# DEVELOPMENT OF HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS METHODS FOR THE SEPARATION AND DETECTION OF SOME POLLUTANTS IN WATER SAMPLES

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## TECHNICAL REPORT

### Statement of the Problem

In the State of Oklahoma as well as in the States of the Southern Plains Region, prior and/or current usage of pesticides and herbicides in agricultural activities and termite extermination are being increasingly recognized as significant sources of ground water pollution. Currently, there are indications that some pesticides and herbicides contamination of drinking water wells does exist. However, the exact nature of these pesticides and their concentrations as well as which wells are contaminated are not known . This may be due to the lack of reliable and sensitive analytical methods.

### Objectives of the Project

The broad objective of the research project entails an integrated approach to the development of rapid, sensitive and efficient capillary electrophoresis methods for the separation of pesticides, herbicides and related compounds. Toward this end the following specific aims were pursued:

(i) Investigation of the potentials of HPCE in the separation of pesticides and herbicides with capillaries having novel hydrophilic coatings over a wide range of conditions.

(ii) Development of capillary preconcentration devices for use in tandem with capillary zone electrophoresis (CZE) in order to provide methods that allow the concentration of dilute samples prior to analysis and separation.

(iii) Introduction of novel buffer systems in order to facilitate the separation of neutral and charged pesticides or herbicides.

### Methodology

Various types of surface chemistry for the modification of the inner surface of fused silica capillaries were examined in order to produce interactive capillaries with hydrophobic or ionic functionalities for the on-line preconcentration of dilute samples prior to separation by capillary electrophoresis. Also, other types of surface treatment were evaluated to produce capillaries with inert coatings for the efficient separation of cationic and anionic species with reduced solute-wall interactions.

### Principal Findings

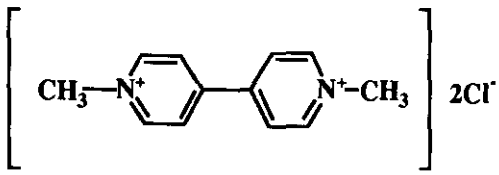
a. Capillary Electrophoresis of Charged Herbicides

Capillary zone electrophoresis with untreated fused-silica capillaries was evaluated in the separation and determination of paraquat and diquat, both are double quaternary ammonium herbicides. As high as 200,000-300,00 theoretical plates per meter were obtained for the separation of the two closely related herbicides over the pH range 3.5-9.5. At relatively high ionic strength in the running electrolyte, electrostatic interactions between the cationic herbicides and the negatively charged surface of the separation capillary were minimized. Under these conditions, the plot of average plate height versus the electroosmotic flow velocity indicated that longitudinal molecular diffusion is the major

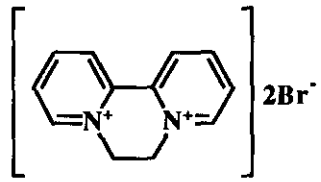
contributor to band broadening. Furthermore, the electrophoretic mobilities of paraquat and diquat were unaffected by the pH of the running electrolyte in the range 3.5-9.5, indicating that solute-wall interaction was lowered by the addition of 0.2 M NaCl to the electrolyte solutions.

The detection limits for paraquat and diquat were 15.4 and 16.8 femtomoles, respectively, with a UV detector. This was favored by the relatively high molar absorptivities of the two herbicides. 2-Aminopyridine was used as an internal standard since it has two absorption bands extending in width over the wavelength region where paraquat and diquat yielded maximum absorbances.

The study has shown the potential of capillary electrophoresis in the determination of minute amounts of charged herbicides [1,2]. Also, the method developed here provided the resolution of two quaternary ammonium ion herbicides having the same charge (i.e., +2) and similar molecular weights (184 for diquat and 186 for paraquat). The two ions are only slightly different in shape, see below.



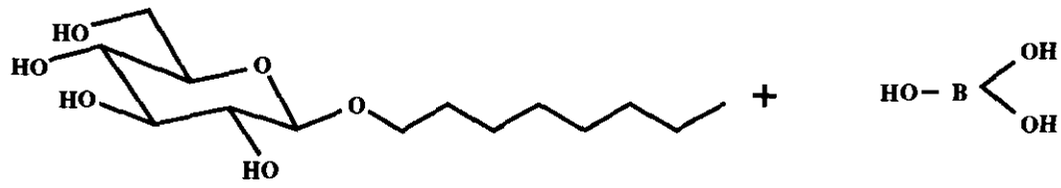

**Paraquat**



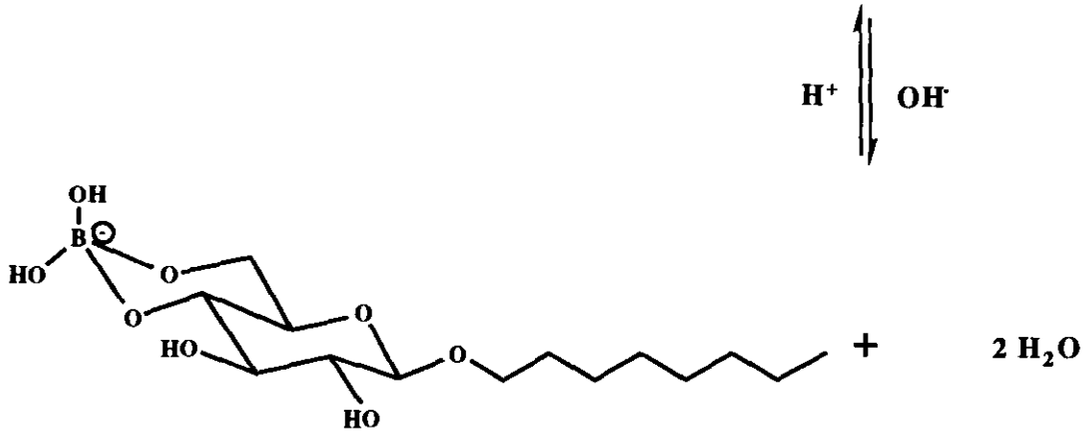

**Diquat**

b. Novel Buffer Systems for the Separation of Neutral Herbicides

Novel micelles with adjustable surface charge density were introduced for micellar electrokinetic capillary chromatography. These micelles are based on the complexation between octylglucoside surfactant and alkaline borate, as shown below [2,3].



**Octylglucoside**



**Octylglucoside-borate complex**

The alkylglucoside-borate complexation is a reversible reaction, and has an equilibrium constant, $K_{eq}$, given by

$$K_{eq} = \frac{[\text{OG-Borate}]}{[\text{OG}][\text{Borate}][OH^-]} \qquad (1)$$

where [OG] and [OG-Borate] stand for the total concentrations of the uncomplexed octylglucoside surfactant and octylglucoside-borate surfactant, respectively, and [Borate] and [$OH^-$] are the borate and hydroxide ions concentrations, respectively. Presumably, it is the negatively charged OG-Borate which migrate in zone electrophoresis. The concentration of OG-Borate in aqueous boric acid is low, and an increase in pH would be expected to raise their concentration and, concomitantly, to result in an increased electrophoretic mobility of the micelle.

As a result of the complexation, the overall surface charge density of the micelle, $\rho_{mc}$, can be expressed as

$$\rho_{mc} = \frac{[\text{OG-Borate}]}{[\text{OG-Borate}] + [\text{OG}]}\ \rho_{mc\text{-}c} = \frac{\rho_{mc\text{-}c}}{1 + \dfrac{[\text{OG}]}{[\text{OG-Borate}]}} \qquad (2)$$

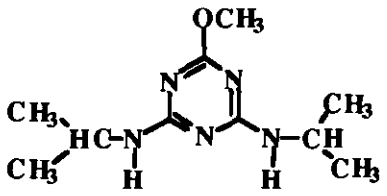
where $\rho_{mc\text{-}c}$ is the limiting charge density of the octylglucoside-borate micelle. The higher the charge density the more negative the micelle. There are several operational parameters

that can alter $\rho_{mc}$. These are the concentrations of the surfactant and borate, and pH of the running electrolyte. According to eqn 1, at constant surfactant concentration, any increase in the borate concentration or pH will result in a decrease in the ratio $\frac{[OG]}{[OG\text{-}Borate]}$, and therefore a larger $\rho_{mc}$, see eqn 2. At constant pH and borate concentration, an increase in the surfactant concentration will yield an increase in the ratio $\frac{[OG]}{[OG\text{-}Borate]}$, and as a result, $\rho_{mc}$ will decrease, see eqns 1 and 2. These readily tuned features of the micelles would allow the tailoring of the elution range for a given separation problem.

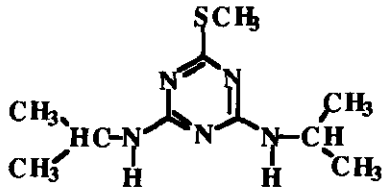
In fact, increasing the pH at constant borate and surfactant concentrations resulted in increasing the retention window and in turn peak capacity and resolution. Similarly, increasing the borate concentration in the running electrolyte at constant pH and surfactant concentration yielded a monotonic increase in the retention window of the new micellar systems.

The above features permitted the tuning of the elution range parameter that largely influences the peak capacity and resolution in micellar electrokinetic capillary chromatography. This represents an advantage over the traditionally used micelles such as sodium dodecyl sulfate (SDS) or other charged micelles. In fact, with SDS the surface charge density of the micelle is independent of pH, and therefore the retention window is predetermined and can not be varied which lead to increased analysis time when such a window is not needed. With SDS and other ionic surfactants window optimization is most often achieved through altering the retention times of unretained and completely dissolved species by the micelles by the adding methanol to the running electrolytes. Such conditions lead to disrupting the structure of the micelles and consequently lower separation efficiencies are obtained.

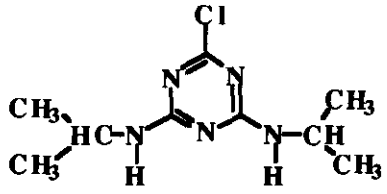
Furthermore, with its balanced hydrophile-lipophile character, the octylglucoside-borate micellar system allowed the separation of herbicides, e.g., prometon, prometryne, propazine and butachlor (see structures below) and some other hydrophobic species including polyaromatic hydrocarbons. High separation efficiencies were obtained over a wide range of elution conditions, and consequently the detection limit for the herbicides was in the range of 18-52 femtomoles using UV detection. The significance of this contribution resides in providing improved micellar phases for the separation of neutral and hydrophobic species by micellar electrokinetic capillary chromatography, a variant of capillary zone electrophoresis [2,3].



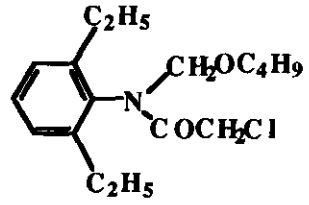
**Prometon**



**Prometryne**



**Propazine**



**Butachlor**

c. On-Line Preconcentration of Dilute Samples

On-line preconcentration with interactive capillaries is an adsorption process that can be described by an adsorption isotherm. An adsorption isotherm provides a relationship between the concentration of the solute in the bulk solution and the amount of solute adsorbed onto the sorbent when the two phases are in equilibrium. Two types of adsorption isotherm are typically used in describing liquid-solid adsorption; Langmuir and Freundlich. The Langmuir isotherm is convenient for the quantitative analysis of adsorption processes and has a physico-chemical basis while the Freundlich model is purely empirical. For the discussion of on-line preconcentration data, the Langmuir model is considered, which can be expressed by the equation :

$$q = \frac{ac}{1 + bc}$$

where q is the amount of solute in the adsorbed phase, c is the concentration of the solute in the bulk liquid phase, and b is an empirical constant related to the energy of adsorption. At low solute concentration, $bc << 1$ and $q \approx ac$. Under these conditions, a linear isotherm is obtained with a slope equal to parameter a, which is the equilibrium constant for the sorption process, K. At high solute concentration, $bc >> 1$ and the Langmuir isotherm flattens out, i.e., q approaches $q_{max}$. This means that at high solute concentrations the adsorption sites become saturated with adsorbed solute molecules, and the concentration of the solute in the adsorbed phase approaches a maximum value. Since the solute concentrations were relatively low in the on-line preconcentration studies, linear isotherms were expected.

The capillaries used in the studies consisted of two sections connected with a Teflon tube having an inner diameter of the same size as the outer diameter of the two capillaries. The first part is a preconcentration capillary, *i.e.*, an open-tubular reversed-phase

chromatography column with bonded octadecyl functions on the inner wall or a capillary with immobilized metal chelates on the inner surface acting as a metal chelate affinity chromatography column. The second part is a separation capillary, *i.e.*, a CZE capillary.

As the sample is introduced, it first enters the preconcentration capillary, and is accumulated on the interactive walls. Samples are retained by the octadecyl groups by hydrophobic interaction or by the immobilized metal sites by metal interaction. Since hydrophobic interaction is non-selective, octadecyl capillaries can be applied for a wide variety of compounds provided that they have non-polar functions. On the other hand, since metal chelate interaction chromatography is an affinity type, the metal chelate capillaries can be used for selective preconcentration. Similar to metal chelate affinity chromatography, solutes are retained by the immobilized metal through interaction with electron donor side chain groups of the analytes. Therefore, metal chelate capillaries would allow selective preconcentration of a contaminant or a group of contaminants having affinity to the metal chelate walls.

In both cases, i.e., octadecyl-capillaries or metal chelate capillaries, the capillary surface was either etched or etched and then coated with colloidal silica . In the case of the metal chelate capillaries, iminodiacetic acid functions (IDA) were covalently attached to the capillary walls to serve as metal chelating ligands. In all the studies, Zn(II) was immobilized on the capillary surface, and the corresponding metal chelate capillary tubes are denoted by Zn(II)-IDA-Cap. The surface coverage with metal chelating functions as well as with octadecyl functions, increased by coating the etched capillaries with colloidal silica. However, the colloidal support leached out after prolonged use. Therefore, most of the studies were carried out with preconcentration capillaries having etched walls, which exhibited constant performance for longer period of time.

The on-line preconcentration process involves two consecutive steps: the accumulation of the solute onto the walls of the preconcentration capillary, and stripping the accumulated solute off the capillary walls. The accumulation of solutes on the octadecylsilyl walls from dilute samples should be carried out in the presence of an electrolyte (e.g. aqueous solution) that affords the strongest interactions between the analyte and the interactive walls, i.e., a binding electrolyte. In the desorption step, the accumulated solutes on the walls of the preconcentration capillary should be stripped off the walls with a strong debinding electrolyte (e.g. hydro-organic solution) so that they enter the separation capillary as a thin plug whereby separation starts. The binding electrolytes used in this study were sodium phosphate solutions, whereas the debinding electrolytes were sodium phosphate solutions containing acetonitrile. Acetonitrile served as the debinding agent [4].

Also, in the case of capillaries with immobilized iminodiacetic acid metal chelating functions, the on-line preconcentration involved two steps: the accumulation followed by the desorption of the solutes, in which binding and debinding electrolytes are used successively. The accumulation step was best carried out by gravity-driven flow , whereas the desorption step was achieved by electromigration. Since the accumulated solute on the inner walls should be stripped off the walls and introduced in the separation capillary as a thin plug, the debinding electrolyte should contain a strong competing agent that desorbs the metal and the protein from the binding sites on the surface of the metal chelate capillary. In this regard, ethylenediaminetetraacetic acid (EDTA) is an excellent candidate since it forms stronger complex with metals than the covalently attached IDA functions on the surface of the capillaries. Thus, the binding electrolytes used in this study were sodium phosphate solutions, whereas the debinding electrolytes were sodium phosphate containing EDTA. Besides that, the concentration of the debinding agent is also very important to

ensure a fast desorption kinetic and minimize band broadening during the process of debinding [5].

The performance of tandem octadecyl capillaries- capillary zone electrophoresis was evaluated with two herbicides, prometon and prometryne (see structures above). The on-line preconcentration was best achieved when oligomeric octadecyl capillaries having roughened walls were employed. The coupled configuration enhanced the detectability in terms of solute concentration by a factor of 10 to 35 as compared to that obtained by capillary zone electrophoresis alone. Large volumes of samples could be introduced without affecting separation efficiency. This contribution has provided miniaturized on-line preconcentration techniques in capillary electrophoresis that are potentially useful in the area of environmental analytical chemistry [2,4].

The metal chelate capillaries with immobilized zinc proved effective in the selective accumulation of detectable amounts of proteins from dilute samples. They permitted the detection of 25 times less concentrated samples than by CZE alone with concentration sensitive detectors. Large sample volumes could be introduced without affecting separation efficiency. Besides demonstrating the effectiveness of metal chelate capillaries in the quantitative determination of dilute samples, the effects of several operating parameters as pH ionic strength etc. on the analytical signal were examined [5]. Although this study utilized proteins as model solutes, the established concept can be transposed to other species including pesticides.

d. Determination of Ionization Constants of Weak Electrolyte Pollutants

Capillary zone electrophoresis in untreated fused silica capillaries has proved suitable for the determination of the ionization constants of weak electrolytes, e.g., aniline, *p*-anisidine, *p*-aminobenzoic acid. Several fundamental equations relating the electrophoretic mobilities of ionized solute to hydronium ion concentration in the running electrolyte have been verified experimentally. The observed dependence of the electrophoretic mobilities of weak bases and ampholytes on the pH of the electrolyte showed good agreement with predicted behavior. The $pK_a$ values calculated from electrophoretic mobilities data obtained by capillary zone electrophoresis were reasonably close to those reported in the literature. The significance of this study resides in providing capillary electrophoresis methodology that has several advantages over traditional potentiometric methods: It requires small amounts of sample; it enables measurements to be made on several species simultaneously; and the samples do not even need to be pure since the impurities can be separated from the solute of interest [6].

The basic theory behind this study can be summarized as follows [6]. In capillary zone electrophoresis, the electrophoretic mobility of charged species can be conveniently adjusted by varying the pH of the running electrolyte, which alters their charge densities. This renders CZE adequate for the determination of ionization constants of weak acids, bases and ampholytes. For the protonation of a base, B , the net electrophoretic mobility, $\mu_e$, of the analyte is given by :

$$\mu_e = \frac{[BH^+]}{[BH^+] + [B]} \mu_{ob}$$

where $\mu_{ob}$ is the electrophoretic mobility of the fully protonated species, $BH^+$. The above equation can be rearranged into:

$$\mu_e = \frac{[H^+]/K_a}{1 + [H^+]/K_a}\mu_{ob} \tag{3}$$

or

$$\frac{1}{\mu_e} = \frac{K_a}{\mu_{ob}} \cdot \frac{1}{[H^+]} + \frac{1}{\mu_{ob}} \tag{4}$$

or

$$pH = pK_a - \log \frac{\mu_e}{\mu_{ob} - \mu_e} \tag{5}$$

where $K_a$ is the equilibrium ionization constant of the conjugate acid.

Eqn 3 shows a sigmoidal relationship between electrophoretic mobility and $[H^+]$ or pH. Eqn 4 shows that a plot of $\frac{1}{\mu_e}$ versus $\frac{1}{[H^+]}$ is a straight line with a slope equal to $\frac{K_a}{\mu_{ob}}$ and an intercept equal to $\frac{1}{\mu_{ob}}$. The ratio of slope to intercept is equal to $K_a$. From this plot, $pK_a$, as well as the limiting electrophoretic mobility, $\mu_{ob}$ can be determined. According to Eqn 5, a plot of pH versus $\log \frac{\mu_e}{\mu_{ob} - \mu_e}$ will result in a straight line with a slope equal to -1 and an intercept equal to $pK_a$. However, this plot requires that $\mu_{ob}$ is known.

In a similar fashion, the following equations can be obtained for an acid:

$$\mu_e = \frac{K_a/[H^+]}{1 + K_a/[H^+]}\mu_{oa} \tag{6}$$

$$\frac{1}{\mu_e} = \frac{1}{K_a\mu_{oa}}[H^+] + \frac{1}{\mu_{oa}} \tag{7}$$

$$pH = pK_a + \log \frac{\mu_e}{\mu_{oa} - \mu_e} \tag{8}$$

where $\mu_{oa}$ is the electrophoretic mobility of the fully deprotonated species. The $pK_a$ values of weak acids can be determined in the same way as in the case of weak bases.

For an ampholyte, similar derivation will lead to the following expression:

$$\mu_e = \frac{[H^+]/K_{a1} - K_{a2}/[H^+]}{1 + K_{a2}/[H^+] + [H^+]/K_{a1}}\mu_o \tag{9}$$

where $\mu_o$ is the limiting electrophoretic mobility. Since the fully protonated and deprotonated forms of an ampholyte have the same magnitude of charge densities except that they are of opposite sign, therefore

$$\mu_{ob} = -\mu_{oa} = \mu_o$$

e. Improving the Speed of Separation of Oppositely Charged Species

Although this study utilized proteins as model solutes, its general applicability would find use in the area of capillary electrophoresis of oppositely charged pollutants including pesticides, herbicides and related compounds. In this study, a novel two-dimensional electrophoretic system for the control of electroosmotic flow in capillary zone electrophoresis has been developed and evaluated for rapid separation of proteins. The system comprises uncoated and polyether coated fused silica capillaries coupled in series. An equation relating the average electroosmotic flow velocity in the coupled capillaries to the intrinsic electroosmotic velocities of the connected segments and their corresponding lengths has been derived and verified experimentally. This approach has the advantage of enabling the tuning of electroosmotic flow independently of the applied voltage. As a consequence, rapid separations at relatively low field strength were achieved without sacrificing the high separation efficiencies obtained with surface-modified capillaries. This contribution has improved the methodology of capillary zone electrophoresis in terms of speed of separation [7,8].

The following describes the basic principles behind the control of the speed of separation with coupled capillaries. In a system of n coupled capillaries having the same inner diameter but differing in their $\zeta$ potentials, the average electroosmotic mobility, $\mu_{eo}$, across the tandem system is a weighted average of the intrinsic or local electroosmotic mobilities in the individual connected segments as follows [7]:

$$\mu_{eo} = \frac{\sum_{i=1}^{n} \mu_{eo,i} l_i}{l_t} \qquad (10)$$

where $l_i$ is the length of the individual capillary segment, $i$, $l_t$ is the total length of the connected capillaries and $\mu_{eo,i}$ is the intrinsic electroosmotic mobility in each capillary segment, $i$, measured on a separate length $l_t$. Equation 1 can be rearranged as a linear function of the fractional length of a given capillary segment, $i$, in the tandem capillaries as follows:

$$\mu_{eo} = (\mu_{eo,i} - \sum_{j=1,j\#i}^{n} \mu_{eo,j}) \frac{l_i}{l_t} + \sum_{j=1;j\#i}^{n} \mu_{eo,j} [1 - \frac{\sum_{k=1;k\#i,j}^{n} l_k}{l_t}] \qquad (11)$$

where $j$ and $k$ are random variables. It follows then that the average electroosmotic flow or bulk flow across the tandem capillary system can be in principle controlled to any desired value bordered by the lowest and highest intrinsic electroosmotic mobilities in the individual capillaries. The slope of these curves, $\mu_{eo,i} - \sum_{j=1,j\#i}^{n} \mu_{eo,j}$, is indicative of the range over which the EOF can be varied. The higher the slope, the wider is the range of variation.

Equation 11 was verified experimentally by measuring the EOF in 3 different tandem capillary systems, i.e., F-2000 → I-200, F-2000 → untreated and I-200 → untreated, using phenol as the inert tracer. F-2000 denotes capillaries with fuzzy polyether coating while I-200 denotes capillaries with interlocked polyether coatings. In all cases linear curves were obtained when the electroosmotic mobility was plotted against the fractional length of the capillary segments.

The F-2000 and I-200 capillaries can be described as follows [ 9, 10]. The F-2000 coating consists of two layers: a glyceropropylpolysiloxane sublayer covalently attached to the inner surface and a polyether top layer. In the I-200 coating, the capillary wall was coated with polysiloxane polyether chains the monomeric units of which at both ends were covalently attached to the capillary inner surface with possible interconnection.

As an extension to the above study, a post-column multiple capillary device, which allowed the switching between several coupled capillary systems was designed and constructed in-house. The utility of the multiple capillary device was also demonstrated and extended to fraction collection in short capillary segments. The fraction collection in capillaries facilitated the quantitative transfer of the collected fractions to high performance liquid chromatography for further analysis or to mass spectrometry for structural determination. The off-line combination of capillary zone electrophoresis with HPLC or MS utilized commercial instruments without the need of expensive interfacing designs [11].

f. Capillary Surface Modification

A series of capillary surface modifications entailing multilayered coatings were introduced to capillary zone electrophoresis of a wide range of species. In one set of surface modification, large molecular weight hydroxypropyl cellulose (HPC) afforded "zero" flow capillaries which were used as precursors for developing anodal flow capillaries. The zero flow capillaries yielded more than 1 000 000 theoretical plates/m indicating that the HPC layer was effective in minimizing solute wall interaction by effectively shielding the surface silanols of the fused silica. However, because of the absence of the electroosmotic flow, the analytes must be highly charged in order to bring about their elution past the detection point. This requirement necessitated the use of extreme pH, i.e., very low pH for basic species and alkaline pH for acidic species.

Other surface modification were developed to generate capillaries with quasi-constant anodal electroosmotic flow to facilitate the separation of acidic species. This was achieved *via* the zero flow capillary precursors the surface of which were covered with a cellulose network in order to minimize the magnitude of the negative zeta ($\zeta$) potential of the silica surface. Next, a cross linked network of polyethyleneimine was covalently attached to an inner cellulose bilayer, and the a hydrophilic layer of polyether chains was then bonded to the polyethyleneimine moieties. These polyether chains have markedly reduced solute-coating interactions. The resulting anodal electroosmotic flow was relatively weak due to the high viscosity of the coated wall imparted by the hydroxypropylcellulose layer [12].

Still other capillary surface modification that provided tubes with constant anodal electroosmotic flow (i.e., the electroosmotic flow is not a function of electrolyte pH) were also developed. These capillaries with relatively stronger and constant anodal electroosmotic flow were obtained by coating the inner capillary surface with methylated polyethyleneimine hydroxymethylated, i.e., a polymer layer containing a high density of quaternary ammonium groups. The ammonium groups provided constant positive charge to the capillary surface, and therefore a constant positive z potential. The hydroxyl groups of the charged polymeric layer permitted the covalent attachment of polyether chains, which minimized electrostatic interaction between the positive charge of the coatings and the negative charge of acidic solutes. Under these conditions rapid transport of acidic species past the detection point in the separation capillary could be achieved, and relatively high plate counts were obtained [12].

g. Development of Novel Column Packing Materials for Liquid Chromatography

Along the lines of surface modification for generating well defined media for liquid microcolumn separation, we have also initiated a small project for rapid high performance liquid chromatography with short columns packed with non porous media. In this regard, microspherical zirconia particles were synthesized and surface modified with octadecylsilane compounds for reversed-phase high performance liquid chromatography (HPLC). Monomeric and "polymeric" octadecyl-zirconia bonded stationary phases were obtained by reacting the support with octadecyldimethylchlorosilane or octadecyltrichlorosilane, respectively. The surface coverage of the zirconia-based stationary phases with octadecyl functions was approximately the same as that of octadecyl-silica sorbents. These phases were packed in short column of 3 x 0.46 cm I.D., and were evaluated in terms of reversed-phase chromatographic properties with non polar, slightly polar and ionic species over a wide range of mobile phase composition and pH. Monomeric octadecyl-zirconia with end-capping exhibited some metallic interactions with both basic and acidic solutes, but these interactions were greatly reduced in the presence of competing agents (e.g., tartarate ions) in the mobile phase. The polymeric octadecyl-zirconia sorbents exhibited higher retention than the monomeric ones with the various solutes investigated, and their residual adsorptivities toward acidic solutes were much lower. The retention of non polar and slightly polar aromatic compounds was quasi homoenergetic on both types of octadecyl-zirconia stationary phases. Stability studies conducted at extreme pH conditions (pH 2.0 and pH 12.0), have shown that polymeric octadecyl-zirconia are more stable than their monomeric counterparts. These stationary phases were quite useful in the separation of polycyclic aromatic hydrocarbons, alkylbenzene and phenylalkylalcohol homologous series, oligosaccharides, dansyl-amino acids, peptides and proteins [13].

To further understand the chromatographic behavior of charged solutes on zirconia based stationary phases, an additional study was undertaken. In effect this study is an extension to the above work in which a series of non-porous, microspherical octadecyl-silica were introduced and compared to octadecyl-zirconia bonded stationary phases in the HPLC of dansyl-amino acids over a wide range of elution conditions. Polymeric octadecyl-silica columns afforded virtually no solute-support interaction, whereas polymeric octadecyl-zirconia bonded stationary phases exhibited metallic interaction with some dansyl amino acids, and their residual adsorptivities toward the separated analytes were comparable to those observed on monomeric octadecyl-silica columns without end-capping. These metallic interactions, which are of the electron donor-electron acceptor (EDA) type, predominate in the acidic pH region. However, the presence of small amounts of tartrate or phosphate ions in the eluent greatly reduced EDA interaction, and consequently allowed the high resolution separation of dansyl amino acids (Dns-AA). Under optimal gradient elution conditions, eleven or fourteen different Dns-AA could be separated in less than 6.0 min on short polymeric octadecyl-zirconia or octadecyl-silica columns, respectively [14].

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