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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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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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A new polymeric anion-exchange resin with triamine functional groups was prepared, and its performance for separating common inorganic anions was studied in detail. Small ionic additives, such as ethanesulfonic acid and protonated triethylamine, were proven to be effective in improving the separation of substituted anilines by capillary electrophoresis. Compared with additives of larger molecules, such as surfactants and polymers, these small additives appears to form thin layer of coatings on the silica capillary surface, thus are easier to remove. Nonaqueous capillary electrophoretic method was developed for separation of nonionic organic compounds with methanol as separation medium. Depending on their structure, these neutral compounds can move at different apparent velocities by interacting with anionic surfactants to varying degrees. Their apparent mobilities are also affected by the properties of the separation media, including dielectric constant and viscosity. Fast and efficient separations of both organic and inorganic anions were achieved by Ion Chromatography - Capillary Electrophoresis (IC - CE) with a water soluble ion-exchange polymer added to the CE background electrolyte. This technique combines the separation

mechanisms of IC and CE, and anions with a variety of structures and mobilities can be well resolved. For example, excellent resolution was achieved for positional isomers of phthalates and tri-benzenecarboxylates. And 17 inorganic and organic anions were separated within 6 min, and no peak distortion was observed for any of these sample ions. The applications of polymer and moderately high concentration of added salts in the background electrolytes also provided very good reproducibility for anion analysis (1.0% RSD for migration time).

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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 (ν) 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}):

$$\mu = \mu_{ep} + \mu_{eo} \quad (5)$$

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 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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