; ● SCN^-; □ CrO_4^{2-}; ■ MoO_4^{2-}.

by the resin, shows a larger change in k' with pH. The decrease in k' is particularly great going from approximately pH 2.2 to pH 3.4.

Chromium(VI) shows a more complicated pattern with respect to changing pH. The k' of chromate ion decreases at pH 2 to pH 3.5, then increases at pH of 3.5 to 6, and decreases again as pH increases further. The possible reason is that chromate ion undergoes some chemical reactions at acidic conditions, e.g.: $CrO_4^{2-} + H^+ = HCrO_4^-$ (for chromic acid, H_2CrO_4: $pKa_1 = 0.74$, $pKa_2 = 6.49$), and the resultant monovalent anions are retained more weakly than chromate ions due to the charge difference. Another possible reaction is the conversion of chromate ions to dichromate ions ($Cr_2O_7^{2-}$), as shown by the following reaction: $2CrO_4^{2-} + 2H^+ = Cr_2O_7^{2-} + H_2O$, which can be protonated in acidic solution to produce the monovalent ions, such as $HCr_2O_7^-$. That would also give a decreased retention time. A spectral shift, presumably from dichromate to chromate or hydrogen chromate, occurs with rising pH and particularly between about pH 5 - 6 (Figure 5).

Molybdate ions were not detected at pH below 4.5. The most likely explanation for this is that molybdenum(VI) is retained too strongly on the stationary phase to be eluted in a reasonable time.

3.3. Other anion separations

Gold(III) and the platinum group elements form stable chloro anions in acidic solutions containing chloride. With conventional anion exchangers, these complex anions are retained so strongly that chromatographic separation is difficult. But with the new triamine resin, a good separation of $AuCl_4^-$, $RhCl_6^{3-}$ and $PtCl_6^{2-}$ was obtained at pH 2.6 (Figure 6). At a higher

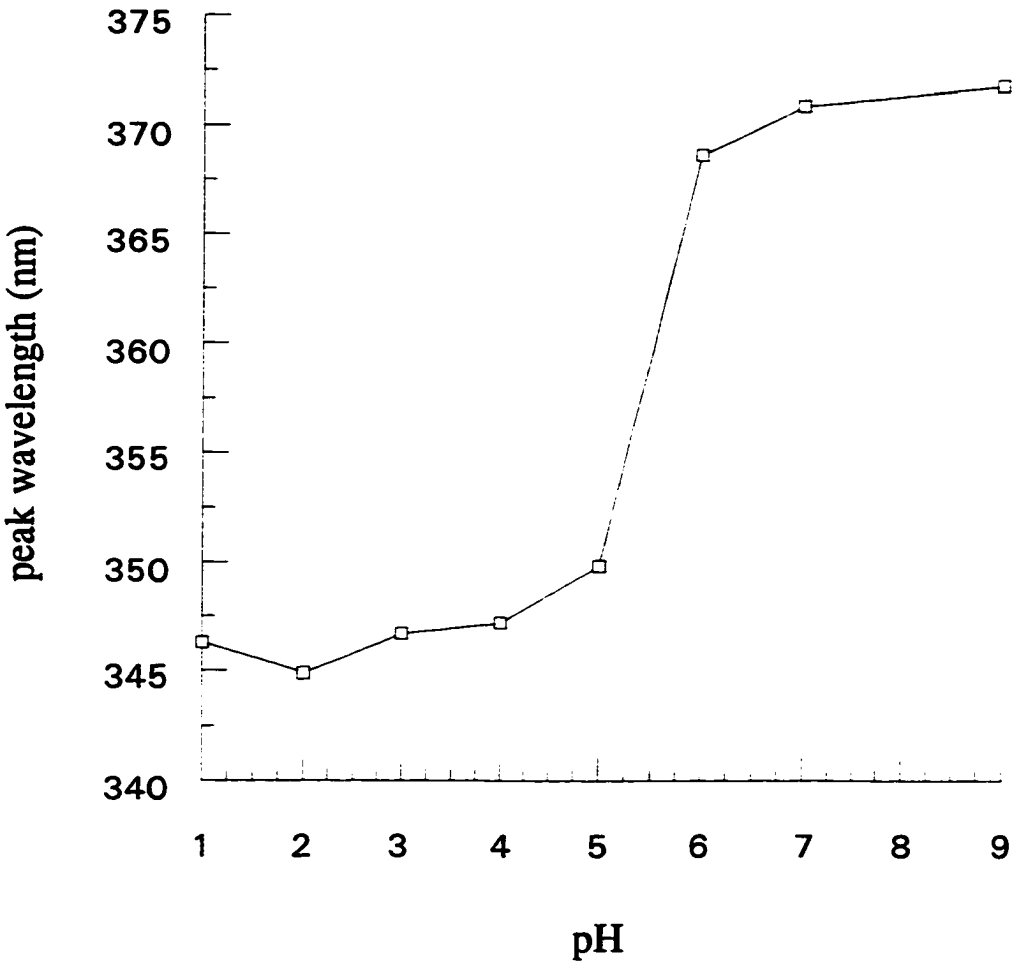


Figure 5. Effect of solution pH on the wavelength at peak absorbance for chromium (VI).

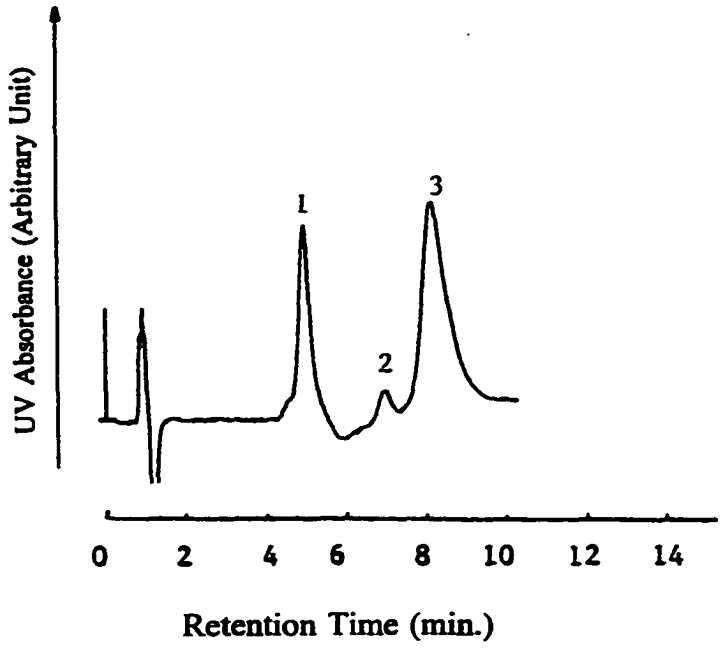


Figure 6. Separation of anionic chloro-metal complexes. Column: 100 x 4.6 mm packed with PS-DVB triamine resin; eluent: 15 mM sodium perchlorate at pH 2.58; detection: UV at 200 nm. Peaks: 1 = $AuCl_4^-$ (4.0 ppm); 2 = $RhCl_6^{3-}$ (3.2 ppm); 3 = $PtCl_6^{2-}$ (0.5 ppm).

pH, the baseline became more noisy.

The separation of major anions in a tap water sample is shown in Figure 7. The bicarbonate in the water was undoubtedly converted to carbonic acid by the acidic eluent (pH 3.8) or by the acidic protonated resin. The interesting feature of this separation is the very large difference in retention times between the chloride and sulfate peaks.

3.4. Separation of organic compounds

The new resin consists of a hydrophobic resin backbone which is modified by the presence of very polar triamine groups. A 10-cm column packed with the triamine resin was washed with 50 mM aqueous hydrochloric acid to protonate the amine nitrogen. Then the column was washed with water and finally with the aqueous-acetonitrile solution to be used as the mobile phase. Excellent separations of alkylphenols and alkylbenzenes were obtained using 50% and 60% aqueous acetonitrile, respectively (Figure 8 and 9). The average theoretical plate number for the alkylphenols was approximately 2,100 for the 10-cm column.

Chromatograms of both sample mixtures were run again after treating the column with dilute sodium hydroxide to eliminate any protonation of the nitrogens. The peaks were significantly broader and retention times were slightly longer. Thus, better results were obtained with the more polar, protonated resin.

4. Conclusions

A polymeric resin with triamine functional groups is an efficient material for ion-chromatographic separation of inorganic anions. A unique feature of this resin is that

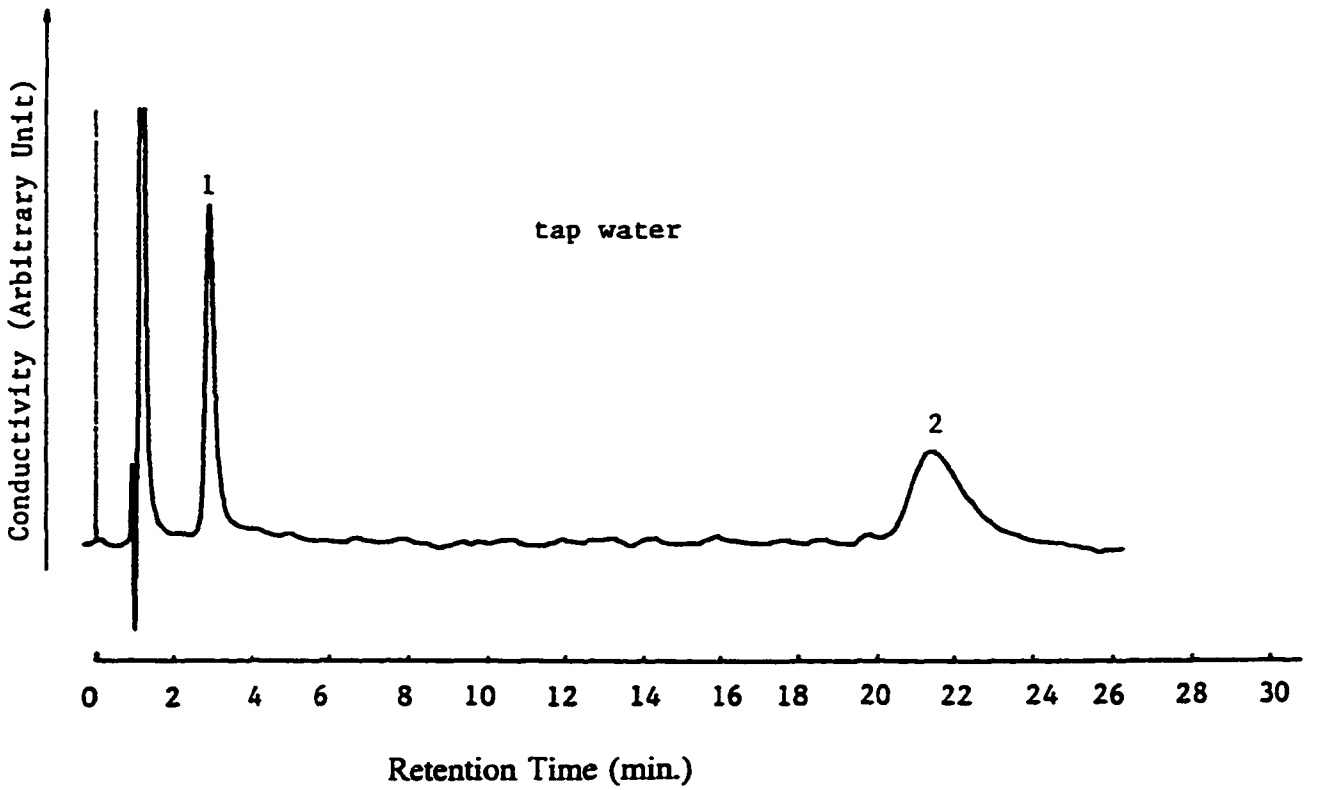


Figure 7. Application of the new stationary phase for analysis of anions in tap water. Column: 100 x 4.6 mm packed with PS-DVB triamine resin; eluent: 2.5 mM potassium hydrogen phthalate at pH 3.80; detection: conductivity. Peaks: 1 = chloride; 2 = sulfate.

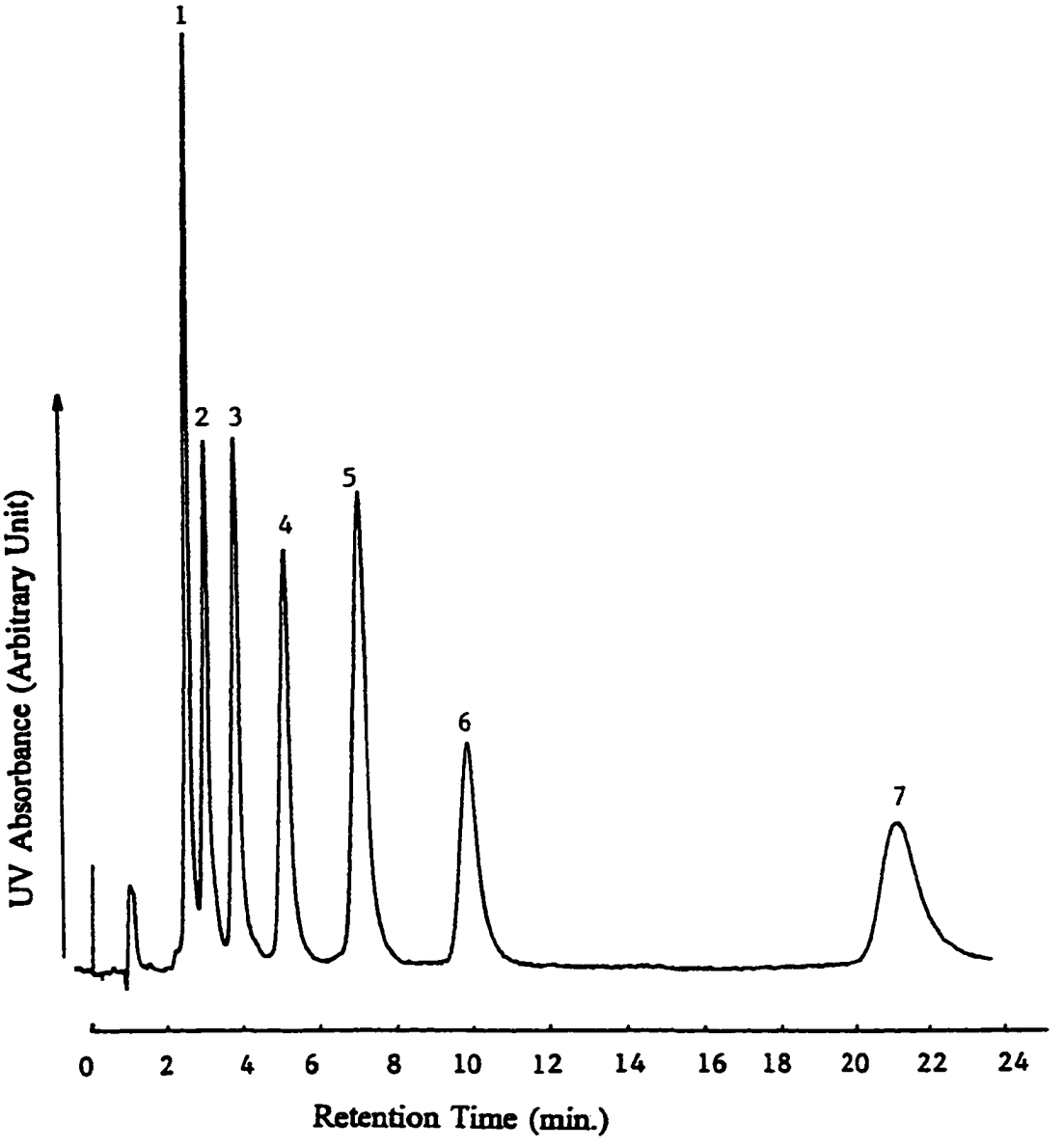


Figure 8. Separation of alkylphenols. Column: 100x4.6 mm packed with PS-DVB triamine resin; eluent: acetonitrile-water (50:50); detection: UV at 254 nm. Peaks: 1 = phenol (7.0 ppm); 2 = p-cresol (10 ppm); 3 = 4-ethylphenol (10.0 ppm); 4 = 4-n-propylphenol (8.0 ppm); 5 = 4-n-butylphenol (15 ppm); 6 = 4-n-amylphenol (20 ppm); 7 = 4-n-heptylphenol (25 ppm).

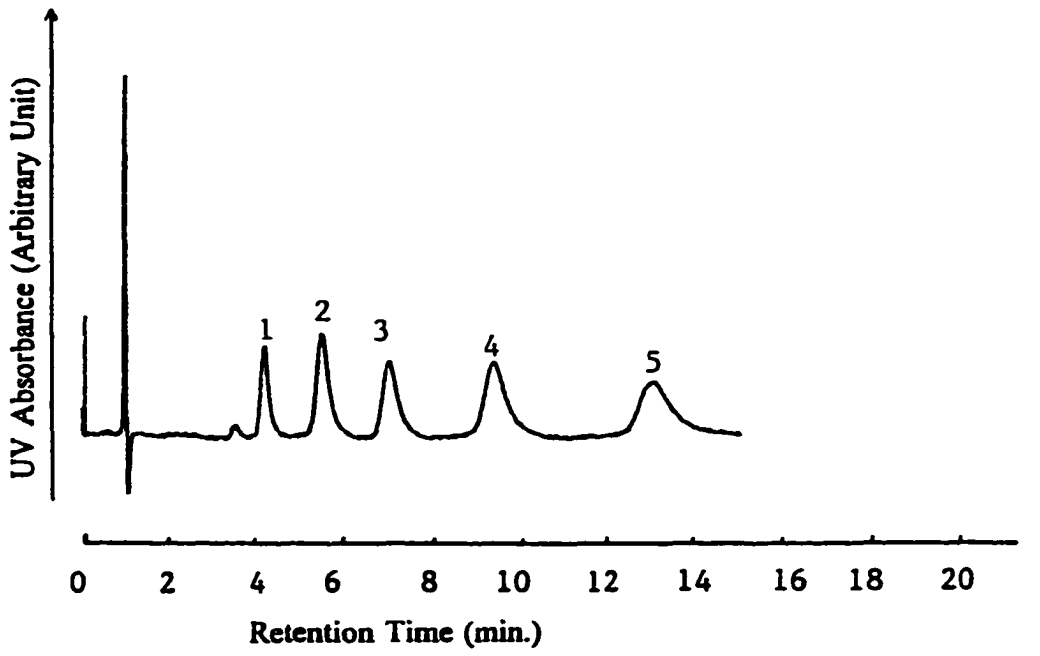


Figure 9. Separation of alkylbenzenes. Eluent: acetonitrile-water (60:40); other conditions same as Figure 8. Peaks: 1 = benzene (35 ppm); 2 = toluene (35 ppm); 3 = ethylbenzene (45 ppm); 4 = propylbenzene (60 ppm); 5 = butylbenzene (60 ppm).

retention times of sample anions can be varied widely simply by changing the pH of the mobile phase. In acidic solutions 2- anions are much more strongly retained than anions with 1- charge. The new resin is also an effective hydrophilic column packing material for separation of phenols and alkylbenzenes by HPLC with an aqueous-acetonitrile mobile phase.

5. Acknowledgements

We wish to thank Doug Gjerde of Sarasep, Inc. (Santa Clara, CA) for a gift of the PS-DVB resin used in this work. This work was performed in the Ames Laboratory, which is operated by Iowa State University for the US Department of Energy under Contract W-7405-Eng-82. This work was supported by the Director of Energy Research, Office of Basic Energy Sciences.

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CHAPTER 3. NONAQUEOUS MEDIA FOR SEPARATION OF NONIONIC ORGANIC COMPOUNDS BY CAPILLARY ELECTROPHORESIS

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Summary

For the separation of neutral compounds by micellar electrokinetic chromatography, separations are usually carried out in predominantly aqueous solution in order to preserve the charged micelle necessary for the separation. We now show that PAH compounds can be separated efficiently by capillary electrophoresis in pure methanol or in aqueous-organic mixtures containing a high percentage of methanol. Sodium tetradecyl sulfate was the preferred surfactant. The effects of pH, solvent composition, surfactant structure and surfactant concentration on the separations were studied. Reproducible migration times and linear calibration plots were obtained.

1 Introduction

Since its inception, capillary electrophoresis (CE) has developed into a powerful technique for the separation of a wide variety of neutral compounds as well as inorganic and organic ions [1-7]. Most CE applications have been carried out in aqueous buffers because water is easy to handle and dissolves most chemicals used in CE. Extensive knowledge about chemistry in aqueous phases is available. However, the attainable diversity in the background electrolyte properties in water is limited and is largely determined by the physicochemical parameters of water. Organic solvents have different physical and chemical characteristics from water and from each other. Therefore, when a nonaqueous medium is substituted for water, all important properties of CE separations, such as resolution, selectivity, efficiency and analysis time, can be greatly influenced.

CE applications in nonaqueous media have been gaining popularity over the last several years. For example, nonaqueous media have been successfully applied for chiral separations [8] and drug purity tests [9, 10]. Valkó, et al concisely reviewed CE in nonaqueous media [11]. The important properties of organic solvents and their potential in CE were discussed. Several commonly used nonaqueous solvents include acetonitrile [12, 13], methanol [14-17], formamide [18-21], N-Methylformamide [22], and N, N-dimethylformamide [23]. Compared with aqueous media, nonaqueous solvents offer wider ranges of dielectric constant, polarity, viscosity, and acid/base chemistry. Besides, currents are generally lower in nonaqueous media than they are in aqueous buffers of the same ionic strength [18]. An additional advantage about CE in nonaqueous media is the better compatibility with electrospray mass spectrometry detection because organic solvents of low viscosity can improve the spray

efficiency, as shown by several reports in this area [24-28]. CE systems using solvent mixtures have also been employed to alter the selectivity [9, 14, 21].

Virtually all CE separations in nonaqueous solvents have been concerned with ions or ionizable compounds where their intrinsic charges could be used for separation. Separation of neutral analytes is possible only when the analytes are associated with a charged surfactant or other additive in solution or in a micellar pseudo phase. A high fraction of an organic solvent in the background electrolyte solution (BGE) has tacitly been assumed to prevent the interaction between surfactant and analytes necessary for separation of neutral analytes. However, Walbroehl and Jorgenson [29] were able to separate six neutral organic compounds in aqueous-organic solutions containing 50% or more of acetonitrile by the addition of a tetrahexylammonium salt to the BGE. They attributed the separation to association in solution between the analytes and the charged additive. More recently, Shi and Fritz [3, 7] and Ding and Fritz [1, 6] have used various charged additives to separate neutral analytes in solutions containing up to 40% acetonitrile, and polymerized surfactants have also been employed for separation of neutral organic compounds [30].

In the present work it is shown that neutral organic compounds can be separated successfully in aqueous-organic solutions containing 70% to 100% methanol. An anionic surfactant was added to the BGE to form charged association complexes with the analytes through solvophobic interactions. The effects of several parameters on the separations were studied, including the type and concentration of surfactant, the percentage of methanol in the BGE, and the apparent pH.

2 Materials and methods

2.1 Chemicals

All chemicals were of the best grade available. Organic solvents, phosphoric acid (85%), hydrochloric acid (HCl), acetic acid, ammonium acetate and sodium dodecyl sulfate (SDS) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Sodium n-tetradecyl sulfate (STS) and sodium n-hexadecyl sulfate (SHS) were obtained from Lancaster Synthesis (Windham, NH, USA). Deionized water (18.2 MΩ) was made with a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). Background electrolytes were prepared by mixing desired buffer species and surfactant in pure methanol or solvent mixture, and the solutions were filtered through a 0.45 μm syringe filter (Costor, Cambridge, MA, USA) when necessary. The apparent pH of the electrolytes was measured with a Corning 440 pH meter (Corning, NY, USA).

The standards of PAH compounds were purchased from Aldrich (Milwaukee, WI, USA). Acidic and basic drugs were purchased from Sigma (St. Louis, MO, USA). All stock solutions were made in acetonitrile at concentrations of 1000 - 2000 ppm. Before injection, sample solutions were diluted with 5% background electrolytes in methanol.

Because a lot of organic substances with different toxicities were involved in this work, safety was paid much attention. Throughout the work, safety gloves and protective clothing were used, and all solutions were prepared in the hood.

2.2 Instrumentation

A Waters Quanta 4000 capillary electrophoresis system (Millipore Waters, Milford, MA, USA) was employed throughout this work. Uncoated fused silica capillaries had the

dimension of 45 cm × 50 μm, and the length from injection end to the detector was 37 cm. Unless specified, a voltage of -20 kV was applied for all experiments, and the current generated was kept below 30 μA. Direct UV detection was performed at 254 nm unless indicated. Electropherograms were collected at the speed of 15 points/s and plotted by Chromperfect data acquisition system (Justice Innovations, Mountain View, CA, USA).

Each new capillary was conditioned with 0.1 M sodium hydroxide and deionized water for 10 min each. When changing the electrolytes, capillaries were rinsed with deionized water for 10 min, followed by methanol rinsing for 1 min. Before each sample injection, capillaries were rinsed with the running electrolyte for 1.5 min. Due to solvent evaporation, electrolyte reservoirs were frequently replenished between runs.

3 Results and Discussion

Several organic solvents, including methanol, acetonitrile and isopropyl alcohol, were tried initially as the separation medium. These solvents were chosen because of their purity, ready availability and relatively minor toxicity. Methanol was found to better dissolve the buffer and surfactants than the other two, so it was employed as the separation solvent for all of this work.

3.1 Selection of the BGE

In methanol and in aqueous-methanol solvent mixtures containing a high proportion of methanol, the pH of common buffers is apt to be significantly different from predominantly aqueous solutions. The pH measured by standard electrodes in these largely organic solutions is often referred to as "apparent pH" (pH*).

The effect of apparent pH on the migration and separation of perylene and anthracene was studied initially using SDS as the additive and methanol as the solvent. With 10 mM ammonium acetate as the buffer the apparent pH of the BGE was about 8.0. At this apparent pH the electroosmotic mobility should be reasonably high. In this case, a counter EOF occurred: EOF toward the cathode, and the analytes moved to the anode by assuming negative charges through the association with SDS. Since EOF was relatively high at pH* ~ 8.0, the migration time was quite long; about 30 min for perylene and 32 min for anthracene. Addition of some acetic acid in the BGE resulted in lower pH* and somewhat shorter migration times; but the improvement was slight. This observation led us to take another approach. By using acidic conditions, EOF would be reduced or eliminated, and the analysis could be much faster. Hydrochloric acid (HCl) and phosphoric acid (H_3PO_4) at 10 mM were each tried for this purpose. The BGE containing HCl had pH* ~ 1.0, and the one containing H_3PO_4 had pH* ~ 2.8. As expected, the migration times were significantly shorter with both HCl and H_3PO_4. Comparing HCl with H_3PO_4, HCl gave faster analysis (16.6 min vs 17.3 min for perylene, 17.7 min vs 18.5 min for anthracene), but H_3PO_4 produced lower current because of its smaller conductance. So H_3PO_4 was selected over HCl as the preferred buffer.

The concentration effect of H_3PO_4 in the BGE was also studied, and the plot of analyte migration time vs. H_3PO_4 concentration was shown in Figure 1. From this figure, it is obvious that as H_3PO_4 content was changed from 10 mM to 50 mM, there was a slow but steady increase in migration times for all the test compounds although the resolution did not change much. This increase was thought to be the result of the increased ionic strength as

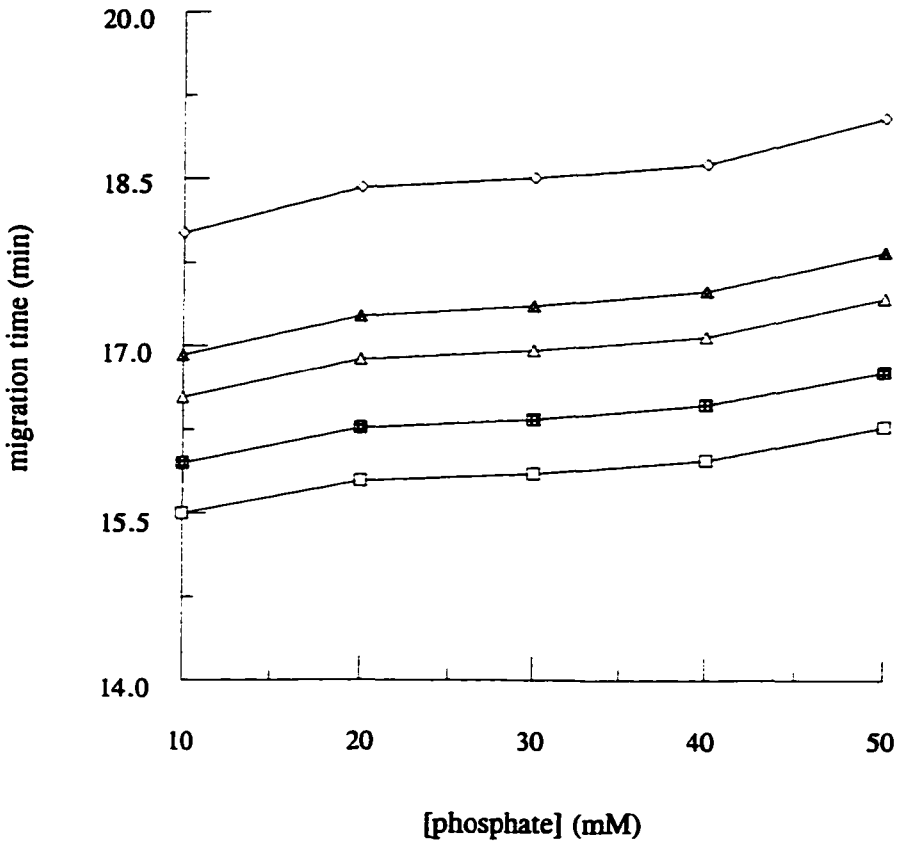

Figure 1. Effect of H_3PO_4 concentration on the migration time. Electrolyte contained H_3PO_4 and 50 mM SDS in methanol; electrokinetic sampling, 15s * 3kV. Samples: □ Benzo[a]perylene; ■ Perylene; △ Pyrene; ▲ Anthracene; ◇ Benzophenone.

more H_3PO_4 was included in the BGE. Similar reports have been published about the influence of ionic strength on the migration behavior of the analytes in NACE [8]. For this work, low H_3PO_4 concentration was chosen to keep current low. A separation of five PAH compounds was shown in Figure 2 using 10 mM H_3PO_4 and 50 mM SDS in methanol.

3.2 Types and concentration of anionic surfactant

In order to separate the neutral compounds by CE, ionic additives which can put charges onto the analytes through various interactions must be included in the BGE. This work employed long-chain anionic surfactants in the NACE running buffers. Surfactants with different chain length, including SDS, STS and SHS, were compared. Figure 3 shows the separations of five PAH compounds with 50 mM of STS and SHS in methanol. Compared with Figure 2, the resolution was obviously better with longer-chain surfactants. Longer surfactants have stronger solvophobic interactions with the analytes, and this is beneficial for their separations.

Figures 4 - 6 illustrate the relationship between electrophoretic mobilities of five PAH-complexes and the concentration of the surfactants. Electrophoretic mobilities were measured in methanol containing 10 mM H_3PO_4 and varying content of surfactants. Migration times were used directly to calculate the mobilities because EOF was negligible at the conditions applied. SDS and STS concentration were varied from 10 to 70 mM, while SHS concentration was increased from 10 to 50 mM only due to its limited solubility at higher concentrations. The figures show that as the concentration of surfactants increases, so do the mobilities of the solutes. The slopes of the plots are greater for larger compounds and smaller for smaller, more polar compounds. This indicates stronger interactions between

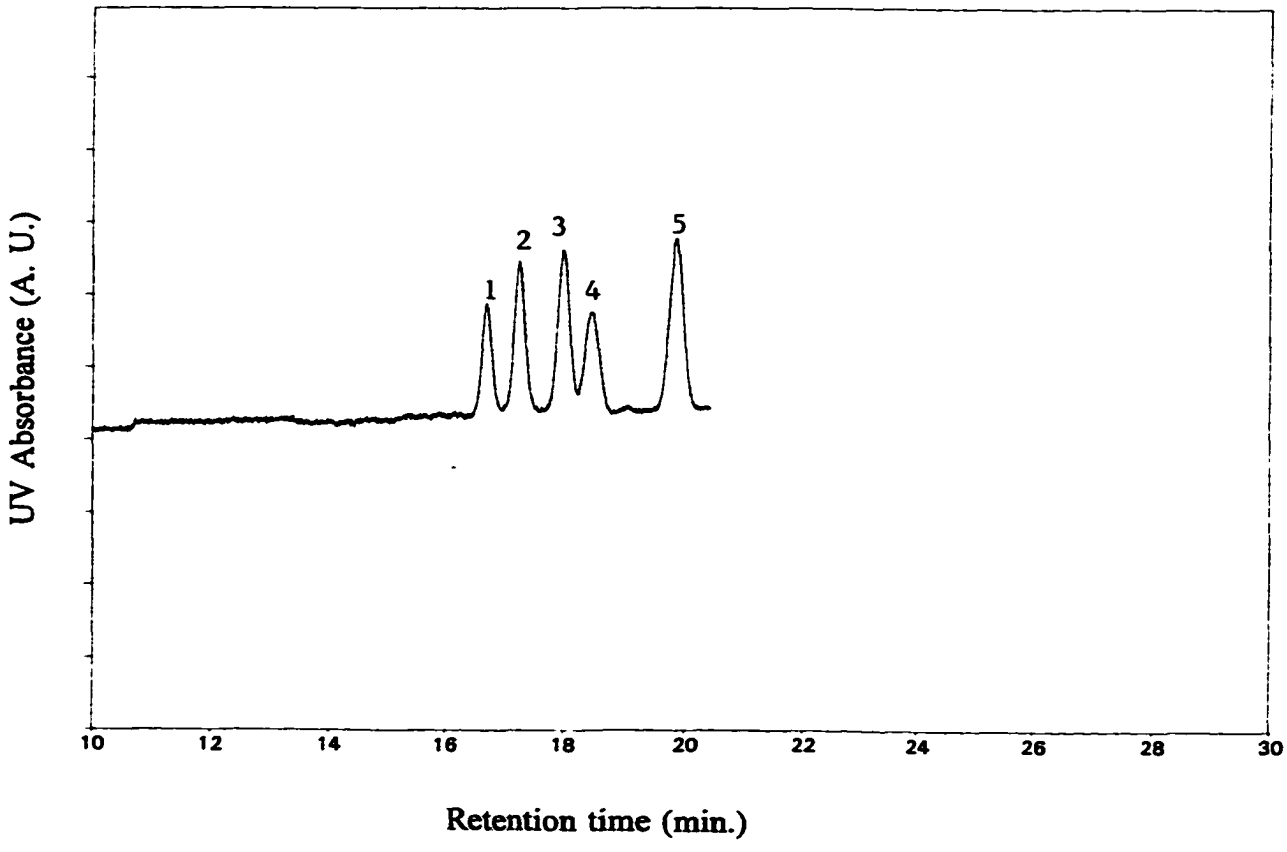

Figure 2. Separation of five PAH compounds. Electrolyte: 10 mM H_3PO_4 and 50 mM SDS in methanol; sample injection: -2kV * 15s. Peaks: 1. Benzo[a]perylene; 2. Perylene; 3. Pyrene; 4. Anthracene; 5. Benzophenone.

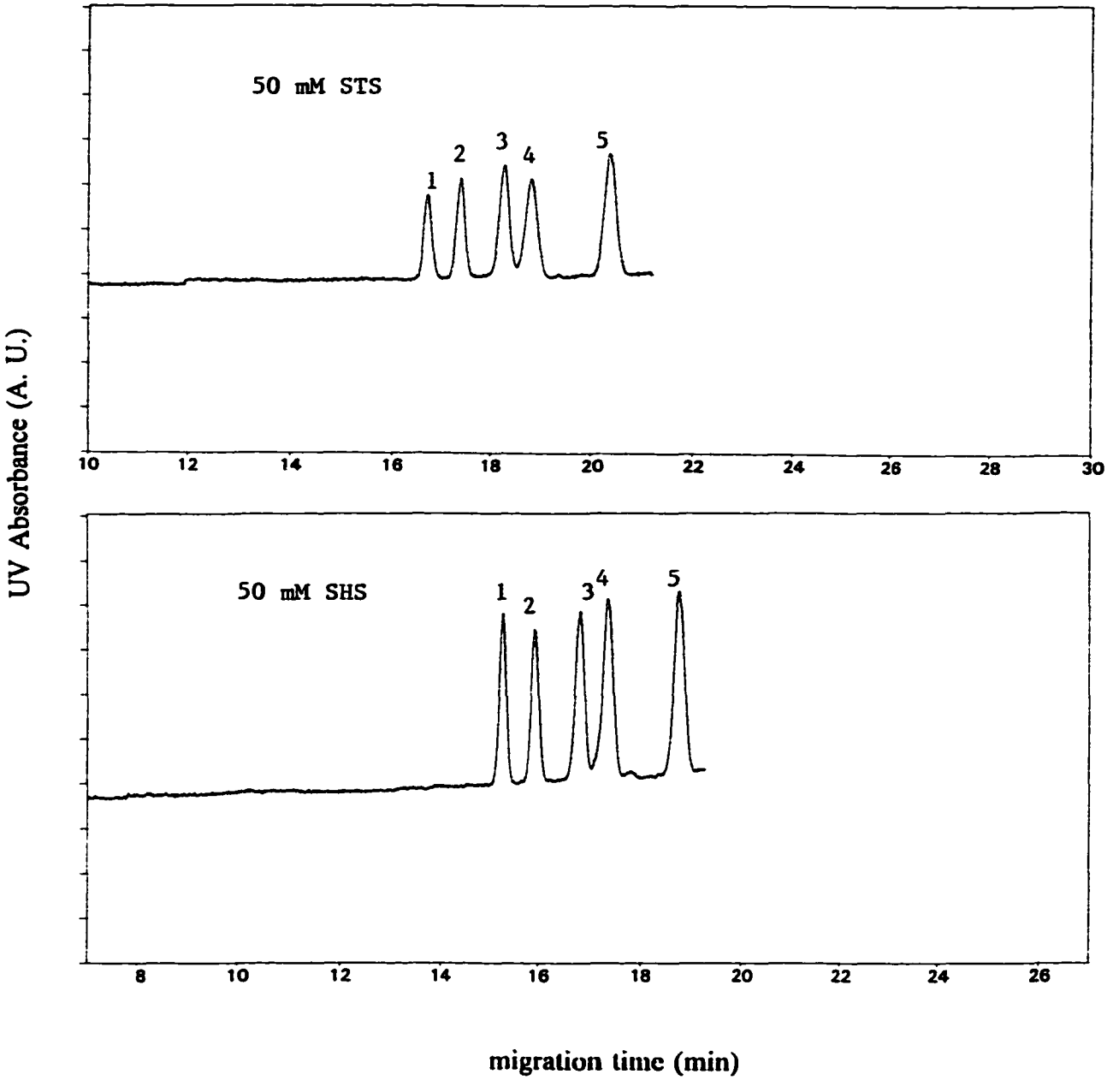

Figure 3. Separation of five PAH compounds. Electrolyte: 10 mM H_3PO_4, 50 mM STS or 50 mM SHS in methanol; sample injection: -2kV * 15s. Peaks: same as Figure 2.

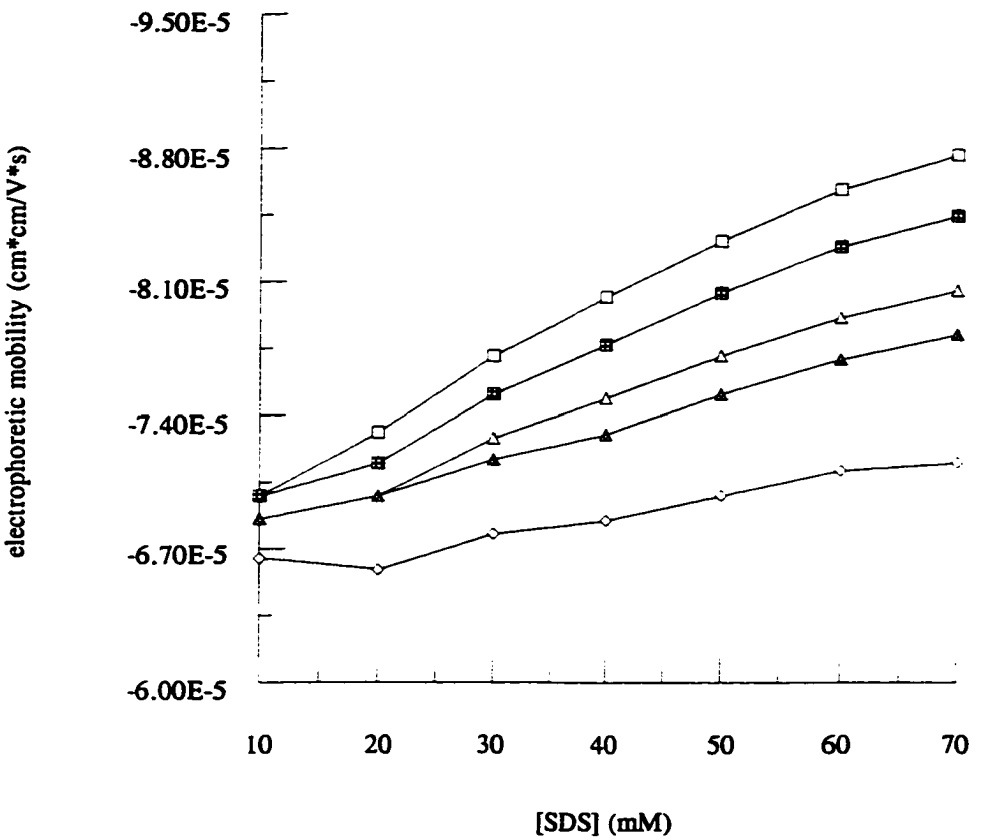

Figure 4. Electrophoretic mobility vs SDS concentration. Electrolyte: 10 mM H_3PO_4 and SDS in methanol; sample injection: -2kV * 15s. Samples: □ Benzo[a]perylene; ■ Perylene; △ Pyrene; ▲ Anthracene; ◇ Benzophenone.

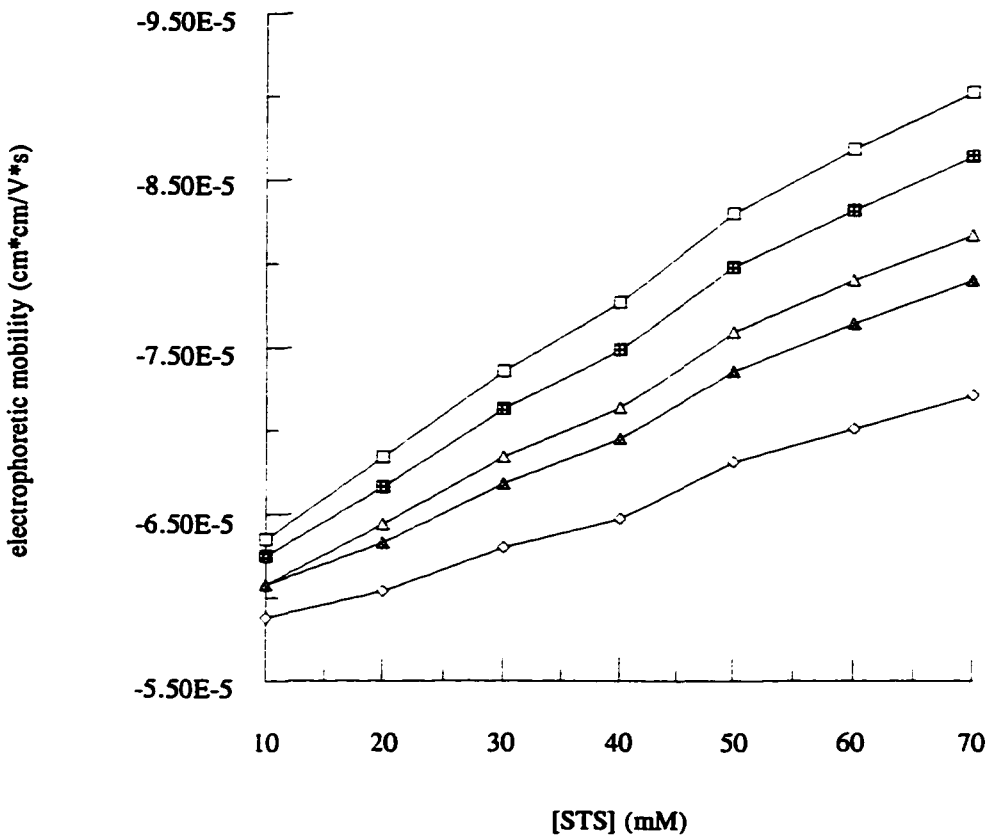

Figure 5. Electrophoretic mobility vs STS concentration. STS was used in the electrolyte; other conditions same as Figure 4. Samples: same as Figure 4.

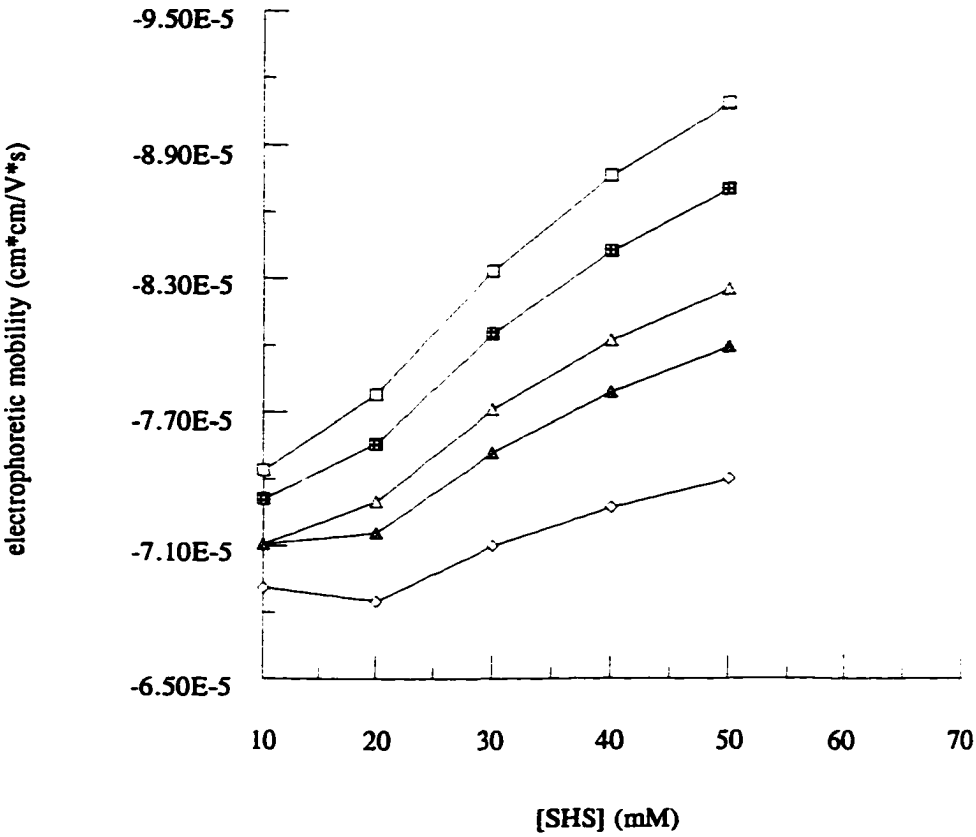

Figure 6. Electrophoretic mobility vs SHS concentration. SHS was in the electrolyte; other conditions same as Figure 4. Samples: same as Figure 4.

larger compounds and the surfactants. On the other hand, the plots with longer chain surfactants are steeper than those with shorter chain surfactants. This again indicates the stronger interactions between the analytes and the surfactants with longer chains. Walbroehl and Jorgenson [29] suggested a dynamic equilibrium mechanism between the associated and the unassociated forms of the analytes to account for their migration. This mechanism is also consistent for our results, as indicated by the steadily increasing mobilities in the plots in Figures 4 - 6. Since the analyte concentration was less than 0.25 mM, the concentration of the surfactants were at least 40-times higher than that of analytes. If an irreversibly bound complex were formed, a 10-fold excess of surfactants would be enough to completely bind the analytes, and further increases in surfactant concentrations would not lead to a large increase in electrophoretic mobilities.

Another anionic surfactant, sodium dioctyl sulfosuccinate (DOSS), was also tested for this study. DOSS has been used successfully for separations of nonionic compounds in aqueous buffers containing up to 40% ACN [31]. However, it did not work for our system with 100% methanol as the medium. Presumably, its branched structure could not provide strong enough interactions with the analytes in methanol for the analytes to come out within a reasonable time. In other words, the surfactant additives with linear long chains were the most effective in separating nonionic compounds in organic solvents. Considering both the solubility and efficiency, STS was the best additive tested.

3.3 Solvent mixtures

The separation window (i.e., the difference in migration times between the first and last analytes) was quite small in pure methanol for the analytes tested. The low boiling point of

pure methanol made it necessary to keep the current low to avoid evaporation of methanol through heating. Because of these concerns, 90/10 (% v/v) mixtures of methanol-formamide, methanol-acetonitrile and methanol-H_2O were tried with nine PAH compounds as sample analytes, and 70 mM STS and 10 mM H_3PO_4 in the BGE. The methanol-formamide mixture gave a much wider separation window, but the separation took much longer ($\sim$38 min for benzophenone) and the efficiency was poor. With methanol-acetonitrile, the separation showed little improvement in the elution window and resolution, and the efficiency was even worse. The mixture of methanol-H_2O provided a wider window and better resolution without prolonging the separation, and the electrophoregram for this separation was included in Figure 7.Compared the separation of nine PAH compounds in 90/10 (%v/v) methanol-H_2O with that in pure methanol, where several compounds comigrated with each other (3 and 4, 5 and 6) or only were partially resolved (6 and 7), baseline separation was obtained in this mixed solvent system.

In order to study the effect of water, the methanol content in the BGE was varied from 100% to 70%. Lower concentrations of methanol were not used because of low solubility of STS in the BGE. Figure 8 demonstrates the change of electrophoretic mobilities with the percentage of methanol. As in Figures 4 - 6, electrophoretic mobilities were calculated from migration time without considering EOF because it was too small. The curved graph of electrophoretic mobilities in Figure 8 was considered to be the combined results of two factors: (a) solvophobic interactions between the analytes and the surfactant additive; (b) the variation of viscosity (η) and dielectric constant (ϵ) as the percentage of methanol was changed. The effect of methanol content on η and ϵ in binary water-methanol mixtures has

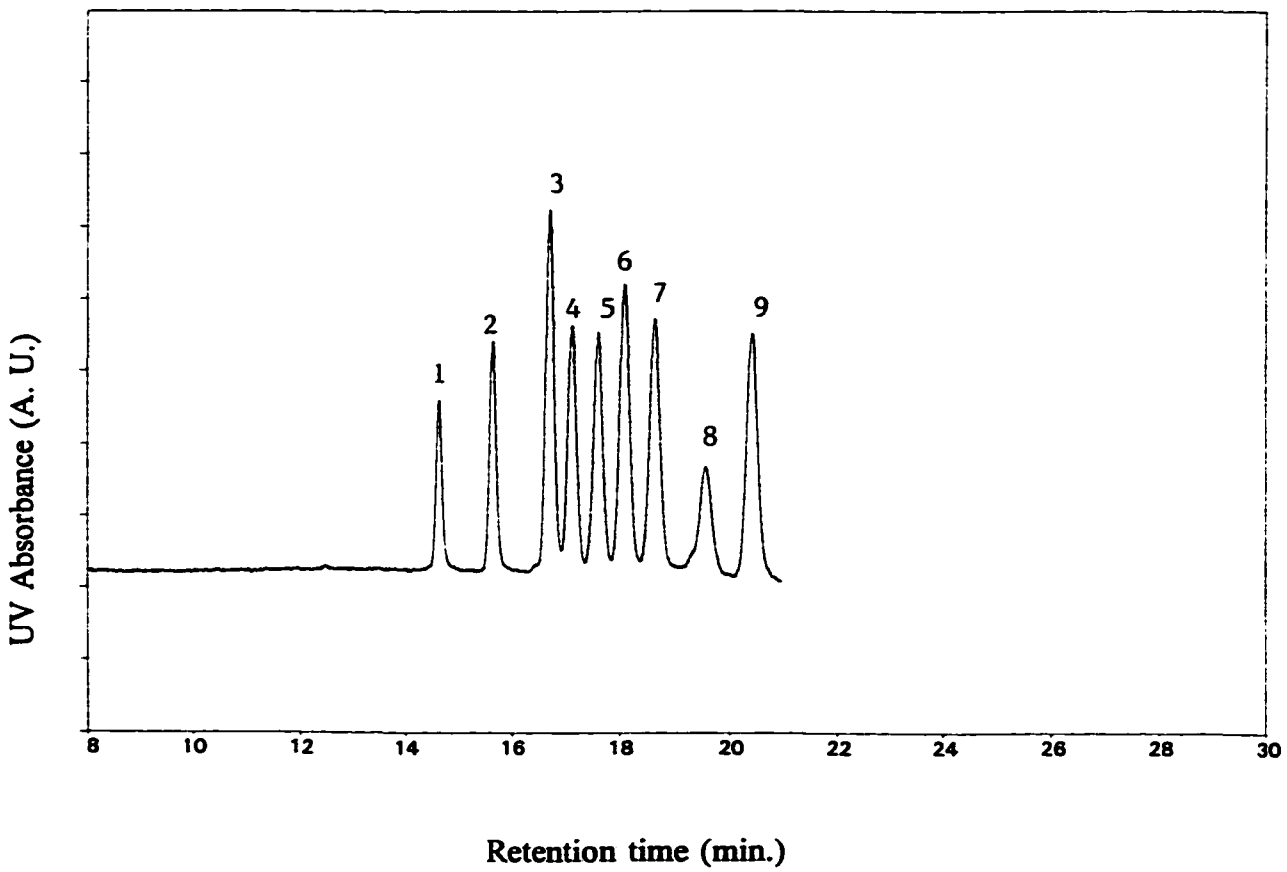

Figure 7. Separation of nine PAH compounds. Electrolyte: 10 mM H_3PO_4 and 70 mM STS in 90/10 (% v/v) methanol - H_2O; sample injection: -3kV * 15s. Peaks: 1. Benzo[a]perylene; 2. Perylene; 3. Benzo[a]anthracene; 4. Pyrene; 5. 9-methylanthracene; 6. Anthracene; 7. Fluorene; 8. Naphthalene; 9. Benzophenone.

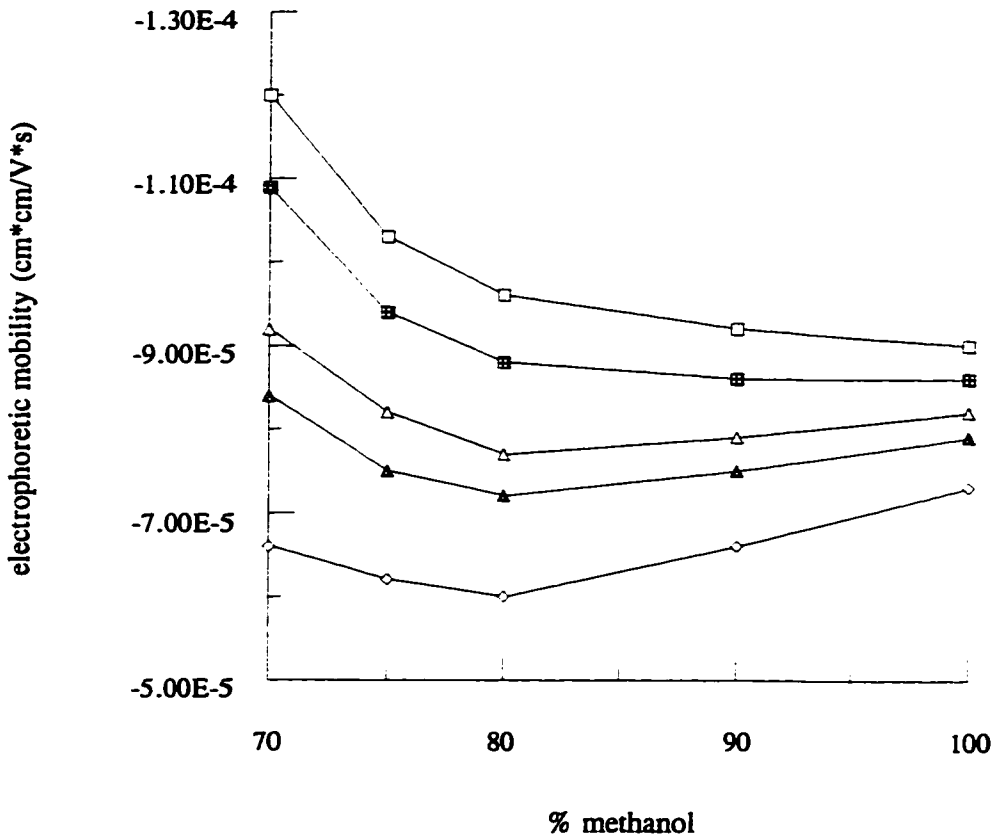

Figure 8. Electrophoretic mobility vs % methanol in the BGE. Electrolyte contains 10 mM H_3PO_4 and 70 mM STS in methanol-H_2O; sample injection: -3kV * 15s. Samples: same as Figure 4.

been very well studied in the literature [11]. It was found that ϵ and η became smaller as methanol concentration in water was increased from 70% to 100%; however, the ratio of ϵ/η grew bigger within the same range. Since electrophoretic mobility is directly proportional to ϵ/η, it should increase at higher concentrations of methanol. On the other hand, the solvophobic interactions become weaker at higher concentration of methanol so the electrophoretic mobility of an analyte due to complex formation will be smaller. Between 70% and 80% methanol, solvophobic interaction was likely the predominant factor, so electrophoretic mobilities decreased. Above 80% methanol, a rising mobility trend was observed due to the greater ϵ/η ratio. Benzo[a]perylene and perylene showed a slight decline in mobility because of their relatively strong interactions with STS. Figure 8 also indicated a wider separation window and better separation at lower methanol content, as illustrated by Figure 9 for the separation of nine PAH compounds in 75/25 methanol-H_2O mixture.

3.4 Separation scope and validation of the method

The high proportion of methanol in the solutions used has a strong tendency to weaken the interactions between the analytes and the surfactant. The success of the separations reported is likely due to the ability of the hydrophobic and fairly bulky test compounds to resist the effect of high methanol content. To test this hypothesis several more polar compounds (acidic and basic pharmaceutical compounds) were tested. Figure 10 shows the separation of six drugs that would be neutral at the acidic apparent pH used. Although the separation was complete, the migration times were quite long. This is caused by weaker association of the relatively polar drugs with STS.

Figure 11 shows the electropherogram for four basic drugs with a positive power supply

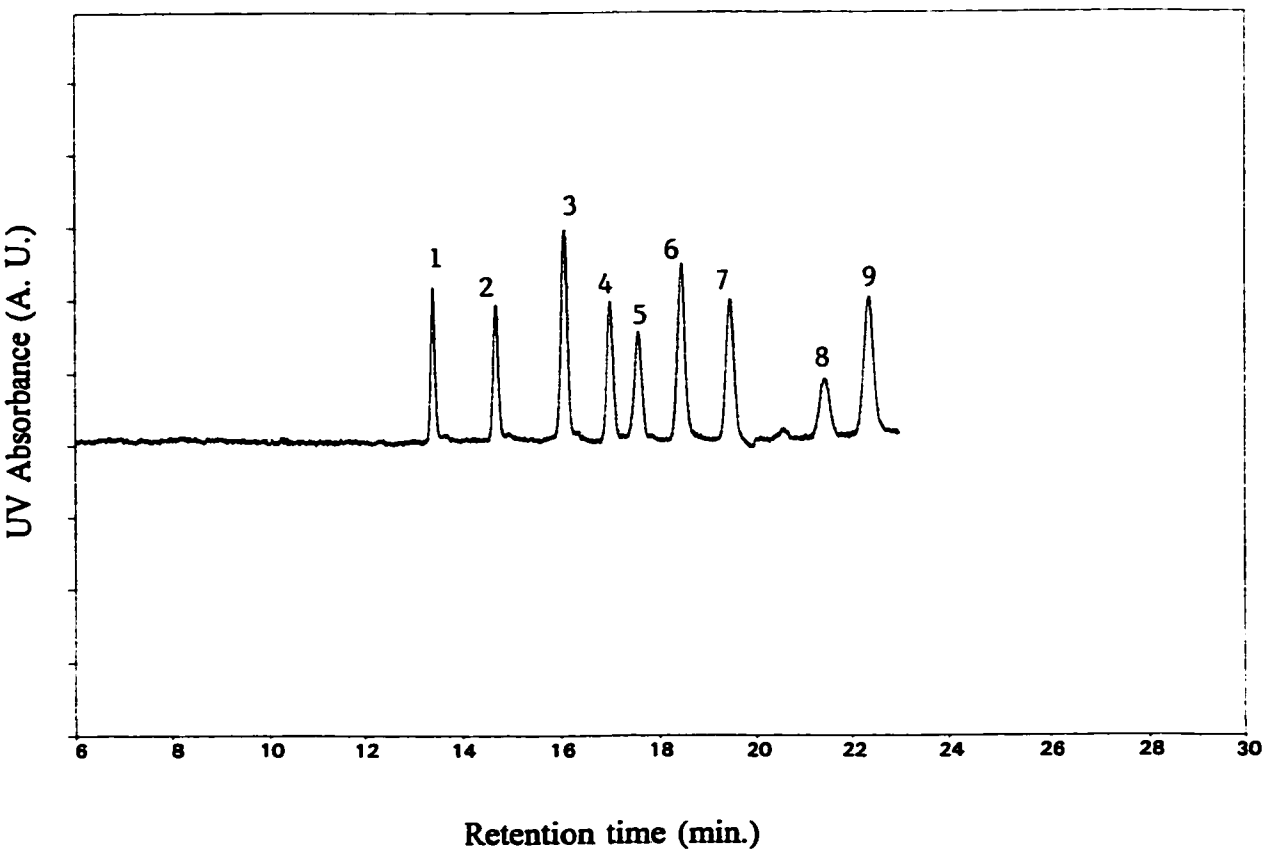

Figure 9. Separation of nine PAH compounds. Electrolyte: 10 mM H_3PO_4 and 70 mM STS in 75% methanol - 25% H_2O; sample injection: -3kV * 15s. Peaks: same as Figure 7.

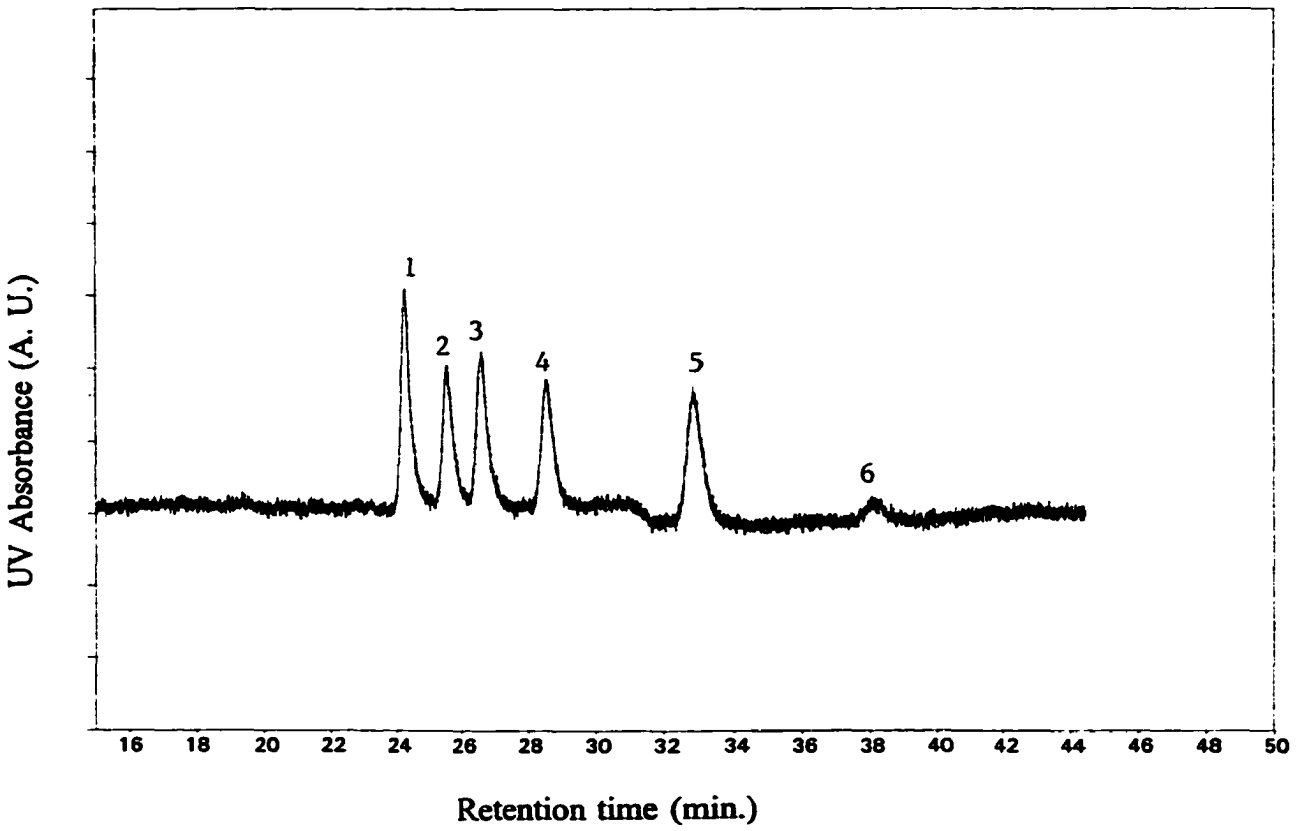

Figure 10. Separations of acidic pharmaceutical compounds. Electrolyte: 10 mM H_3PO_4 and 70 mM STS in 85% methanol - 15% H_2O; sample injection: -2.5kV * 15s; detection: UV@214 nm. Peaks: 1. Indomethacin; 2. Fenoprofen; 3. Ketoprofen; 4. α-hydroxyhippuric acid; 5. 1,7-dimethylxanthine; 6. β-phenylpyruvic acid.

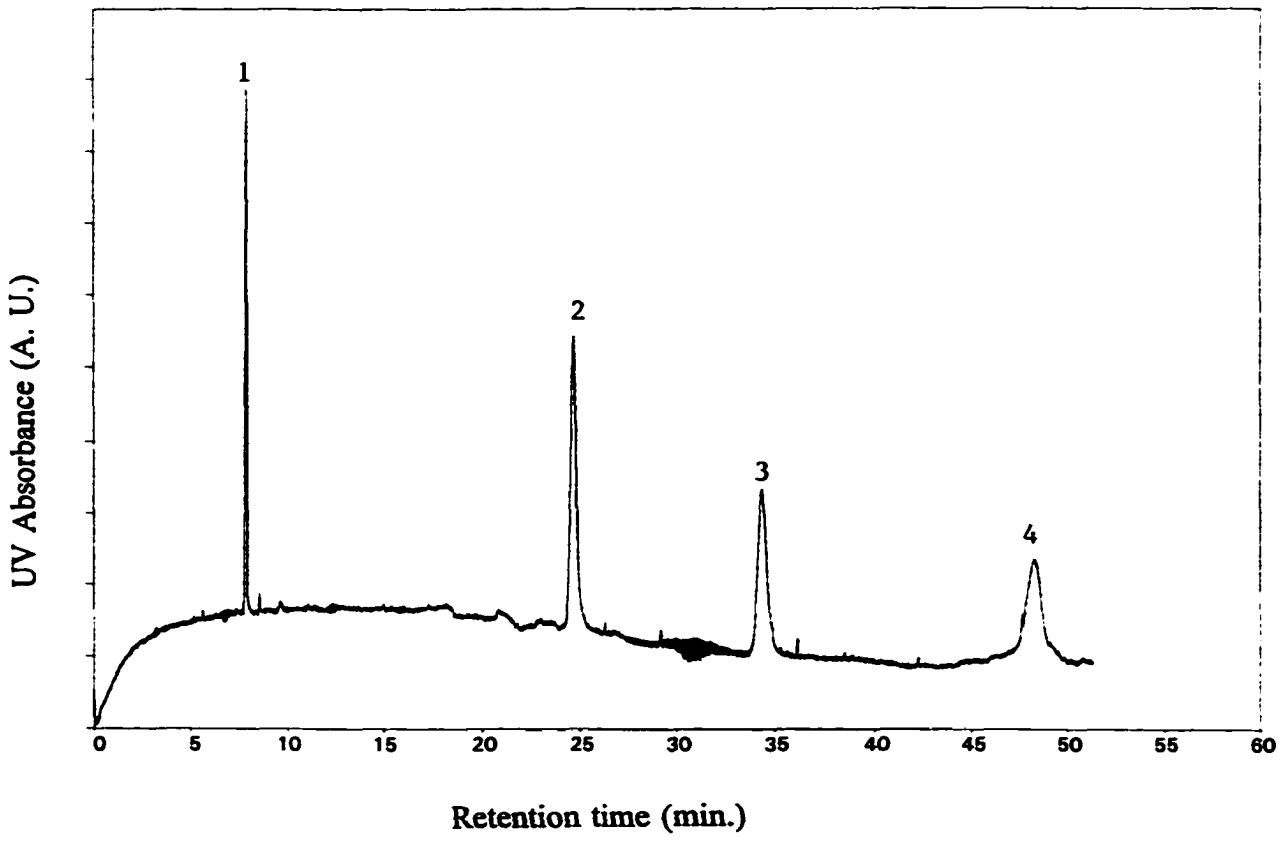

Figure 11. Separation of basic pharmaceutical compounds. Electrolyte: 100 mM H_3PO_4 in 85% methanol - 15% H_2O; sample injection: -2.5kV * 15s; voltage: +20 kV; detection: UV@254 nm. Peaks: 1. Cytidine; 2. Guanosine; 3. Sulfapyridine; 4. Uridine.

and no surfactant present. This separation is due to the intrinsic positive charges on the protonated analytes. However, these compounds are very weak bases and thus may be incompletely protonated.

Tests for reproducibility of migration times and linearity of calibration plots were performed with perylene and benzophenone as sample analytes. The BGE contained 50 mM SDS and 10 mM H_3PO_4 in methanol; samples were injected hydrostatically. Replenishing the BGE between runs, the results from eight consecutive runs showed very good reproducibility for the migration times. The RSD for perylene and benzophenone was 0.55% and 0.73%, respectively.

As ahown in Figures 12 and 13, plots of peak height and peak area as a function of analyte concentration were linear for benzophenone between 0.005 mM and 0.60 mM. The correlation coefficient was 0.9997 for peak height and 0.9995 for peak area. Similar plots for perylene between 0.005 mM and 0.40 mM were slightly curved with a linear correlation coefficient of 0.985 for peak height and 0.995 for peak area. The curvature in the perylene plot was probably due to its limited solubility at the higher concentrations.

4 Concluding remarks

The ability to work in predominantly nonaqueous solutions adds a valuable new dimension to our technology for separation of neutral compounds by CE. Nonaqueous CE with methanol as separation medium has been successfully applied for separation of nonionic organic compounds as well as acidic and basic drugs. Methanol does not provide a wide elution window, but addition of a low percentage of water into methanol largely overcomes

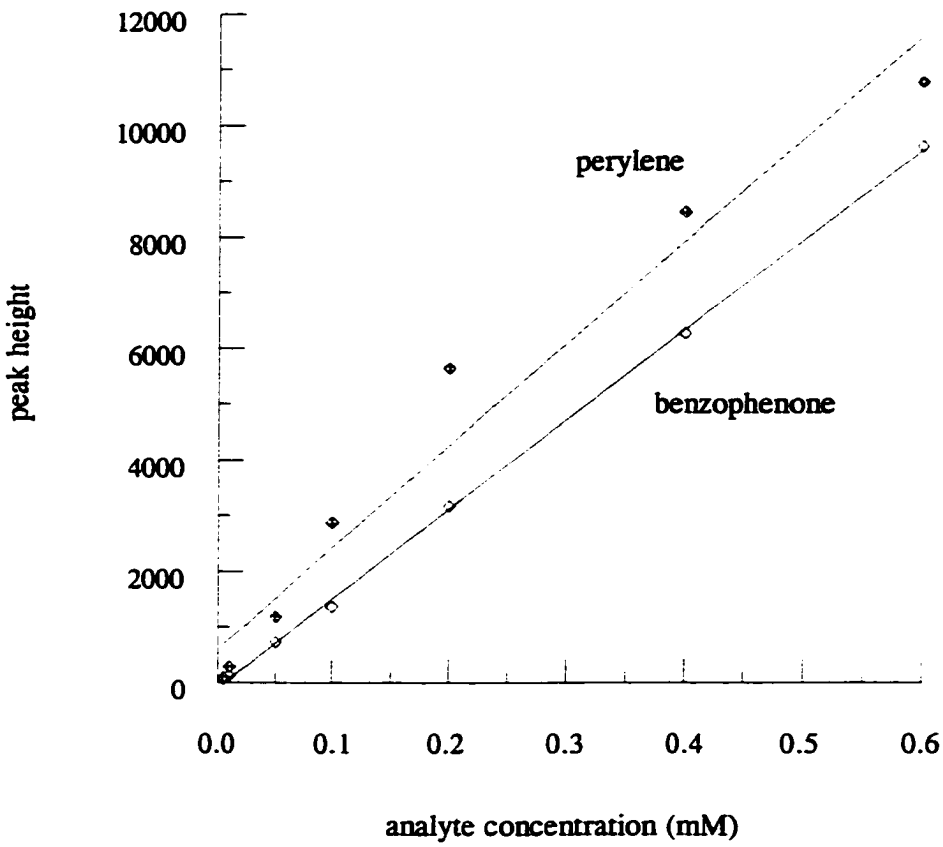

Figure 12. Plot of peak height vs. concentration of analytes. Electrolyte: 50 mM SDS and 10 mM H_3PO_4 in methanol; hydrostatic sampling: 12 s at 10 cm height.

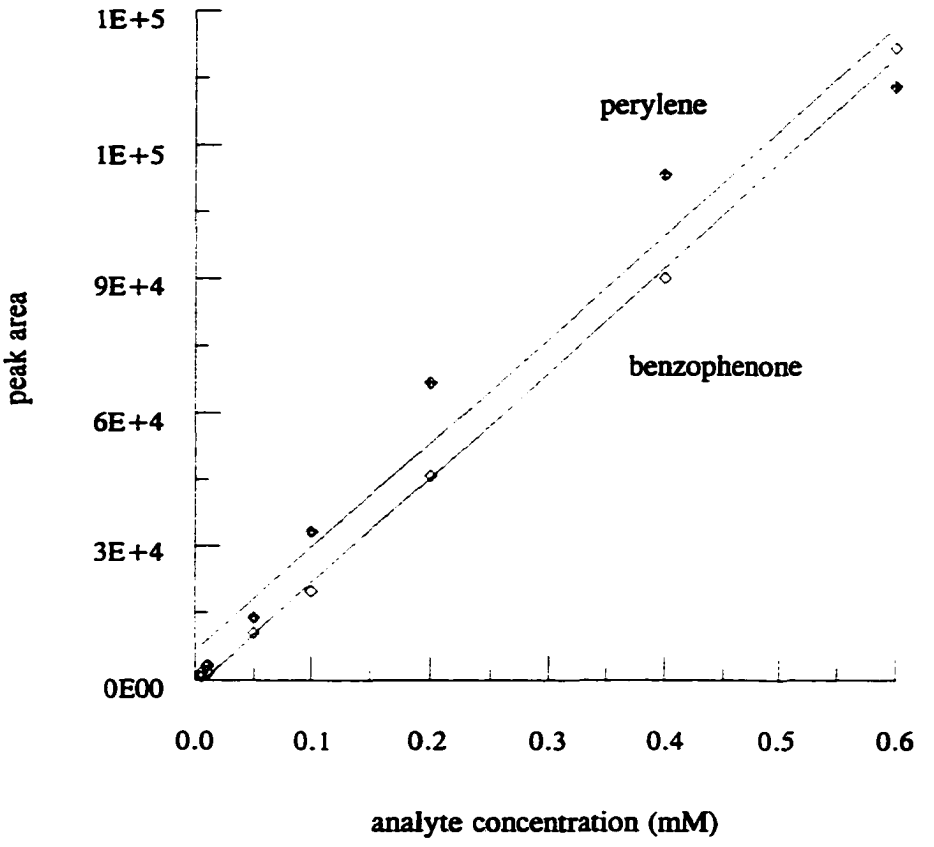

Figure 13. Plot of peak area vs. concentration of analytes. Electrolyte: 50 mM SDS and 10 mM H_3PO_4 in methanol; hydrostatic sampling: 12 s at 10 cm height.

this limitation. In methanol-water mixtures, separations are affected both by the solvophobic interactions between the analytes and the surfactant additives and by the ratio of dielectric constant over viscosity.

Acknowledgements

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CHAPTER 4. SEPARATION OF ANILINES BY CAPILLARY ELECTROPHORESIS WITH SMALL IONIC COMPOUNDS AS BUFFER ADDITIVES

A paper accepted by *J. Chromatogr. A*

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Abstract

Addition of either ethanesulfonic acid or protonated triethylamine to the background electrolyte markedly improves the separation of protonated anilines by capillary electrophoresis. These additives appear to form a thin coating on the capillary surface via a dynamic equilibrium. This results in a change in electroosmotic flow and reduces interactions of the sample cations with the silica surface. A mixture of ten substituted anilines could be separated, including several positional isomers. Migration times of the sample cations were reproducible with a RSD less than 1.0%.

1. Introduction

Organic bases can be separated by capillary electrophoresis (CE) as protonated cations by operating at an acidic pH. Lin et al separated ß-blockers using citrate buffer at a very acidic pH [1]. Basic proteins and peptides have been separated at acidic pH values with a positively-charged surfactant additive in the background electrolyte (BGE) [2, 3]. The surfactant is adsorbed on the capillary walls, giving a positive surface that repels the protein cations and prevents their adsorption. Chiral bases have been resolved by CE using chiral additives such as native and derivatized ß-cyclodextrins [4-6]. Organic bases may also be separated in their molecular form by micellar electrokinetic chromatography. Sodium dodecyl sulfate (SDS) [7], tetraalkylammonium salts [2-4, 8], bile salts [9, 10] and glycopyranoside-based surfactants [11, 12] have been used for this purpose. In some cases, especially for protein separations, polymers were employed to coat capillary surface covalently or adsorptively so that the interactions between the analytes and silanol groups on the capillary surface were decreased or eliminated. Poly(ethylene glycol) (PEG) [13-15], poly(vinyl alcohol) (PVA) [16] and poly(ethylene oxide) [17] are a few examples of the polymers used.

Generally, the additives mentioned above are quite large. While they have usually worked well for the separation of basic compounds, there are some problems with their usage. Surfactants and other large additives may form thick coatings on the capillary surface. Ding and Fritz demonstrated that the coating could continue to build up from run to run, thus causing a gradual increase in migration time [2]. It may be difficult to remove the coating completely when experimental conditions are changed.

Published work suggests that the use of much smaller molecules as BGE additives may

effectively inhibit unwanted interactions between sample cations and the silica surface of the capillary. Incorporation of ethanesulfonic acid in the BGE was found to markedly improve the CE separation of protonated amino acids [18] and basic drugs [19] at an acidic pH. Quang and Khaledi found that tetrabutylammonium salts improved the chiral separation of bases with ß-cyclodextrin at pH 2.5 [4]. Triethylammonium salts have often been used in HPLC to block secondary interactions between analytes and the silica-based stationary phase [20], which indicates its potential as the BGE additive for CE separations.

This research has two main goals. One was to find conditions for a practical CE separation of substituted anilines of very similar chemical structure. Compounds of this type are potential environmental pollutants [21-23]. Several groups reported the separation of chloro- and nitro- substituted anilines by different techniques [24-26]; however, nothing about the separation of alkyl-substituted anilines could be found. The second, and perhaps more important goal, was to study the effect of some small organic ions as CE electrolyte additives on the separation of protonated organic bases. A low concentration of ethanesulfonic acid (ESA) or protonated triethylamine (TEA) in the BGE was found to greatly improve the separation of organic cations and to provide excellent reproducibility of the migration time. Experiments were performed to elucidate the mechanism of ESA and TEA with the silica surface of the capillary.

2. Experimental

A Waters Quanta 4000 capillary electrophoresis system (Millipore Waters, Milford, MA, USA), equipped with a positive power supply, was employed to separate anilines under

acidic conditions and generate all electropherograms. Polyimide-coated fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 45 cm in total length (37 cm effective length) with an I. D. of 50 μm. Direct UV detection was performed at 229 nm. A voltage of +15 kV was applied for all separations. All samples were injected electrokinetically, and electroosmic flows (EOF) were measured with the "accelerated method" introduced by Sandoval and Chen [27] with formamide and DMSO as neutral markers. Electropherograms were collected at speed of 15 points/s and plotted by Chromperfect data acquisition system (Justice Innovations, Mountain View, CA, USA).

Standards of substituted anilines, ethanesulfonic acid (ESA), triethylamine and diethylenetriamine were purchased from Aldrich (Milwaukee, WI, USA). Organic solvents were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were the best grade available. All standards and buffer solutions were prepared with 18.2 MΩ deionized water by a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA, USA). Buffers were made by mixing phosphoric acid and ethanesulfonic acid/amine additives, and adjusting pH with 1 M sodium hydroxide or 1 M phosphoric acid before 2-propanol was added.

Each new capillary was conditioned with 1 M sodium hydroxide for 1 h, followed by 1-h rinsing with deionized water. Each day, prior to use, capillaries were rinsed with 0.1 M sodium hydroxide and deionized water for 10 min each. When different running buffers were used, capillaries were rinsed with the desired buffer for 10 min following the NaOH and D. I. H_2O rinsing. Before each sample injection, capillaries were rinsed with the running buffer for 3 min.

3. Results and Discussion

3.1 Effect of ESA on the separation of anilines

3.1.1 Preliminary experiments

Preliminary experiments at an acidic pH gave very poor CE separations of various protonated anilines when no additive was used in the CE buffer solution. A similar situation had been observed for the CE separation of protonated amino acids [18]. Varying the pH or increasing the phosphate concentration in the buffer improved the resolution somewhat, but separation of the aniline mixture was still far from complete, and the peaks were broad and somewhat tailed. Addition of 30 - 50 mM ESA to the BGE was found to greatly improve the separation of the substituted anilines.

Preliminary results on buffer pH established pH 3 - 4 as the optimum range, so the subsequent experiments were performed within this range.

3.1.2 Selection of an organic solvent

Experiments were performed in which the BGE contained 50 mM ESA in addition to a phosphate buffer, and part of the water in the buffer was replaced with an organic solvent. Three types of organic solvent, acetonitrile, 2-propanol and 1-butanol, were studied, and each solvent was tested with several concentrations in the buffer composition: 2.5%, 5.0%, 7.5% and 10% (2.5% and 5.0% only for 1-butanol). Electropherograms are given in Figure 1 and 2 comparing acetonitrile and 2-propanol as organic modifiers. The results with 2-propanol showed better resolution and peak shape for the aniline separations, probably due to the better solvation of the analytes by 2-propanol, while the analysis time did not differ much for three solvents. So 2-propanol was chosen as the organic solvent. A concentration

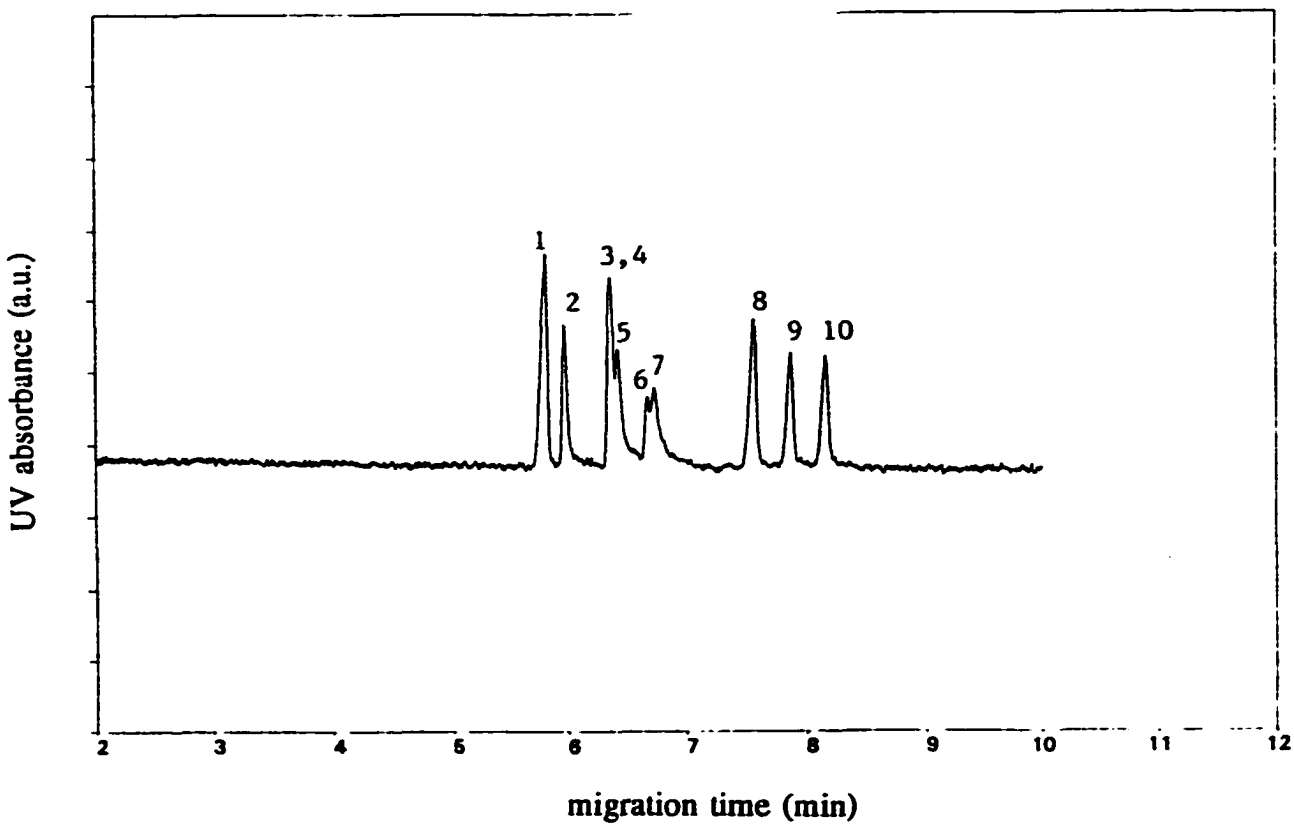


Figure 1. Separation of substituted anilines with acetonitrile as organic modifier. Electrolyte contained 50 mM ESA, 10 mM phosphate and 7.5% acetonitrile at pH 3.70; electrokinetic sampling, 8s * 5kV. Peaks: 1 = aniline; 2 = 4-ethylaniline; 3 = 3-ethylaniline; 4 = 4-propylaniline; 5 = 4-iso-propylaniline; 6 = 4-butylaniline; 7 = 4-sec-butylaniline; 8 = 2-ethylaniline; 9 = 2-iso-propylaniline; 10 = 2-propylaniline. Each analyte was 50 ppm in sample solution.

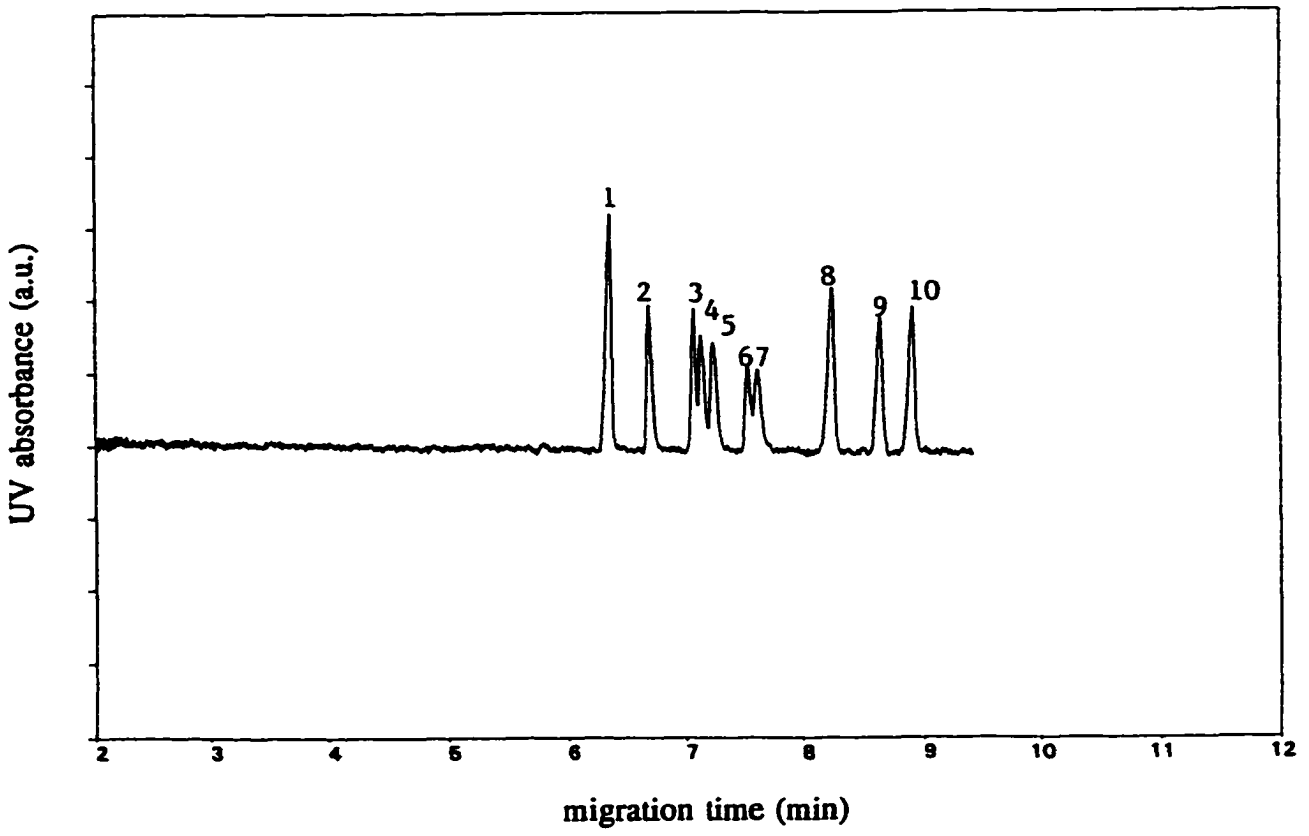


Figure 2. Separation of substituted anilines with 2-propanol. Conditions same as Figure 1 except that acetonitrile was replaced by 2-propanol. Peaks no.: same as Figure 1.

of 7.5% 2-propanol itself in the buffer gave slightly better separation of 10 substituted anilines than other concentrations (Figure 2 and 3). Therefore, 7.5% 2-propanol was added to the electrolytes for all the following studies.

3.1.3 pH effect

The pH study was confined to pH 3.0 - 4.0. Outside this range, the separation was not acceptable; the baseline was noisy and several analytes comigrated. As shown in Table 1, measured or predicted pKa values are available for several of the substituted anilines and ranged approximately from 4.4 to 5.1 [28]. Between pH 3.0 and 4.0, the substituted anilines would be partially protonated to different degrees. This would give different mobilities and contribute to a better separation. Without this effect, the separation could be based only on small differences in analyte structure and would be much more difficult.

Figure 4 shows the effect of buffer pH on the migration behavior of the substituted anilines. It is evident that all the analytes migrated slower at higher buffer pH, as would be expected. The pH for the best separation of all ten of the anilines studied was determined from Figure 4 to be 3.65 with ESA as the additive.

3.1.4 Effect of ESA on EOF and electrophoretic mobility

A more complete picture of the effect of the electrolyte additives can be obtained by measuring the electroosmotic flow (EOF) and the electrophoretic mobilities as a function of the additive concentration. Plots for ESA at pH 3.65 are given in Figure 5. The EOF dropped rapidly and then decreased much more slowly as ESA concentration was increased. This would indicate a decrease in surface negative charge. A thin layer of ESA is most likely adsorbed on the silica surface via a dynamic equilibrium [18]. Adsorption of ESA may

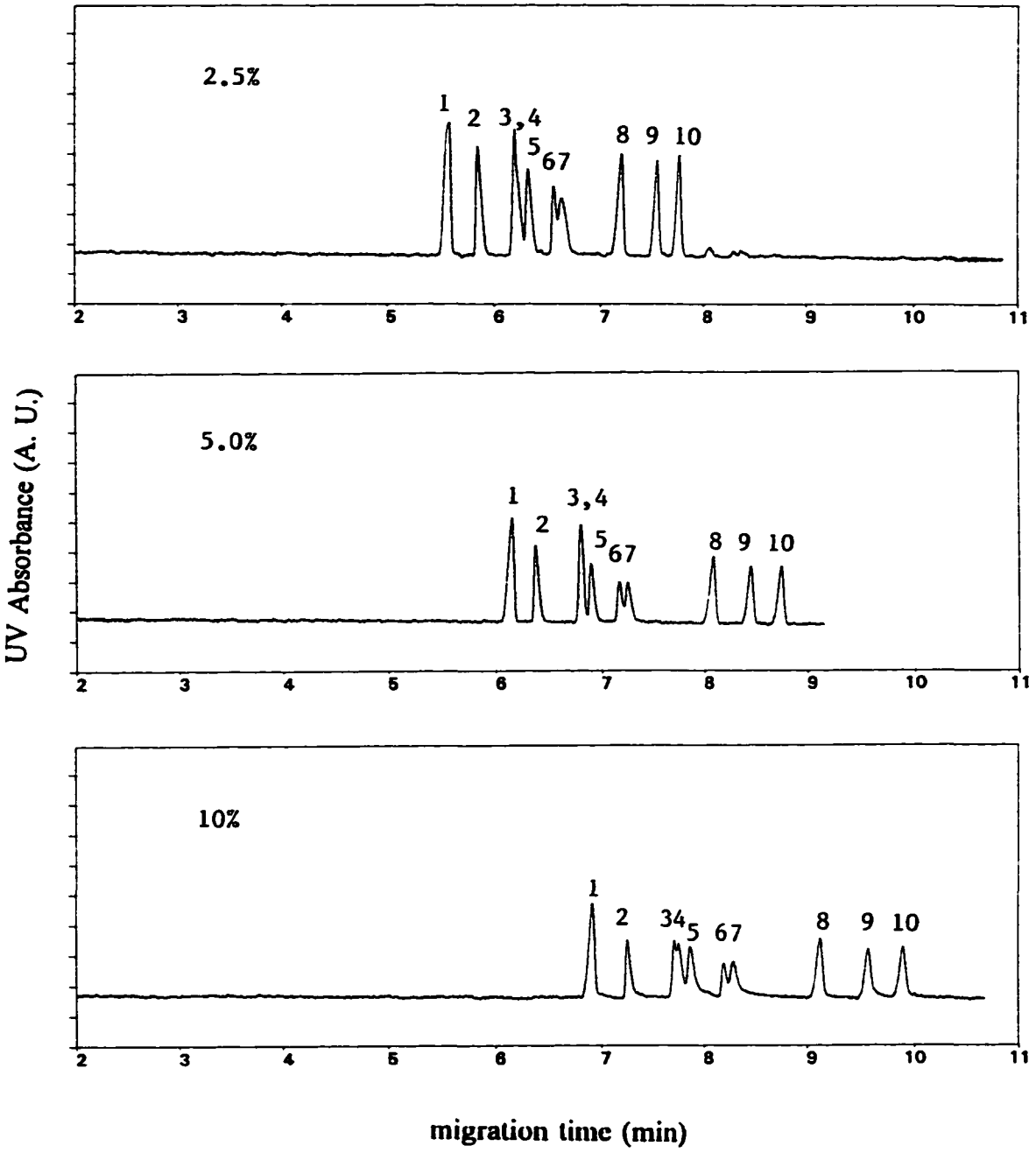


Figure 3. Separation of substituted anilines with 2-propanol at different concentration. Conditions same as Figure 2 except that percentage of 2-propanol in the BGE was changed. Peaks no.: same as Figure 2.

Table 1. pKa values for the analytes used in this study.

No.	Compound	pKa
1	aniline	4.60
2	2-ethylaniline	4.44[a]
3	3-ethylaniline	4.78[a]
4	4-ethylaniline	5.01[a]
5	2-propylaniline	*
6	2-isopropylaniline	4.49[a]
7	4-propylaniline	5.01[a]
8	4-isopropylaniline	5.01[a]
9	2-butylaniline	*
10	2-sec-butylaniline	*
11	4-butylaniline	5.04[a]
12	4-sec-butylaniline	5.13[a]
13	4-tert-butylaniline	5.01[a]

a. predicted value based on ref. 28.

*. insufficient data.

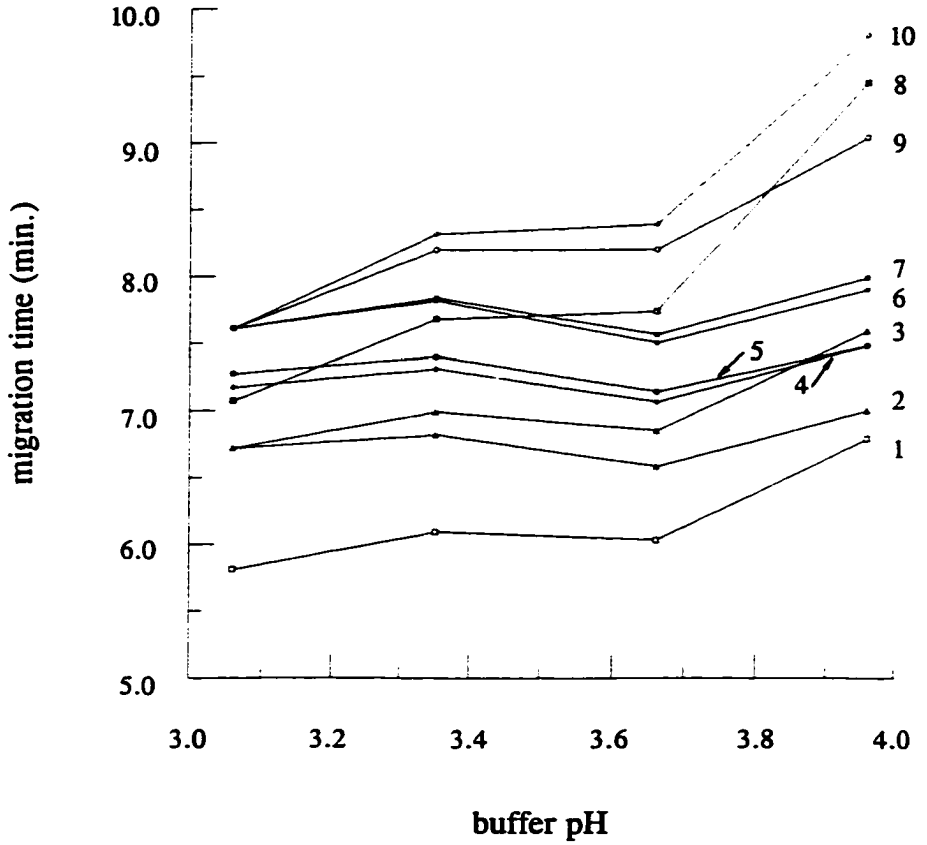
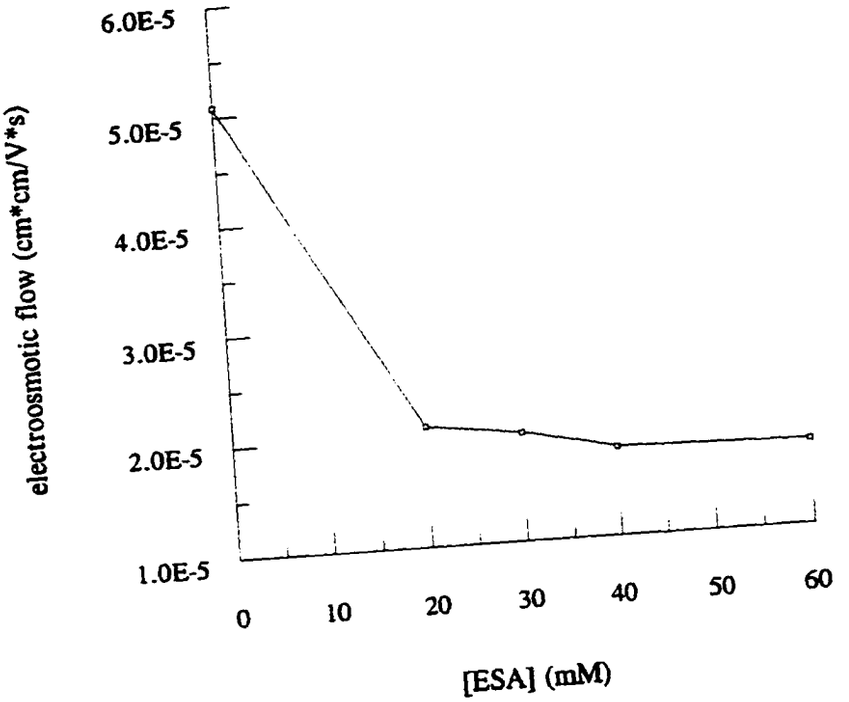
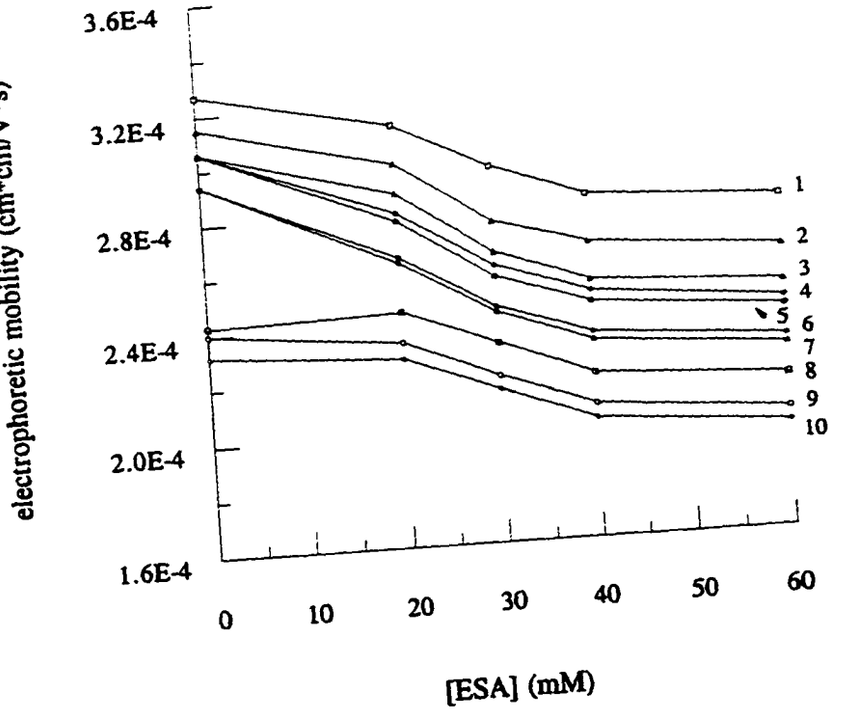


Figure 4. Effect of buffer pH on the migration time of substituted anilines. Electrolyte: 10 mM phosphate, 50 mM ESA, 7.5% 2-propanol; electrokinetic sampling, 8s * 5kV. Sample I.D.: 1 = aniline; 2 = 4-ethylaniline; 3 = 3-ethylaniline; 4 = 4-propylaniline; 5 = 4-iso-propylaniline; 6 = 4-butylaniline; 7 = 4-sec-butylaniline; 8 = 2-ethylaniline; 9 = 2-iso-propylaniline; 10 = 2-propylaniline.

Figure 5. Effect of ESA concentration on EOF and electrophoretic mobilities of substituted anilines. Electrolyte: 10 mM phosphate, 7.5% 2-propanol, pH 3.65; electrokinetic sampling, 8s * 5kV. Sample I.D.: same as Figure 4.


6.0E-5
5.0E-5
4.0E-5
3.0E-5
2.0E-5
1.0E-5
electroosmotic flow (cm*cm/V*s)
0
10
20
30
40
50
60
[ESA] (mM)


3.6E-4
3.2E-4
2.8E-4
2.4E-4
2.0E-4
1.6E-4
electrophoretic mobility (cm*cm/V*s)
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
[ESA] (mM)

involve hydrogen bonding between the sulfonate groups and the surface silanol groups of the silica capillary, so that some of the surface negative charges are covered up by ESA. Adsorption of a second layer of ESA, which would give a more negative surface, seems unlikely because the hydrocarbon chains of the ESA are too short to provide much hydrophobic attraction.

Figure 5 shows that the electrophoretic mobilities also decrease somewhat with the increasing ESA concentration. This is probably due to a certain amount of ion-pair formation between the protonated anilines and the negatively-charged ESA.

$$BH^+ + ESA^- \rightleftharpoons \text{Ion pair}$$

$$K = \frac{[ionpair]}{[BH^+][ESA^-]}$$

A decrease in the fraction of analyte present as the free cation (BH^+) would decrease its electrophoretic mobility. Differences in the equilibrium constants (K) of the various analytes would lead to improved separations.

From Figure 5, an ESA concentration within 40 - 60 mM seems to be optimal for the separation of substituted anilines. Included in Figure 6 was the separation of some anilines with 55 mM ESA in the background electrolyte. Several butylaniline positional isomers were baseline resolved under this condition.

3.1.5 Plate number

Ding found that addition of ethanesulfonic acid (ESA) to the BGE gave much sharper peaks for the separation of basic drugs at pH $\sim$2.5 in aqueous electrolytes containing 10%

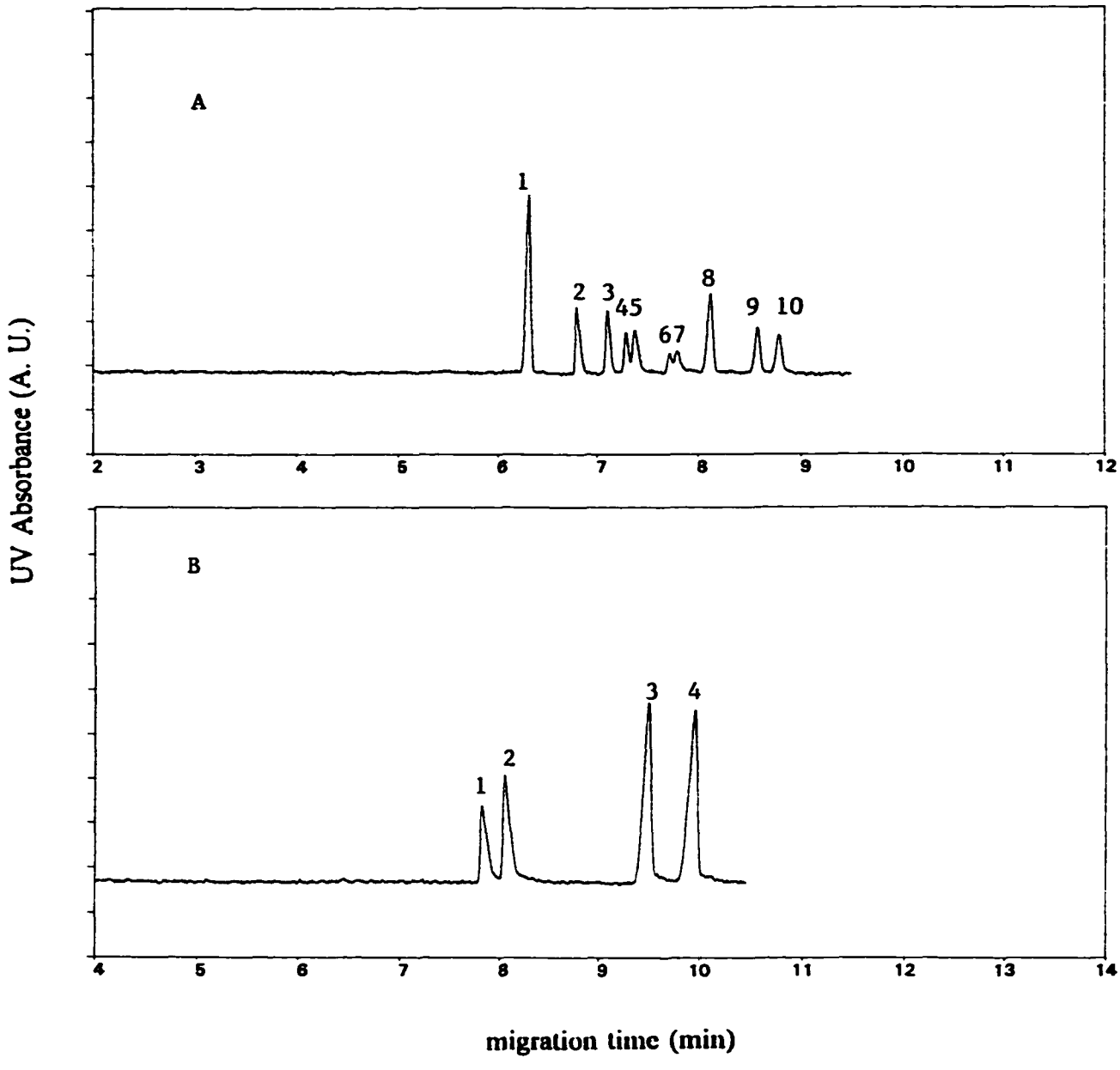


Figure 6. Separation of substituted anilines. Electrolyte: 55 mM ESA, 15 mM phosphate, 7.5% 2-propanol at pH 3.65; electrokinetic sampling: 8s * 5kV. Peaks: (A) same as Figure 1; (B) 1 = 4-butylaniline; 2 = 4-tert-butylaniline; 3 = 2-sec-butylaniline; 4 = 2-butylaniline. Each analyte was 50 ppm.

Table 2. Comparison of plate number (N) and peak asymmetry factor (PAS) for separations with and without ESA as the BGE additive. Electrolyte: 10 mM phosphate, 7.5% 2-propanol, pH 3.65. Electrokinetic sampling, 8s * 5kV.

Compound	N		PAS	
	0 mM ESA	50 mM ESA	0 mM ESA	50 mM ESA
Aniline	22,000	81,000	2.34	0.60
2-propylaniline	54,000	127,000	0.32	0.88

acetonitrile [19]. In the present work, addition of ESA was shown to improve both peak sharpness and peak symmetry. Typical values for CE separation of anilines at pH 3.65 in aqueous solution containing 7.5% 2-propanol with or without ESA as BGE additive were shown in Table 2.

3.1.6 Reproducibility

The run-to-run reproducibility of the aniline migration times was determined by replicate injections (n = 5) on the same capillary. Four anilines were used as sample analytes. No treatment of the capillary was performed between runs except for a brief rinsing with fresh buffer. The data in Table 3 for buffers containing 55 mM ESA gave migration times with an average RSD of 0.9%. These results indicate that ESA in the buffer leads to excellent reproducibility and that no appreciable buildup of ESA occurs on the capillary surface.

Table 3. Reproducibility test based on five consecutive runs (n = 5). For ESA, electrolyte contained 15 mM phosphate, 55 mM ESA and 7.5%2-propanol at pH 3.65; for TEA, electrolyte contained 50 mM phosphate, 40 mM TEA and 7.5% 2-propanol at pH 3.45. Electrokinetic sampling, 8s * 5kV.

compound	ethanesulfonic acid (ESA)		triethylamine (TEA)	
	migration time (min)	RSD (%)	migration time (min)	RSD (%)
aniline	6.39	0.8	9.07	0.4
4-ethylaniline	6.92	0.8	9.99	0.5
3-ethylaniline	7.22	0.9	10.55	0.5
2-ethylaniline	8.27	1.1	12.63	0.7

3.2 Effect of amine additives

3.2.1 pH effect

Addition of 40 mM of protonated triethylamine (TEA) to the BGE instead of ESA also improved the peak sharpness and resolution of the aniline analytes. The pH study in Figure 7 showed that a pH of 3.45 gave the best resolution of the test mixture. Migration times were longer with TEA than with ESA as the additive.

3.2.2 Effect of amine additives on EOF and electrophoretic mobility

The protonated forms of two amine additives were studied: triethylamine (TEA) and diethylenetriamine (DETA). Figure 8 shows a much greater change in EOF for TEA than was observed for ESA. The EOF decreased steadily within the concentration range studied, and its direction was reversed from positive to negative as the amount of triethylamine in the buffer increased. This clearly pointed out the adsorption of triethylamine onto the capillary surface. This adsorption could probably involve several aspects: the electrostatic interactions between the negative capillary surface and the triethylamine cations, the hydrogen bonding between the silanols and the amino groups, and perhaps the hydrophobic interactions between the alkyl chains and the siloxane groups, which are known to exhibit hydrophobic character [29, 30]. The electrophoretic mobilities of the analytes did not vary much within the same concentration range, which indicated the absence of the interactions between the substituted anilines and triethylamine. A higher concentration of TEA gave a better separation of substituted anilines. This occurred because of a higher EOF counter to electrophoretic mobilities, which increased migration times and gave better peak resolution. Figure 9 shows the separation of anilines with 40 mM TEA as the BGE additive.

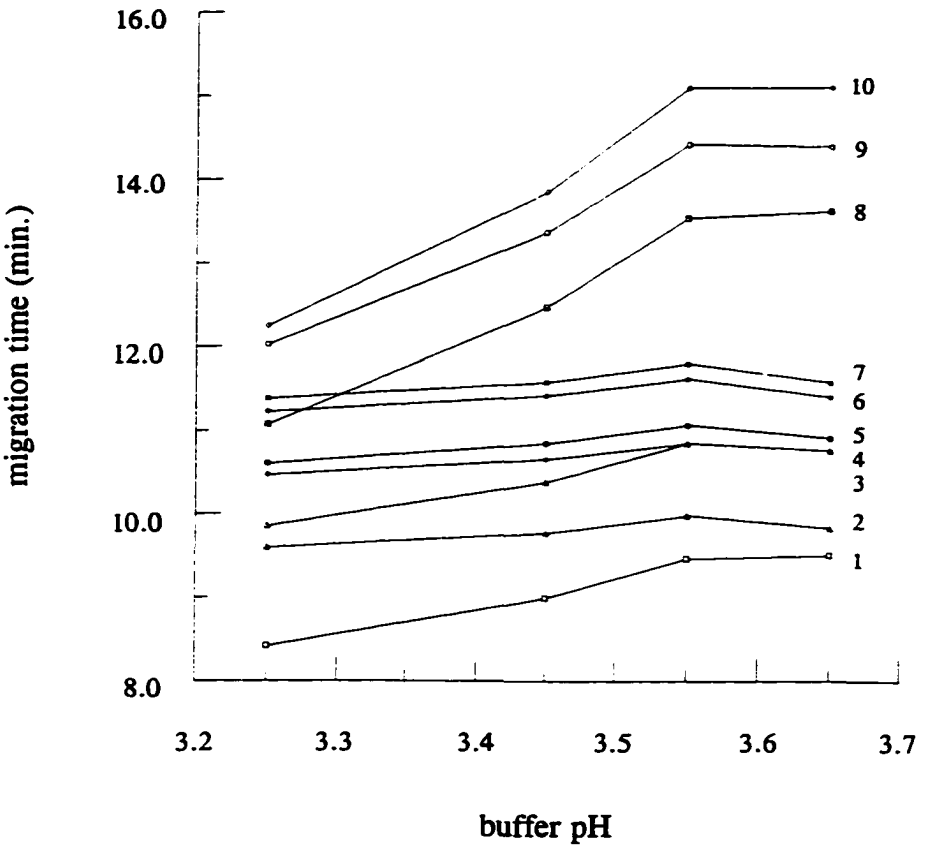
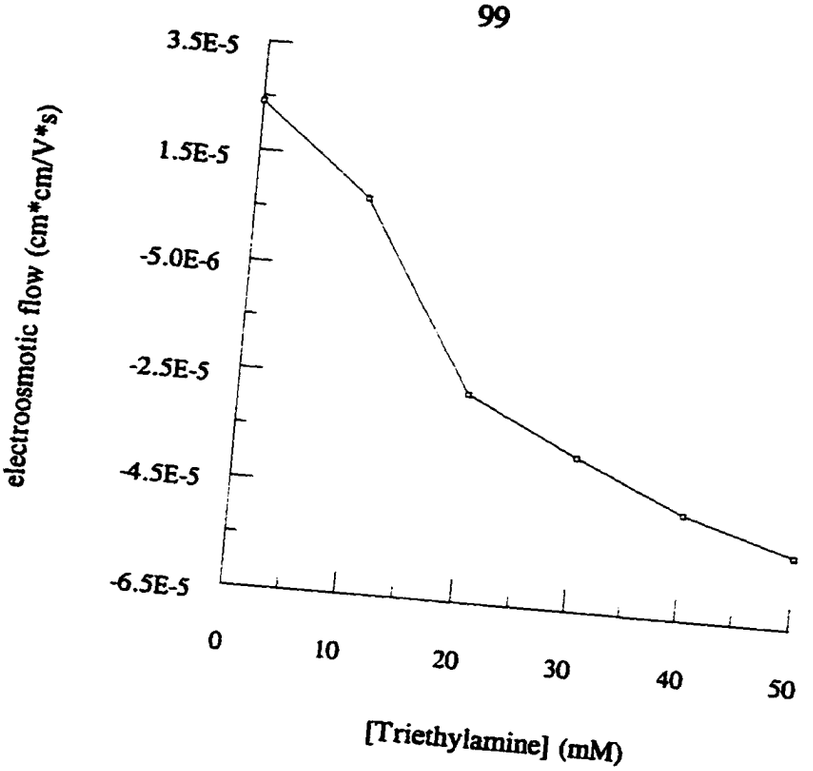
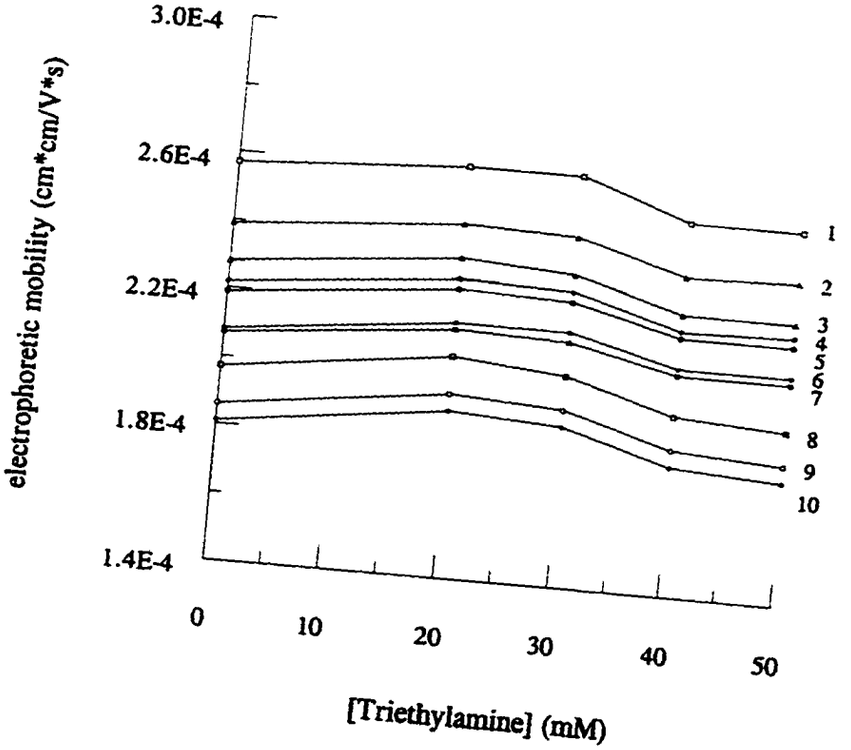


Figure 7. Effect of buffer pH on the migration time of substituted anilines. Electrolyte: 40 mM triethylamine, 50 mM phosphate, 7.5% 2-propanol; electrokinetic sampling, 12s * 3kV. Sample ID.: same as Figure 4.

Figure 8. Effect of triethylamine concentration on EOF and electrophoretic mobilities of substituted anilines. Electrolyte: 50 mM phosphate, 7.5% 2-propanol, pH 3.45; electrokinetic sampling, 12s * 3kV. Sample I.D.: same as Figure 4.


3.5E-5
1.5E-5
-5.0E-6
-2.5E-5
-4.5E-5
-6.5E-5
electroosmotic flow (cm*cm/V*s)
0
10
20
30
40
50
[Triethylamine] (mM)


3.0E-4
2.6E-4
2.2E-4
1.8E-4
1.4E-4
electrophoretic mobility (cm*cm/V*s)
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
[Triethylamine] (mM)

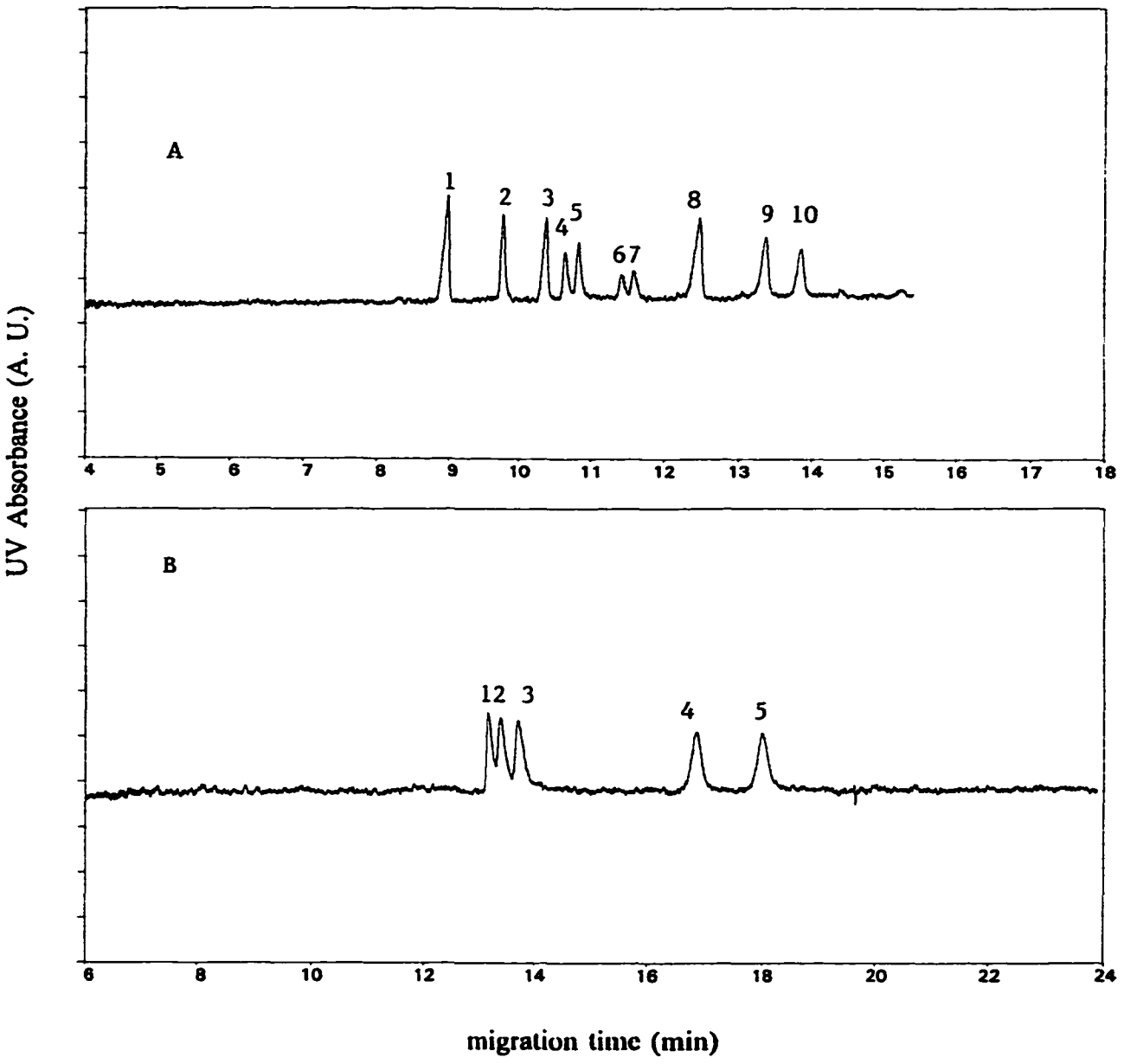


Figure 8. Separation of substituted anilines. Electrolyte: 40 mM triethylamine, 50 mM phosphate, 7.5% 2-propanol, pH 3.45; electrokinetic sampling, 10s * 4kV. Peaks: (A) same as Figure 1; (B) 1 = 4-butylaniline; 2 = 4-sec-butylaniline; 3 = 4-tert-butylaniline; 4 = 2-sec-butylaniline; 5 = 2-butylaniline. Each analyte was 50 ppm.

Diethylenetriamine (DETA) was also briefly investigated as a buffer additive. Under the conditions for this study (pH 3.65), each DETA molecule possesses more than two positive charges, so it is more polar than both ESA and triethylamine. As DETA concentration was varied from 0 to 30 mM, the EOF decreased and reached a certain value instead of being reversed. Probably, DETA could interact with the surface only through hydrogen bonding. Unlike triethylamine, its high polarity prevented the hydrophobic interaction between DETA molecules and the siloxane groups, and its structure made it impossible to form DETA-bilayer on the surface and reverse the EOF. However, the electrophoretic mobilities did not change much, which is similar to the variation with triethylamine. Therefore, it is likely that there is no interaction between the analytes and these cationic additives.

3.2.3 Reproducibility

Run-to-run reproducibility (n = 5) of migration times was determined at pH 3.45 in CE buffer solutions containing 7.5% 2-propanol and 40 mM protonated TEA. The average RSD (Table 3) was 0.5%.

4. Conclusions

Small anionic or cationic buffer additives, such as ESA and protonated TEA, were shown to improve the separation of protonated organic bases. These additives appear to form a thin coating on the capillary surface which modifies the electroosmotic and electrophoretic mobilities. Most likely, the additives reduce or prevent interaction of the sample cations with the capillary surface, thereby giving sharper sample peaks. Excellent reproducibility (< 1%

RSD) of migration times was obtained.

A mixture of ten substituted anilines was separated with near baseline resolution. The compounds separated included several positional isomers as well as isomers with primary, secondary and tertiary butyl groups.

Acknowledgements

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CHAPTER 5. SEPARATION OF ORGANIC AND INORGANIC ANIONS BY ION CHROMATOGRAPHY - CAPILLARY ELECTROPHORESIS

A paper submitted to *Analytical Chemistry*

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Ames, Iowa 50011, U. S. A.

Abstract

Capillary electrophoresis with a water-soluble ion-exchange polymer in the background electrolyte is very efficient for the separation of organic and inorganic anions because the ion-exchange selectivity, as well as differences in electrophoretic mobility, can be used for separating sample ions. Poly(diallyldimethylammonium chloride) (PDDAC) was employed for this purpose. A very stable electroosmotic flow was obtained between pH 2.3 - 8.5 due to the strong adsorption of PDDAC onto the capillary wall. The effect of ion exchange on the migration of sample anions and their separation was controlled by varying the concentration of PDDAC, the concentration and the type of salt used in the CE background

electrolyte (BGE). Addition of organic solvent (e.g., acetonitrile) could also modify the sample migration and the separation. Baseline separations were obtained for anions with very similar mobilities, such as bromide and iodide, naphthalenesulfonates, and bi- and tri-carboxylic acids. Typical separation efficiencies were between 195,000 and 429,000 theoretical plates per meter. Ten replicate separations gave an average RSD of 1.0% for migration times of the sample anions studied. Excellent separations were obtained for a variety of samples, including a separation of 17 inorganic and organic anions in less than 6 min.

1. Introduction

Since its introduction in the late 1970s, ion chromatography (IC) has become the dominant analytical method for separating and determining inorganic anions and small organic anions[1-8]. Separations in IC are based on differences in the affinity of sample anions for the exchange sites on a solid anion exchange material in the separation column. Movement of the sample anions along the column is caused by pumping a mobile phase containing a competing anion through the column. Samples containing several anions can usually be resolved satisfactorily by IC.

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

to modify the mobilities of anions and thereby improve their separation. These include cationic surfactants[10,16-18], polyvalent metal cations[19,20] and various cyclodextrins[21].

The method now proposed, IC-CE, combines two mechanisms for separation of anions. The separation is carried out in a fused silica capillary using standard CE instrumentation. However, a water-soluble anion-exchange polymer is added to the BGE to impart an ion exchange component to the separation. Thus, ordinary CE is based only on differences in sample ion mobility while IC is based on differences in affinity of sample anion and eluent anion (the BGE anion) for the ion-exchange sites. The migration orders of the two mechanisms may be different. For example, the CE migration order for halogens is I^-, Br^- > Cl^- > F^- whereas the IC migration order is F^- > Cl^- > Br^- > I^-.

A limited amount of work in this area has already been reported. Combining ion exchange with capillary electrophoresis was first proposed by Terabe and Isemura[22-24] for separating organic anions with almost identical mobilities. Cassidy and coworkers also studied the effect of cationic polymers on the CE separation of both inorganic and organic anions using indirect photometric detection[25-27]. Polymers with different chemical structures and molecular weights were compared. Although these publications laid out the basic framework of IC-CE, the effect of BGE salt concentration on the ion-exchange equilibrium was apparently not considered. By varying the anion concentration in the BGE, the interaction of the sample anion with the anion-exchange polymer can be either increased or decreased so that migration times may be kept within a desired range. Contrary to previous expectations, Ding and Fritz[28] reported that practical CE separations are possible in BGE salt solutions as high as 5 M. A moderately high salt concentration (e.g., 100 - 250 mM) in the

BGE was shown to significantly improve peak sharpness, apparently by providing conditions favorable for electrostacking.

The present work describes the separation of both inorganic and organic anions using a cationic polymer, poly(diallyldimethylammonium chloride) (PDDAC, or simply as P^+), and an added salt at moderately high concentration. The system used provides a valuable new parameter for achieving rapid, practical separations of anions including those of similar chemical structures.

2. Experimental section

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

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

All standard and electrolyte solutions were prepared with 18.2 MΩ deionized water from a Barnstead Nanopure II system (Sybron Barnstead, Boston, MA). Stock analyte solutions were made with deionized water at 5,000 ppm, and diluted in 10% buffer solutions to desired concentration prior to injection. Electrolyte solutions were prepared by mixing lithium sulfate, PDDAC, 20 mM boric acid, and acetonitrile when needed, and adjusting pH with 2 M sodium hydroxide or 1 M hydrochloric acid. All pH values were measured with a Corning 440 pH meter (Corning, NY) calibrated immediately prior to use.

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

3. Theory

Terabe and Isemura[23] derived an equation for the difference in velocity of two ions (Δv) when a soluble polymeric anion exchanger is added to the BGE.

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

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

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

$$P^+Cl^- + A^- \rightleftharpoons P^+A^- + Cl^- \quad (2)$$

for which the equilibrium constant (K) is:

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

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

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

Combining equation (3) and (4), and rearranging:

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

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

4. Results and Discussion

4.1. Choice of polymer. Preliminary experiments were performed with each of several polymers added to the BGE at a pH value of 8.5. A relatively high concentration (120 - 150 mM) of a salt, such as sodium chloride or lithium sulfate, was found to markedly improve the sharpness of sample anion peaks. Poly(diallyldimethylammonium chloride), abbreviated as PDDAC, at a concentration of 0.05% or 0.3% was the most satisfactory of the polymers tested. Its structural formula is given in Figure 1. A mixture of bromide, iodide, nitrate, nitrite, chromate, thiocyanate and molybdate was baseline resolved in a BGE solution containing 150 mM lithium sulfate, 20 mM borate and 0.05% PDDAC at pH 8.5 (Figure 2). In the absence of PDDAC it is not possible to separate bromide and iodide because their electrophoretic mobilities are almost identical.

The other polymers tested did not perform as well as PDDAC. Polyethyleneimine (PEI), polyacrylamide and polyvinylpyrrolidone provided incomplete resolution of the seven

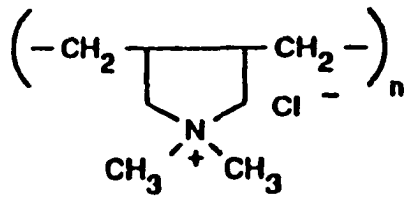

Figure 1. Structure of PDDAC. Molecular weight: low: 100,000 - 200,000; medium: 200,000 - 350,000; high: 400,000 -500,000.

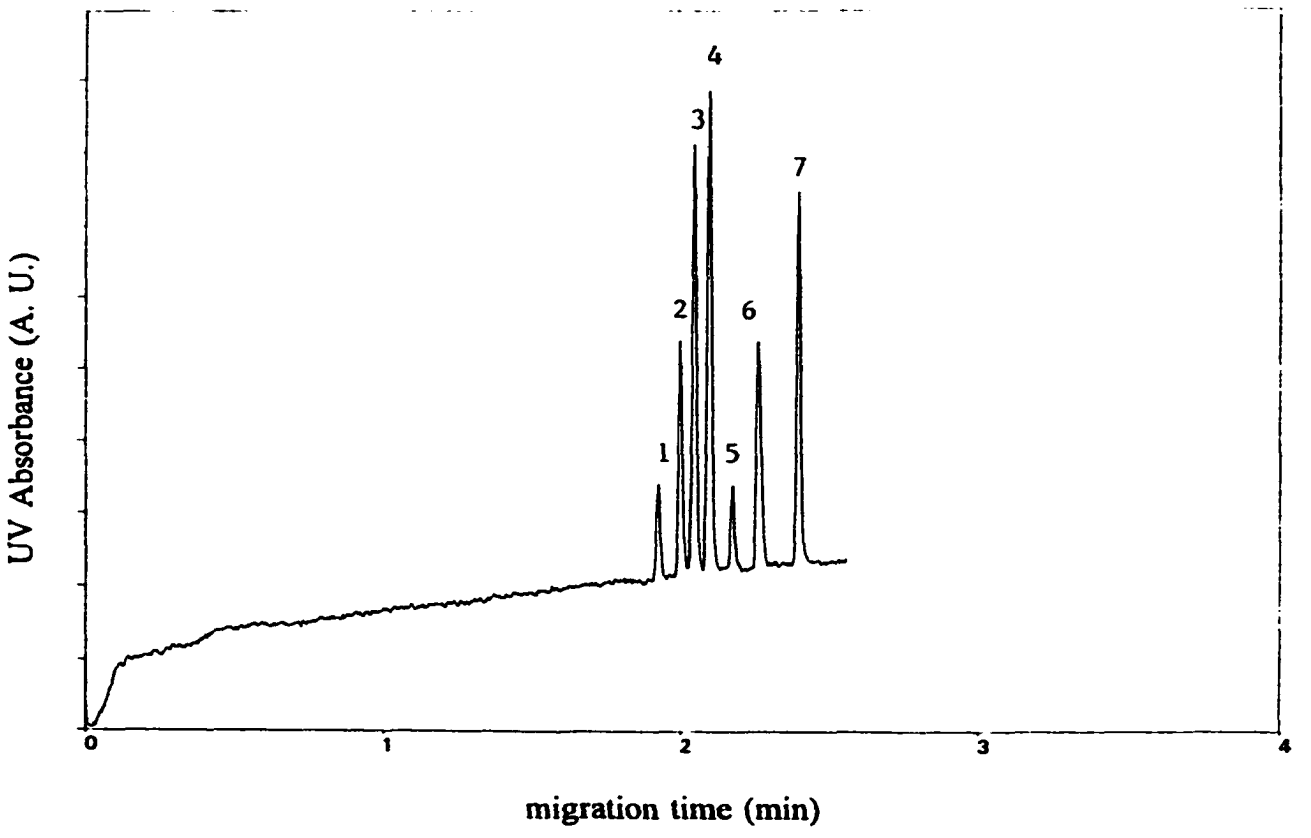

Figure 2. Separation of inorganic anions. Electrolyte: 150 mM Li_2SO_4, 20 mM borate, 0.05% PDDAC, pH 8.5; other conditions as specified in experimental section. Peaks: 1 = Br^-; 2 = I^-; 3 = NO_2^-; 4 = NO_3^-; 5 = CrO_4^{2-}; 6 = SCN^-; 7 = MoO_4^{2-}.

Table 1. Effect of electrolyte pH on the reversed EOF and migration time of inorganic anions. Electrolyte solution contains 150 mM Li_2SO_4, 0.05% PDDAC and 20 mM borate or acetate or hydrochloric acid for desired pH. EOF marker: water. Other conditions as described in experimental section.

pH	EOF ($cm^2/V \cdot s$)	Migration Time (min)			
		Br^-	I^-	NO_3^-	SCN^-
2.3	-2.46×10^{-4}	1.98	2.06	2.17	2.34
5.0	-2.65×10^{-4}	1.95	2.03	2.13	2.30
8.5	-2.74×10^{-4}	1.93	2.00	2.10	2.26

inorganic test anions. The latter two polymers also gave a rather high background absorbance at the wavelength used.

4.2 Effect of BGE pH. Cationic polymers are known to adsorb strongly onto the capillary wall so that electroosmotic flow (EOF) can be reversed from positive to negative[25]. For the present work, the EOF reversal was also observed after the addition of PDDAC in the BGE. To check the stability of PDDAC adsorption on the silica surface, the effect of pH on EOF was studied with PDDAC concentration controlled at 0.05%, and the results were summarized in Table 1. As pH was increased, there was only very minor change in EOF, indicating a strong and stable adsorption of PDDAC. A direct result of this strong adsorption is the residual free silanol groups are insignificant in affecting EOF.

As the pH increases, sample anions showed a slight decrease in migration times due to the small increase of EOF. Further work was all performed at pH 8.5 because anions

from both strong and weak acids can be analyzed under alkaline conditions.

4.3 Effect of PDDAC concentration. The effect of PDDAC concentration on both EOF and electrophoretic mobility of 9 sample anions is reported in Figure 3. In the range covered, 0.1% to 1.0% PDDAC, the EOF was virtually unchanged. This indicates either that the capillary surface is more or less completely covered by the polymer even at the lowest concentration, or that any increase in zeta potential was compensated by increased viscosity as the bulk PDDAC concentration was increased.

Equations 2 and 3 predict that increasing concentration of PDDAC (abbreviated as P^+) will result in a higher fraction of a sample anion (A^-) being associated with the ion-exchange polymer. This in turn will result in a slower rate of migration for the sample anion (equation 5). The electrophoretic mobilities of the sample anions in Figure 3 do become smaller as the concentration of PDDAC is increased. The decrease in mobility of sample anions 6 - 9 is much greater than that of anions 1 - 5. The naphthalenesulfonates (6 and 7) are more bulky than anions 1 - 5 and would therefore interact more strongly with the anion-exchange polymer. Anions 8 and 9 would also interact strongly with the polymer by the virtue of their higher charges (2- or 3-) at pH 8.5.

The data in Figure 3 predict that resolution of this mixture of anions would be very poor without the ion-exchange effect of the PDDAC. Separation of 1- and 2-naphthalenesulfonic acids is, for example, not possible by CE alone. Complete resolution of all 9 anions except for p-hydroxybenzoic acid and p-aminobenzoic acid in a solution containing 0.3% PDDAC is shown in Figure 4A. Baseline resolution of all 9 anions was obtained with 1.0% PDDAC, as shown in Figure 4B, although the separation takes longer

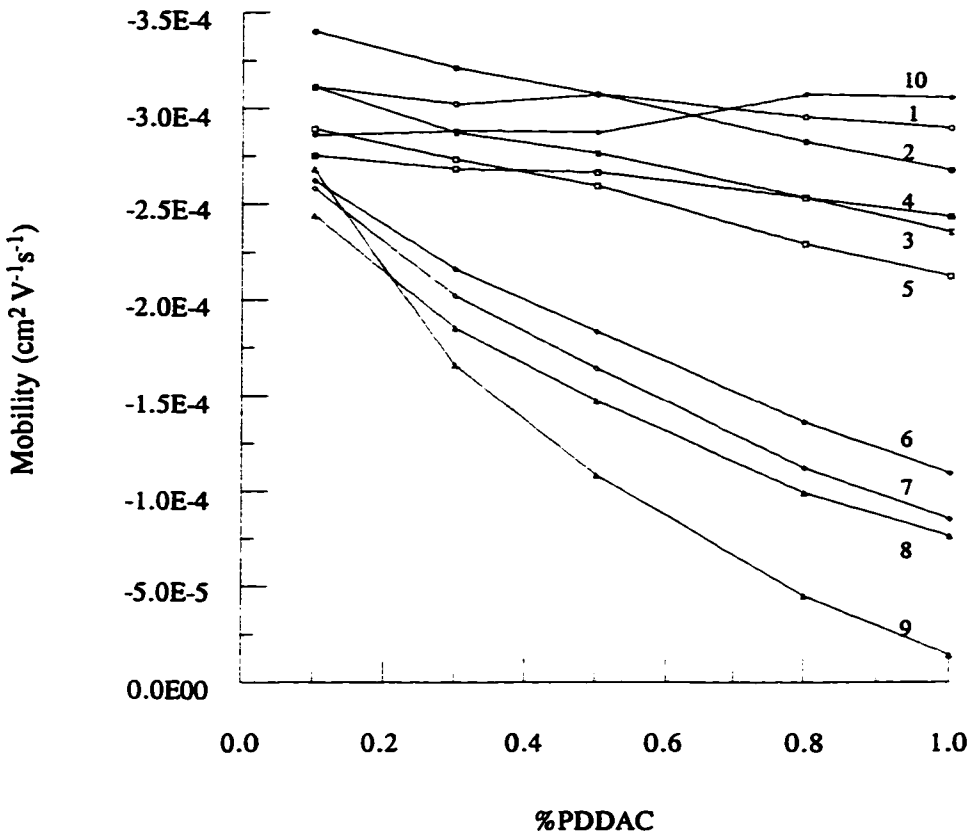

Figure 3. Effect of PDDAC concentration on EOF and electrophoretic mobilities of organic anions. Electrolyte contains 150 mM Li_2SO_4, 20 mM borate and PDDAC at pH 8.5. Other conditions as specified in experimental section. Samples: 1 = benzoate; 2= benzenesulfonate; 3 = p-toluenesulfonate; 4 = p-aminobenzoate; 5 = p-hydroxybenzoate; 6 = 2-naphthalenesulfonate; 7 = 1-naphthalenesulfonate; 8 = 3,5-dihydroxybenzoate; 9 = 2,4-dihydroxybenzoate; 10 = water for EOF.

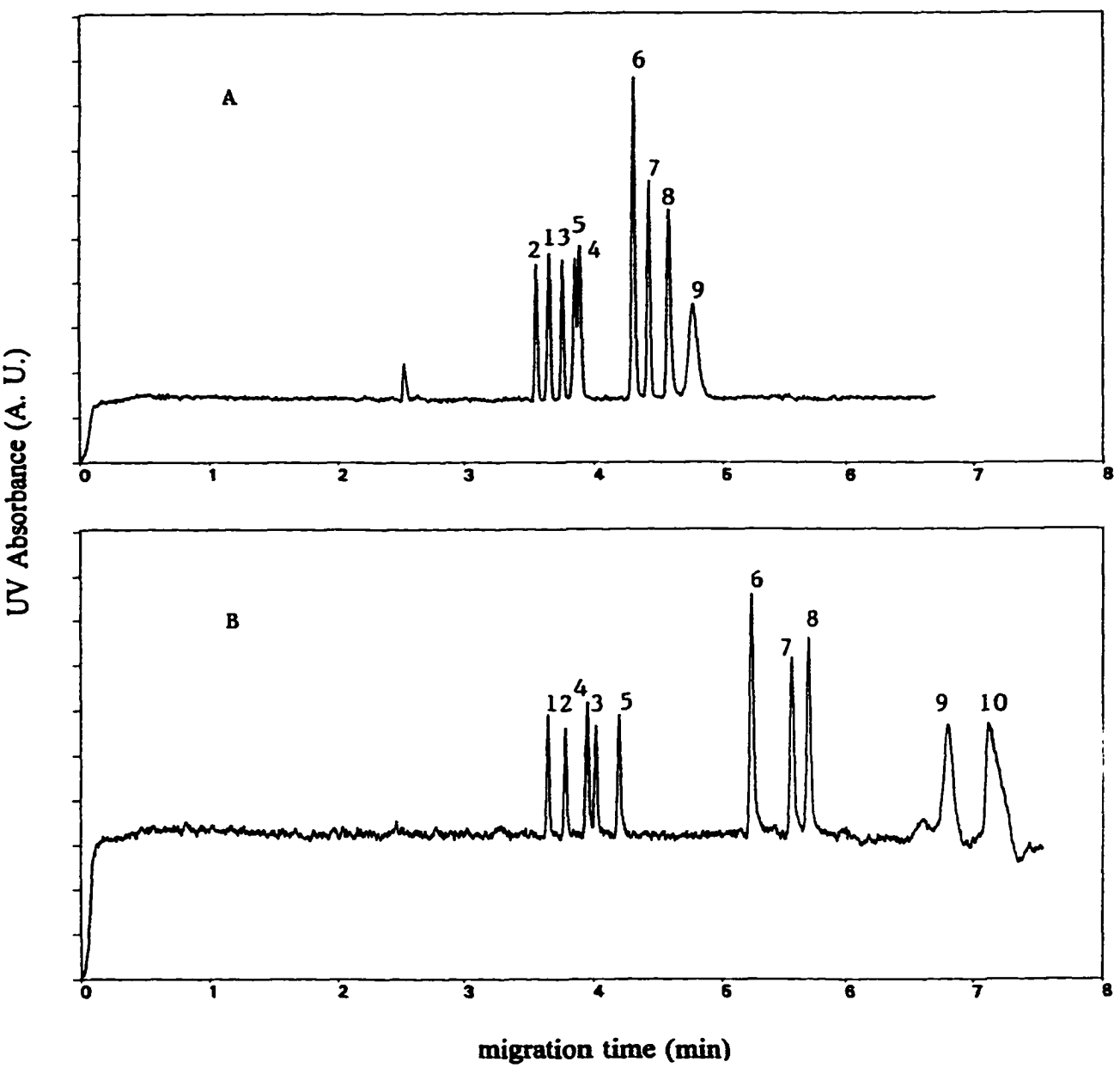

Figure 4. Separation of nine organic anions. Electrolyte contains 150 mM Li_2SO_4, 20 mM borate, 0.3% (A) or 1.0% (B) PDDAC at pH 8.5; other conditions as specified in Experimental. Peak numbers same as Figure 3.

and the baseline is noisier. Comparison of Figures 4A and 4B shows several changes in elution order as predicted by crossovers for anions 1 and 2, and 3, 4 and 5 in Figure 3.

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

4.5 Effect of added salt. Increasing concentrations of a salt added to the BGE will decrease the ion-exchange effect and cause sample anions to migrate more rapidly (equation 5). This is demonstrated in Figure 5 where higher NaCl concentrations are shown to repress ion-exchange interactions and lead to more rapid migration rates. The change in electrophoretic mobility is more pronounced for iodide, which undergoes a stronger ion exchange effect than bromide. Similar effects were noted for other salts added to the BGE.

The effect of salt concentration was also studied by comparing separation of several inorganic anions in 50, 100 and 150 mM lithium sulfate in the BGE. While the EOF was virtually unchanged at different lithium sulfate concentrations, the best resolution was

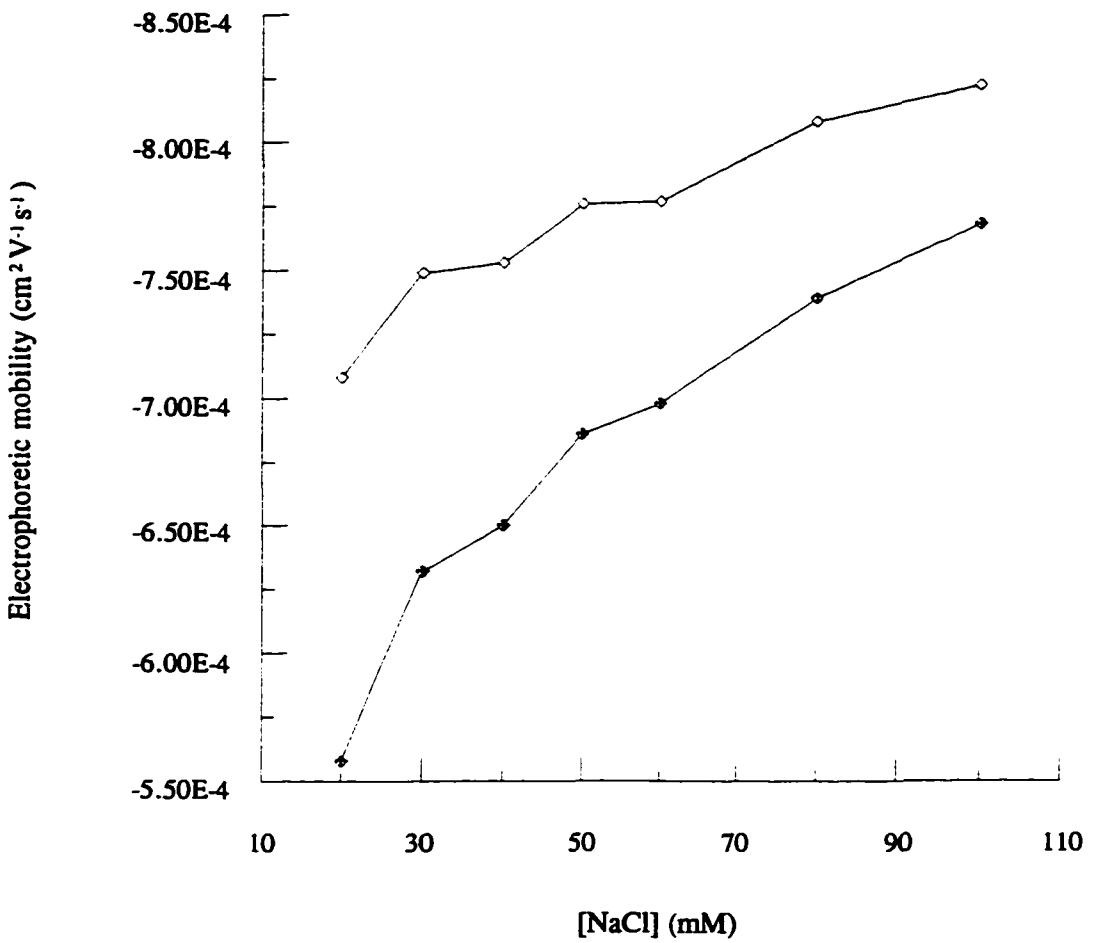

Figure 5. Effect of NaCl concentration on EOF and electrophoretic mobilities of bromide and iodide. Electrolyte contains NaCl, 20 mM borate, 0.05% PDDAC at pH 9.0; other conditions as specified in experimental section. EOF marker: D. I. H_2O. Symbols: ◇ = Br^-; ◆ = I^-.

Table 2. Comparison of plate number (N) for inorganic anions at different salt concentrations. Electrolyte: Li_2SO_4, 20 mM borate, 0.05% PADDC, pH 8.5. $N = 5.54(t_r/w_{1/2})^2$.

Concentration of Li_2SO_4 (mM)	Plate Number (N)			
	Br^-	CrO_4^{2-}	SCN^-	MoO_4^{2-}
50	80,000	69,000	49,000	60,000
100	107,000	143,000	71,000	138,000
150	100,500	153,000	86,000	152,000

obtained at 150 mM concentration (Figure 2). In 50 mM lithium sulfate, the sample anions migrate more slowly (2.2 to 2.7 min compared to ca. 1.9 to 2.4 min in 150 mM) but resolution of nitrite, iodide and nitrate was very poor. Increased salt concentration also favored the separation efficiency. Theoretical plate numbers for several of the sample anions on a 40-cm capillary in 50 and 150 mM lithium sulfate were compared in Table 2, and larger plate numbers were ontained with higher salt concentration. More peak focusing through electrostacking is a likely reason for the better peak efficiency at the higher salt concentrations.

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

PDDAC than the same concentration of sulfate. The migration times of bromide and iodide in 150 mM lithium sulfate are 5.74 min and 6.88 min, respectively (α = 1.20). In 150 mM sodium acetate the migration times are 6.08 min for bromide and 8.77 min for iodide (α = 1.44) (other conditions: 0.3% PDDAC, pH 9.0, injection-to-detection length of 52.5 cm). The stronger ion-exchange interactions of bromide and iodide in sodium acetate lead to longer migration times and a larger separation factor for bromide and iodide.

The counter ion of the BGE salt, as well as the particular anion, can also affect sample ion migration in IC-CE. Examples are shown in Figure 6 and 7. Migration of anions 6 - 9 is slower in chloride than in sulfate due to stronger ion-exchange interactions. However, a comparison of 6a with 7a, and 6b with 7b also shows longer migration times in the lithium salt than the sodium salt of the same anion. It is known that lithium salts can form weak complexes or ion-pairs with carboxylates. This would tend to reduce further the fraction of sample analytes present as the free anions.

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

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

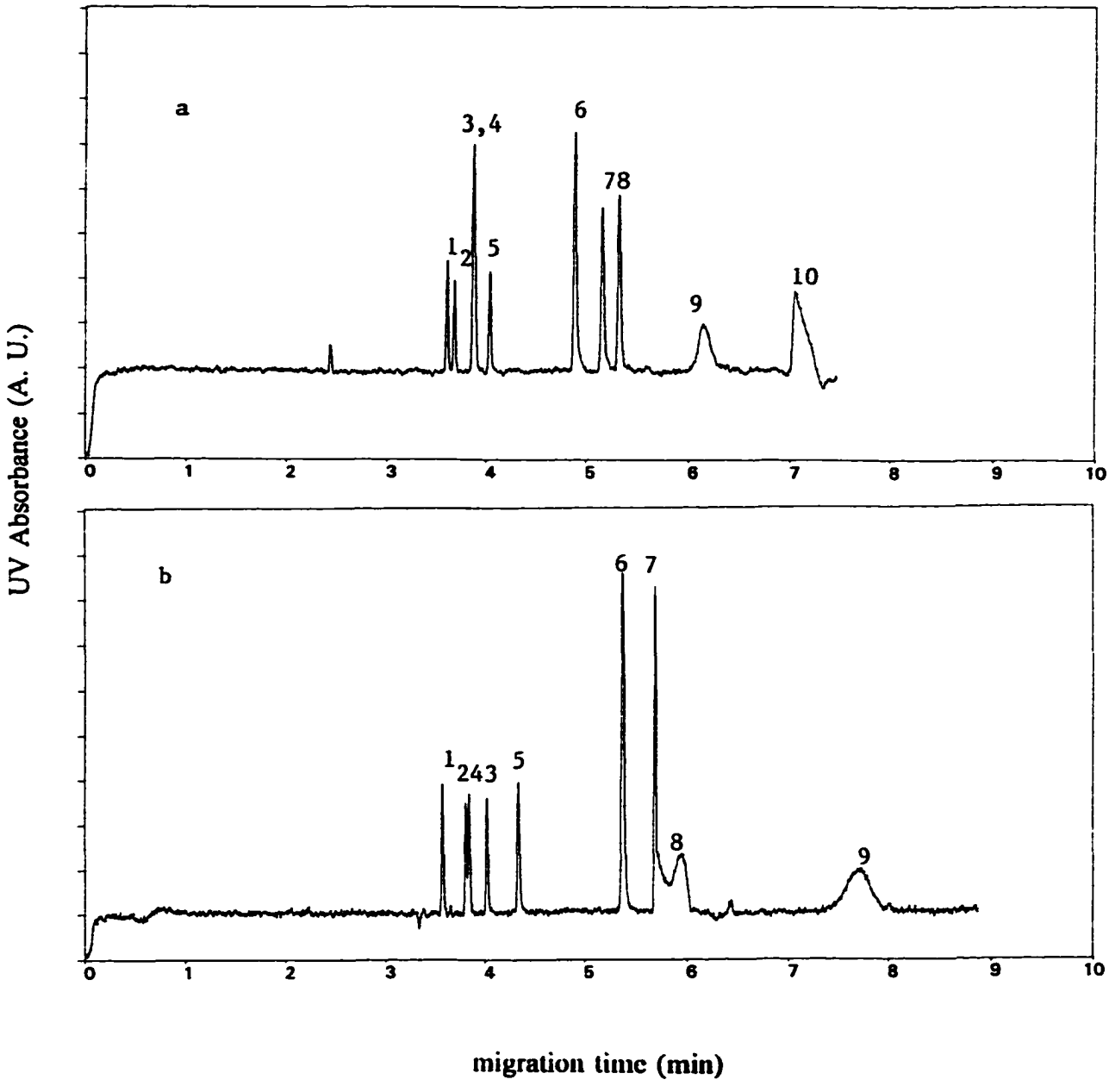

Figure 6. Separation of nine organic anions with lithium salt. Besides 20 mM borate and 0.8% PDDAC, electrolyte also contains Li_2SO_4 (a) or LiCl (b) at 150 mM and pH 8.5; other conditions as specified in Experimental. Peak numbers same as Figure 3.

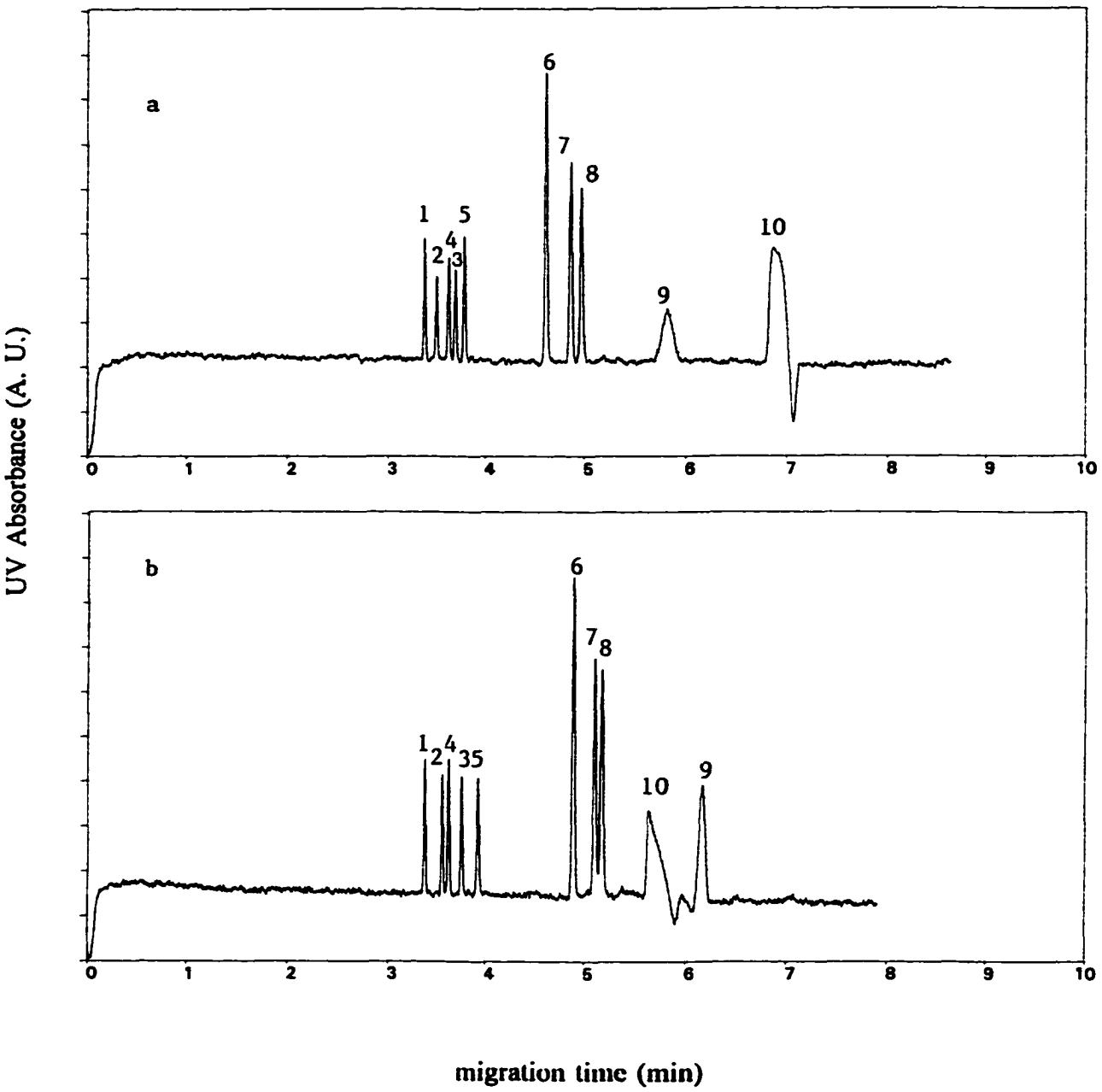

Figure 7. Separation of nine organic anions with sodium salt. Conditions same as Figure 6 except that Na_2SO_4 (a) or NaCl (b) replaced lithium salt in the BGE. Peak numbers same as Figure 3.

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

Figure 8 shows the effects of adding acetonitrile to the BGE at concentrations of 15% and 30%. Concentrations much above 30% caused PDDAC to partially separate from the liquid phase. Some reduction in the EOF is generally observed in CE when acetonitrile is added to an aqueous electrolyte, but the sharper reduction between 15% and 30% acetonitrile is likely the result of a decreased adsorption layer of PDDAC on the capillary surface.

The effect of acetonitrile on the electrophoretic mobility is rather small for anions 1 - 5 in Figure 8. However, anions 6 and 7, which contains bulkier naphthalene groups rather than phenyl groups, have significantly higher electrophoretic mobilities in the solutions containing acetonitrile. This would be the result of decreased ion-exchange and/or hydrophobic interactions with PDDAC. The ability to resolve the peaks of these two anions has in fact been destroyed by the incorporation of 30% acetonitrile in the BGE.

Addition of some acetonitrile to the BGE in IC-CE can be beneficial in some cases. For example, 3- and 4-hydroxycinnamic acids could not be separated in aqueous solution, whereas addition of 7.5% acetonitrile to the BGE gave an excellent separation with

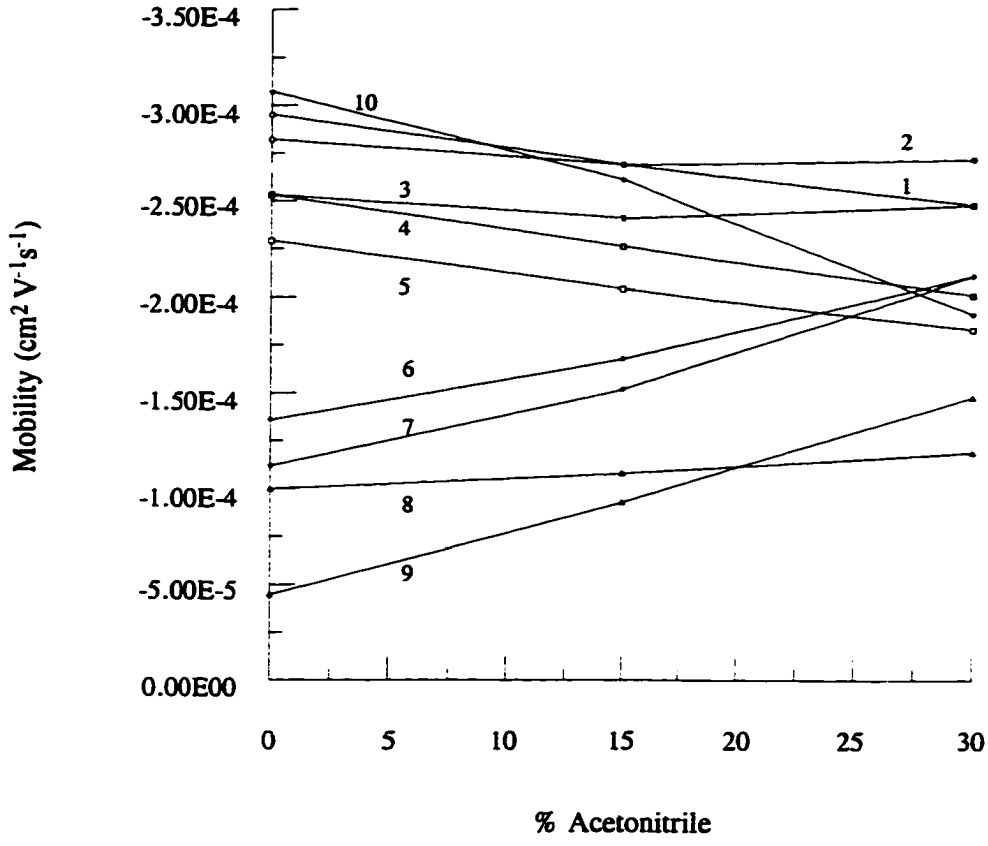

Figure 8. Effect of acetonitrile concentration on EOF and electrophoretic mobilities of organic anions. Electrolyte contains 150 mM Li_2SO_4, 20 mM borate, 0.8% PDDAC and acetonitrile at pH 8.5; other conditions as specified in Experimental. Sample numbers same as Figure 3.

resolution of approximately 10.

4.7 Scope and reproducibility of IC-CE separations. Inorganic anions tend to have significantly shorter migration times than the larger aromatic carboxylate and sulfonate anions. Mixtures of both of these general types can be separated in a single run by IC-CE, as shown in Figure 9. All 17 peaks were well resolved and, with the exception of 2,4-dihydroxybenzoate, the peaks were very sharp and narrow. Data from 10 consecutive runs gave actual plate numbers ranging from 63,000 to 140,000. The average plates per meter for the anions in this table ranged from 195,000 for iodide to 429,000 for phthalate, with an overall average of 292,000 (Table 3). The reproducibility of migratin time for each and every peak was excellent with a RSD of 1.0% or 1.1%. The RSD of peak areas had an overall average of 5.5%. However, no special precautions were taken to ensure that a precise amount of sample was introduced each time.

Several additional sample mixtures of organic anions were separated under a variety of conditions to illustrate the broad scope of IC-CE. As shown in Figure 10, three isomeric phthalic acids were baseline separated in about 3.8 min using an electrolyte solution containing 0.3% PDDAC, 120 mM lithium sulfate and 20 mM borate at pH 8.5; and a separation of three isomeric benzenetricarboxylic acids was also obtained under the same conditions, with 1,2,3-tricarboxylic acid eluted at 3.47 min followed by 1,2,4- at 3.81 min and 1,3,5- at 4.36 min. A mixture of six different cinnamic acids, two pairs of which are positional isomers, was completely resolved provided 7.5% acetonitrile was added to the BGE that contained 0.8% PDDAC and 150 mM lithium sulfate at pH 8.5 (Figure 11). Separations of valine and norvaline, and of leucine and norleucine are considered to be

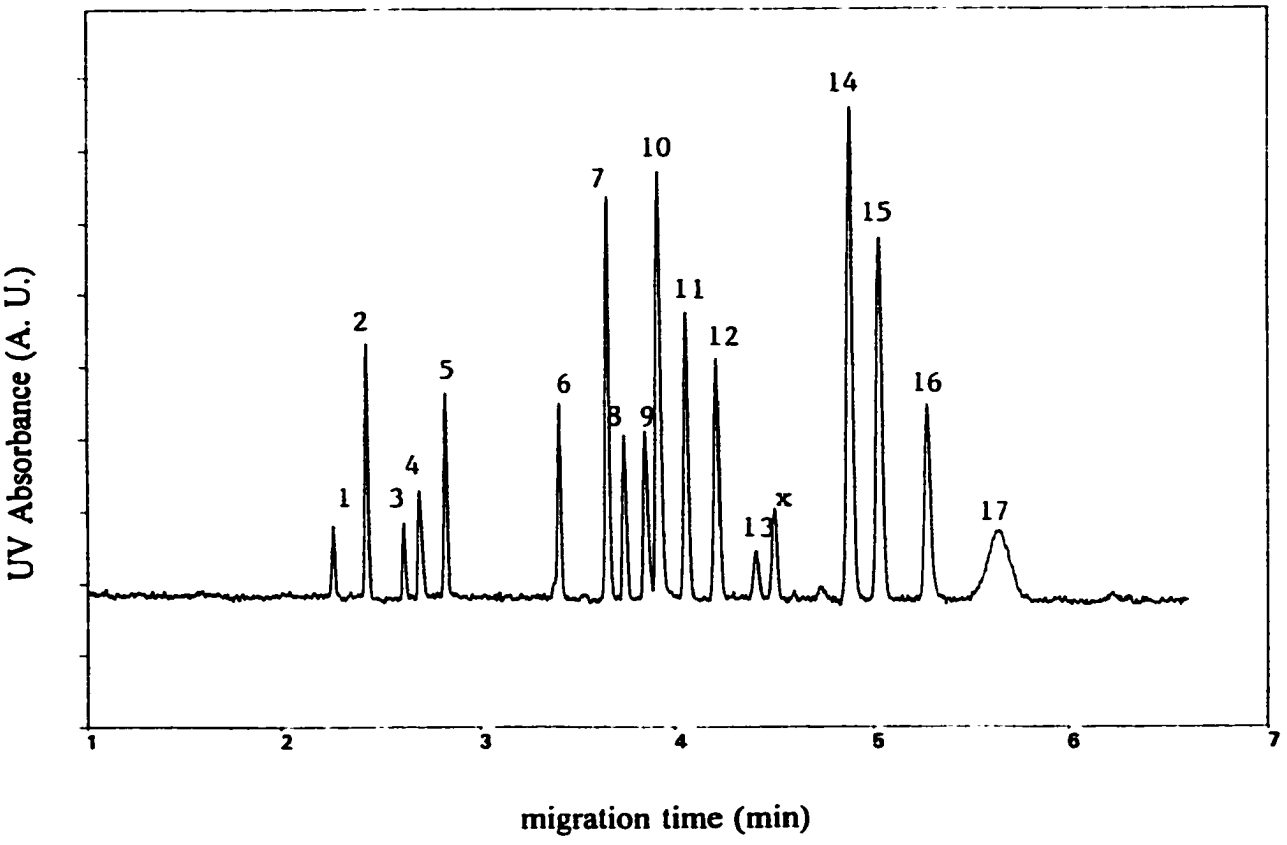

Figure 9. Separation of 17 inorganic and organic anions. Electrolyte: 120 mM Li_2SO_4, 20 mM borate, 0.3% PDDAC, pH 8.5; other conditions as specified in Experimental. Peaks: 1 = bromide; 2 = nitrate; 3 = chromate; 4= iodide; 5 = molybdate; 6 = phthalate; 7 = 1,2,3-tricarboxylate; 8 = 1,2-benzenedisulfonate; 9 = terephthalate; 10 = isophthalate; 11 = benzoate; 12 = p-toluenesulfonate; 13 = 1,3,5-tricarboxylate; 14 = 2-naphthalenesulfonate; 15 = 1-naphthalenesulfonate; 16 = 3,5-dihydroxybenzoate; 17 = 2,4-dihydroxybenzoate; x = unidentified impurity.

Table 3. Reproducibility study for inorganic and organic anions. Conditions same as Figure 9. Data from 10 consecutive injections (n = 10) were for calculation of RSD.

anion	plate number per meter (N)	RSD (%)		
		migration time	peak area	peak height
bromide	263,000	1.1	9.6	5.1
nitrate	276,000	1.1	4.3	7.4
iodide	194,500	1.1	3.1	4.7
benzoate	362,400	1.1	6.2	8.7
p-toluenesulfonate	331,700	1.0	6.2	9.1
phthalate	429,400	1.0	7.2	10.4
terephthalate	313,000	1.1	6.2	6.1
isophthalate	278,200	1.0	3.2	10.7
1,2-benzenedisulfonate	363,000	1.0	5.0	7.8
1,2,3-tricarboxylate	408,000	1.1	4.6	10.9
1-naphthalenesulfonate	333,700	1.1	6.2	7.6
2-naphthalenesulfonate	357,500	1.1	3.7	7.6

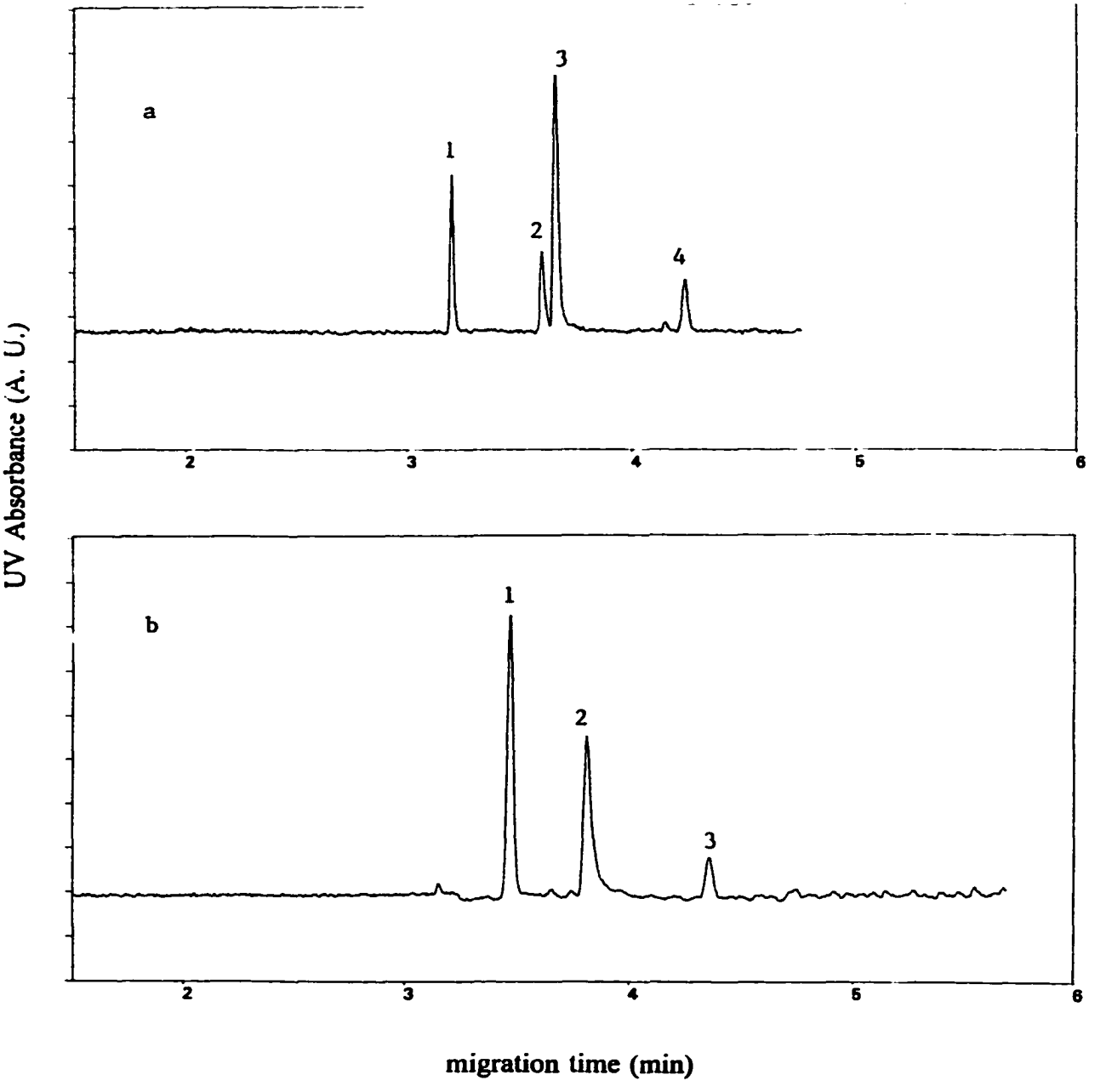

Figure 10. Separation of bi- and tri- carboxylic acids. Same conditions as Figure 9. Peaks: (a) 1 = phthalic acid; 2 = terephthalic acid; 3 = isophthalic acid; 4 = unidentified impurity; (b) 1 = 1,2,3-tricarboxylic acid; 2 = 1,2,4-tricarboxylic acid; 3 = 1,3,5-tricarboxylic acid.

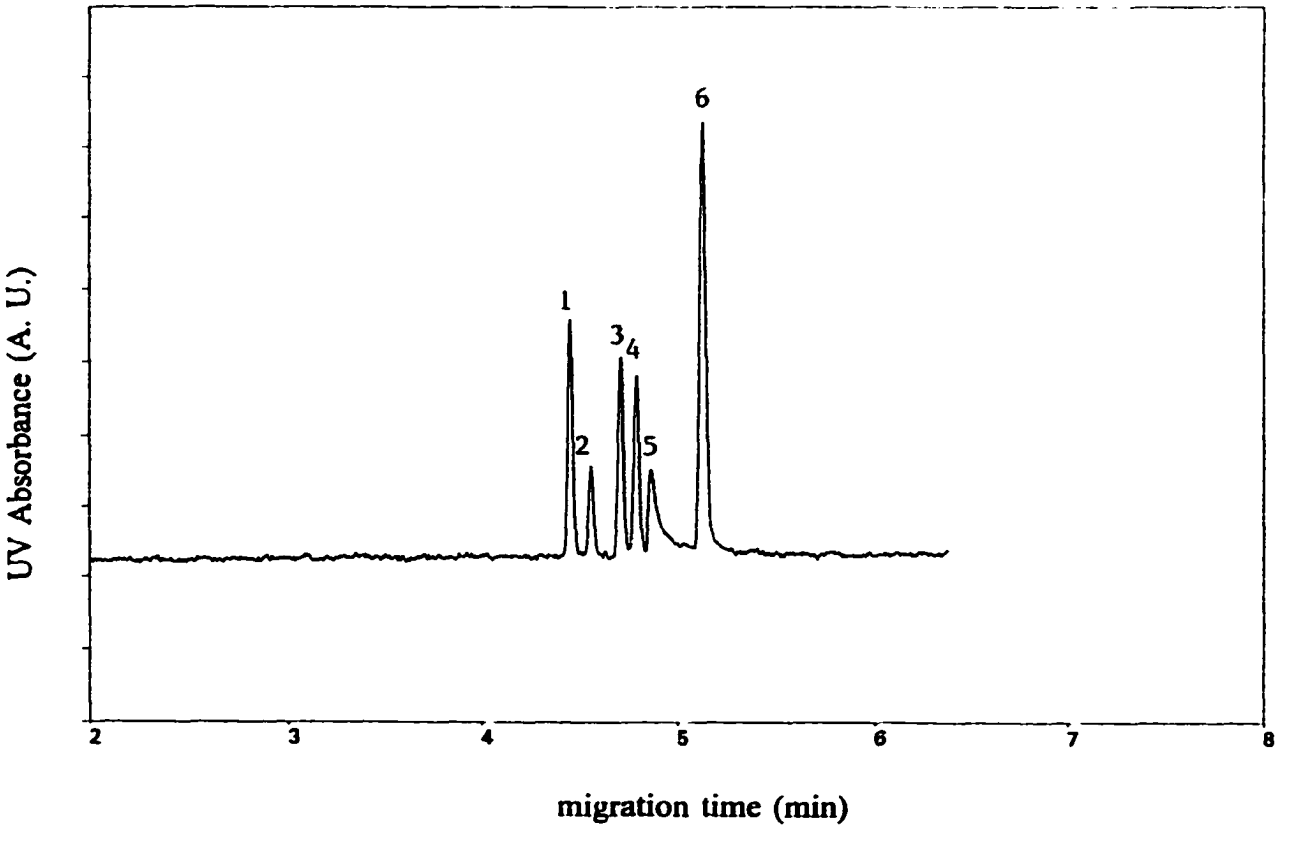

Figure 11. Separation of cinnamic acids. Electrolyte contains 150 mM Li_2SO_4, 20 mM borate, 0.8% PDDAC and 7.5% ACN at pH 8.5; other conditions as specified in Experimental. Peaks: 1 = trans-cinnamic acid; 2 = 4-hydroxycinnamic acid; 3 = 3-methoxycinnamic acid; 4 = 2-methoxycinnamic acid; 5 = 3, 4-dihydroxycinnamic acid; 6 = 3-hydroxycinnamic acid.

difficult, yet these were completely resolved within a few minutes by IC-CE using a 33-cm capillary and 0.3% PDDAC in the BGE. A mixture of six nucleotides, i.e., UMP, CMP, AMP, ADP, GMP and ATP, was also separated successfully with 0.3% PDDAC on a 60-cm capillary. In general, the resolution of these compounds of biological interest requires careful choice of electrolyte pH and added salt. For instance, while valine and norvaline were best separated with 80 mM potassium fluoride at pH 9.53, and leucine and norleucine were near baseline separated with 60 mM lithium sulfate at the same pH, the nucleotides were resolved at pH 9.0 using 80 mM lithium sulfate.

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

5. Conclusions

IC-CE combines two methods of separation in a single technique. Electrophoretic migration of sample ions toward the detector in addition to EOF in the same direction combine to give reasonably fast migration times for both inorganic and organic anions. Ion exchange interactions between sample anions and the positively charged polymer slow down the analyte migrations to varying degrees and enhances our ability to separate complex mixtures. A high salt concentration in the BGE decreases the ion exchange effect while a higher concentration of polymer strengthens the ion exchange effect. The relatively high salt concentrations used in this work sharpen sample peaks by electrostacking and also appear to improve reproducibility by reducing sample ion interactions with the silica surface of the

capillary.

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

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CHAPTER 6. GENERAL CONCLUSIONS

A novel polymeric resin with triamine functional groups was prepared and demonstrated to be an efficient material for ion-chromatographic separation of inorganic anions. A unique feature of this resin is that retention times of sample anions can be varied widely simply by changing the pH of the mobile phase. In acidic solution, 2- anions are much more strongly retained than anions with 1- charge. Common anions in tap water as well as anionic chloro-metal complexes are well separated on the column packed with this new anion-exchange resin. This resin is also an effective hydrophilic column packing material for separation of phenols and alkylbenzenes by HPLC with an aqueous - acetonitrile mobile phase.

The ability to work in predominantly nonaqueous solutions adds a valuable new dimension to our technology for separation of neutral compounds by capillary electrophoresis. Nonaqueous CE with methanol as separation medium has been successfully applied for separation organic compounds as well as acidic and basic drugs. These separation are achieved through the addition of anionic surfactants to the CE electrolytes so that nonionic compounds can obtain different apparent mobilities by interacting with the surfactants; larger molecules interact more strongly with the surfactants than smaller molecules, leading to greater mobility and faster migration of these large compounds, such as benzo[a]perylene and perylene. Methanol does not provide a wide elution window, but addition of a low percentage of water into methanol can largely overcome this limitation. Compared with pure solvent, solvent mixtures have different properties, such as dielectric constant and viscosity. In methanol-water mixtures, resolutions are affected by the solvophobic interactions between the

analytes and the surfactant and by the ratio of dielectric constant over viscosity, which has an impact on analyte mobility and electroosmotic flow.

Polymers and surfactants are commonly used as buffer additives to improve CE separations of basic compounds. They often form thick coatings on silica capillary surface, and the coating could gradually build up from run to run. Small ionic additives, such as ethanesulfonic acid (ESA) and protonated triethylamine (TEA), are shown to improve the separation of protonated organic bases. These additives appears to form a thin coating on the capillary surface which modifies the electroosmotic and electrophoretic mobilities. For example, EOF is decreased by ESA, and addition of TEA to the BGE can even reverse the direction of EOF. Most likely, these additives reduce or prevent interaction of the sample cations with the capillary surface, thus giving sharper sample peaks. Separation of substituted anilines, including several positional isomers as well as isomers with primary, secondary and tertiary butyl groups, were obtained with ESA and TEA as additives.

Ion chromatography - capillary electrophoresis (IC-CE) combines two methods of separation in a single technique. Addition of an water soluble anion-exchange polymer to CE electrolyte has dual advantages. The cationic polymer can adsorb onto the capillary surface to produce a reversed EOF, and fast analyses for both inorganic and organic anions are achievable due to the same direction of EOF and electrophoretic migration of sample anions toward the detector. More importantly, ion exchange interactions between sample anions and the positively charged polymer slow down the analyte migrations to varying degrees and enhances the ability to separate complex mixtures. A high salt concentration in the BGE decreases the ion exchange effect while a high concentration of polymer strengthens the ion

exchange effect. The relatively high salt concentrations used in this work sharpen sample peaks by electrostacking and also appears to improve reproducibility by reducing sample ion interactions with the capillary surface. Also, improved reproducibility is possible because adsorption of the polymer onto silica capillary surface provides a well-controlled electroosmotic flow.

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IMAGE EVALUATION
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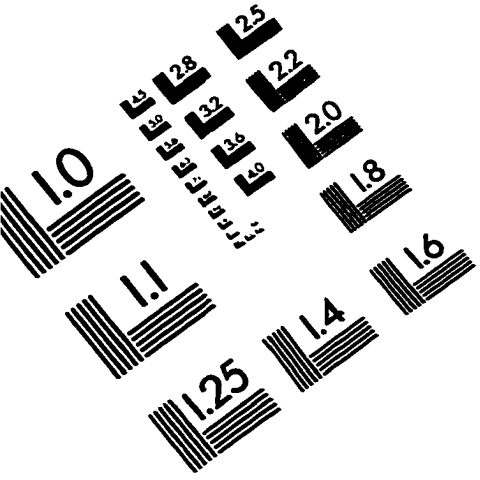

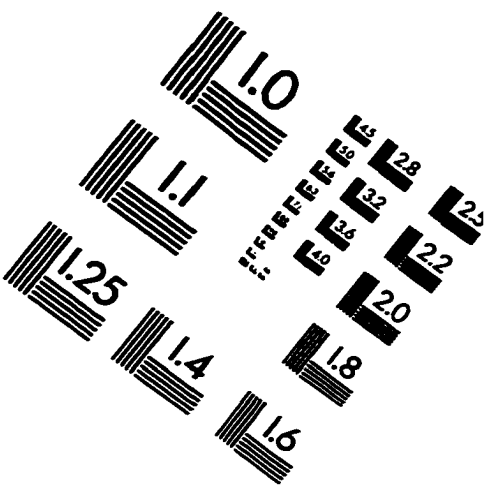

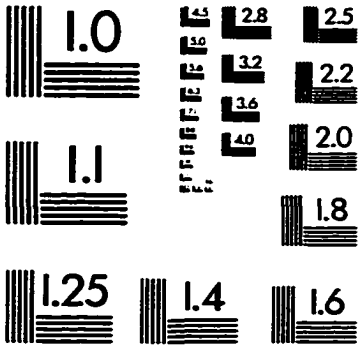

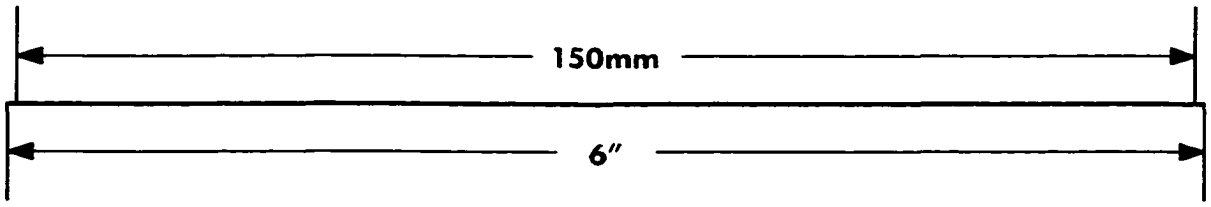

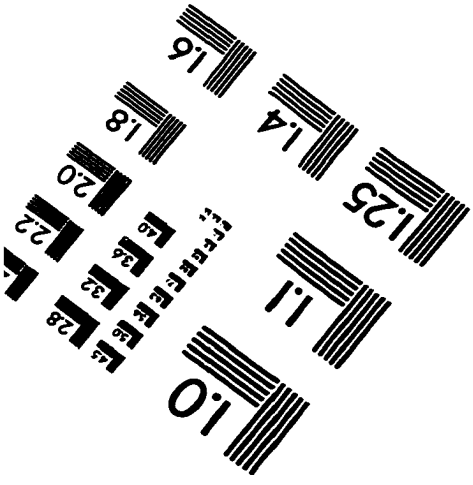

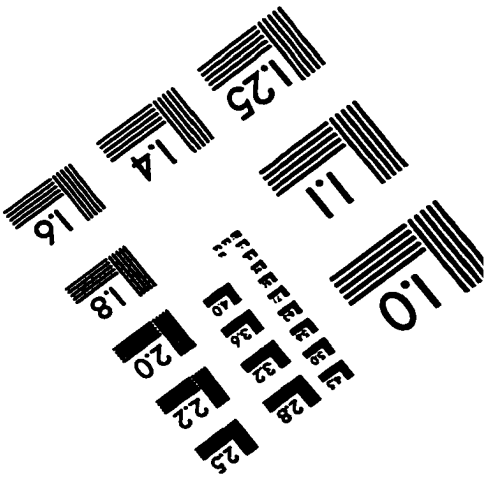

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