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Synthesis and evaluation of capillaries for separation by CEC

Laxmi Iyer
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**SYNTHESIS AND EVALUATION OF CAPILLARIES
FOR SEPARATION BY CEC**

A Thesis

Presented to

**The Faculty of the Department of Chemistry
San Jose State University**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Science**

by

Laxmi Iyer

August 2001

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ABSTRACT

SYNTHESIS AND EVALUATION OF CAPILLARIES FOR SEPARATION BY CEC

By Laxmi Iyer

Capillary electrochromatography (CEC) is an emerging electroseparation technique that is quickly gaining popularity. The synthesis and evaluation of various capillaries for separation by open tubular chromatography (OTCEC) are described. Fused silica capillaries with an i.d of 50 μm are etched and then chemically modified by the silanization/hydrosilation process to attach 1-octadecyl and/or 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) stationary phases. These capillaries are then used for separations of a number of different types of compounds such as mixtures of lysozymes, tetracycline antibiotics etc. In comparison to bare 50 μm capillaries and unetched but chemically modified 50 μm capillaries, the etched and chemically modified capillaries show better separation under identical conditions of buffer pH. Electroosmotic flow (EOF) measurements over the pH range of 2.14 – 7.40 for some of the chemically modified capillaries are presented.

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TABLE OF CONTENTS

LIST OF TABLES	x
-----------------------	----------

LIST OF FIGURES	xi
------------------------	-----------

Chapter I – INTRODUCTION

1	History	1
1.1	Chromatography	1
1.2	Electrophoresis	2
1.2.1	Capillary electrophoresis (CE)	3
1.3	Electrochromatography	6
2	Development of capillary electrochromatography	7
2.1	Capillary Electrophoresis System	8
2.1.1	System Overview	8
2.1.2	Sample Injection	9
2.1.3	Source and Destination Vials	11
2.1.4	Power Supply	12
2.2	Advantages of CEC	12
2.3	Electrochemical phenomena	13
2.3.1	The electrical double layer	13
2.3.2	Electroosmosis	14
2.3.3	Electrophoresis	15
2.4	Measurement of electroosmotic flow (EOF)	17

2.5	Reversing electroosmotic flow (EOF)	18
3	Format of capillaries	18
3.1	Bare capillaries for capillary electrophoresis	18
3.2	Coated capillaries	19
3.3	Packed capillaries	22
3.4	Open tubular capillaries	24
4	Goals of the research	25

Chapter II – EXPERIMENTAL

1	Materials	30
2	Instrumentation	32
2.1	Capillaries	32
2.2	Gas chromatography oven	32
2.3	Capillary electrochromatography	33
2.3.1	Analysis	33
3	Methodology	34
3.1	Etching of the capillaries	34
3.2	Formation of the hydride layer	35
3.3	Apparatus for the synthesis of the bonded material	36
3.3.1	Preparation of the C-18 mixture	37
3.3.1.a	Attachment of the C-18 moiety	37
3.3.2	Preparation of the AMPS mixture	37

3.3.2.a Attachment of the AMPS moiety	38
3.4 Preparation of buffers	38
3.5 Electrochromatographic analysis	39
3.5.1 Making of a detection window on the capillary	39
3.5.2 Preparation of the samples	40
3.5.3 Effects of the buffers and voltage on separations	40
3.5.4 Measurements for electroosmotic flow (EOF)	40

Chapter III – RESULTS AND DISCUSSION

1 Etching and modification of the capillaries	44
2 Choice of test solutes	49
2.1 Caffeine and theophylline	49
2.2 Chicken and turkey lysozymes	49
2.3 Serotonin and tryptamine	49
2.5 Tetracycline antibiotics	50
3 Interpretations of chromatographic data	50
3.1 Separation of caffeine and theophylline	50
3.2 Separation of chicken and turkey lysozymes	59
3.3 Separation of serotonin and tryptamine	67
3.4 Separation of tetracycline antibiotics	76
4 EOF measurements	84
4.1 Effect of pH on EOF	84

4.1.1 Etched capillary modified with C-18 and AMPS	84
4.1.2 Etched capillary modified with AMPS	87
Chapter IV – CONCLUSION	88
REFERENCES	90

LIST OF TABLES

1. Table 1	List of chemicals used in this study along with their chemical abstract service (CAS) registry numbers.	30
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LIST OF FIGURES

Figure 1:	Schematic of CEC instrumentation.	10
Figure 2:	The silanoate groups on the inner wall of the capillary attract the cations forming a fixed layer, while the mobile layer of cations is pulled towards the cathode resulting in electroosmotic flow.	16
Figure 3:	Structure of 1-octadecene (C-18).	28
Figure 4:	Structure of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS).	28
Figure 5:	Structures of the tetracycline antibiotics used in the study.	41
Figure 6:	Structures of the tetracycline antibiotics used in the study(cont'd).	42
Figure 7:	Structures of some other drugs used in the study.	43
Figure 8:	The electronegative fluorine atom causes an electron shift in the neighboring atoms resulting in the weakening of hydroxyl bonds.	46
Figure 9:	Diagram of the reactions on the surface of the capillary wall.	47
Figure 10:	Structures of the capillary surfaces used in the study.	48
Figure 11(a):	Separation of caffeine & theophylline on bare capillary. L = 50 cm, l = 32 cm, V = 25 kV, pH = 8.18 (1:10).	51
Figure 11(b):	Separation of caffeine & theophylline on unetched & C-18 modified capillary. L = 34.4 cm, l = 26.4 cm, V = 10 kV, pH = 8.14 (1:10).	52
Figure 11(c):	Separation of caffeine & theophylline on etched & C-18 modified capillary. L = 47 cm, l = 25 cm, V = 20 kV, pH = 8.18 (1:10).	53
Figure 11(d):	Separation of caffeine & theophylline on etched & C-18 and AMPS modified capillary. L = 35 cm,	54

$l = 27$ cm, $V = 15$ kV, $pH = 8.18$ (1:10).

Figure 11(e): Separation of caffeine & theophylline on etched & AMPS modified capillary. $L = 50$ cm, $l = 25$ cm, $V = 30$ kV, $pH = 8.14$ (1:10).	55
Figure 11(f): Separation of caffeine & theophylline on etched & AMPS and C-18 modified capillary. $L = 49.6$ cm, $l = 25$ cm, $V = 30$ kV, $pH = 8.14$ (1:10).	56
Figure 12(a): Separation of chicken & turkey lysozymes on bare capillary. $L = 50$ cm, $l = 32$ cm, $V = 25$ kV, $pH = 8.18$ (1:10).	60
Figure 12(b): Separation of chicken & turkey lysozymes on unetched & C-18 modified capillary. $L = 34.4$ cm, $l = 26.4$ cm, $pH = 3.70$ (1:10), A. $V = 15$ kV, B. $V = 20$ kV.	61
Figure 12(c): Separation of chicken & turkey lysozymes on etched & C-18 modified capillary. $L = 47$ cm, $l = 25$ cm, $V = 15$ kV, $pH = 3.70$ (1:10).	62
Figure 12(d): Separation of chicken & turkey lysozymes on etched & C-18 and AMPS modified capillary. $L = 35$ cm, $l = 27$ cm, $V = 15$ kV, $pH = 3.70$ (1:10).	63
Figure 12(e): Separation of chicken & turkey lysozymes on etched & AMPS modified capillary. $L = 50$ cm, $l = 25$ cm, $V = 25$ kV, $pH = 3.70$ (1:10).	64
Figure 12(f): Separation of chicken & turkey lysozymes on etched & AMPS and C-18 modified capillary. $L = 49.6$ cm, $l = 25$ cm, $V = 20$ kV, $pH = 3.70$ (1:10).	65
Figure 13(a): Separation of serotonin & tryptamine on bare capillary. $L = 50$ cm, $l = 32$ cm, $V = 20$ kV, $pH = 2.14$ (1:10).	69
Figure 13(b): Separation of serotonin & tryptamine on unetched & C-18 modified capillary. $L = 34.4$ cm, $l = 26.4$ cm, $pH = 2.14$ (1:10), A. $V = 15$ kV, B. $V = 20$ kV.	70
Figure 13(c): Separation of serotonin & tryptamine on etched & C-18 modified capillary. $L = 47$ cm, $l = 25$ cm,	71

V = 15 kV, pH = 2.14 (1:10).

Figure 13(d): Separation of serotonin & tryptamine on etched & C-18 and AMPS modified capillary. L = 35 cm, l = 27 cm, V = 15 kV, pH = 2.14 (1:10).	72
Figure 13(e): Separation of serotonin & tryptamine on etched & AMPS modified capillary. L = 50 cm, l = 25 cm, V = 20 kV, pH = 2.14 (1:15).	73
Figure 13(f): Separation of serotonin & tryptamine on etched & AMPS and C-18 modified capillary. L = 49.6 cm, l = 25 cm, V = 10 kV, pH = 2.14 (1:20).	74
Figure 14(a): Separation of tetracycline antibiotics on bare capillary. L = 50 cm, l = 32 cm, V = 20 kV, pH = 2.14 (1:10).	77
Figure 14(b): Separation of tetracycline antibiotics on unetched & C-18 modified capillary. L = 34.4 cm, l = 26.4 cm, pH = 2.14 (1:10), A. V = 15 kV, B. V = 20 kV.	78
Figure 14(c): Separation of tetracycline antibiotics on etched & C-18 modified capillary. L = 47 cm, l = 25 cm, V = 20 kV, pH = 2.14 (1:10).	79
Figure 14(d): Separation of tetracycline antibiotics on etched & C-18 and AMPS modified capillary. L = 35 cm, l = 27 cm, V = 15 kV, pH = 2.14 (1:10).	80
Figure 14(e): Separation of tetracycline antibiotics on etched & AMPS modified capillary. L = 50 cm, l = 25 cm, V = 10 kV, pH = 2.14 (1:15).	81
Figure 14(f): Separation of tetracycline antibiotics on etched & AMPS and C-18 modified capillary. L = 49.6 cm, l = 25 cm, V = 15 kV, pH = 2.14 (1:20).	82
Figure 15: Effect of EOF as a function of pH in etched capillary modified with C-18 & endcapped with AMPS.	85
Figure 16: Effect of EOF as a function of pH in etched capillary modified with AMPS.	86

Chapter 1

INTRODUCTION

1 History

1.1 Chromatography

Chromatography is the most widely used means of performing analytical separations and finds application to all branches of science. Chromatography includes many diverse and important methods that permit the separation of closely related constituents of complex mixtures. In chromatographic separations the sample is dissolved in a mobile phase, which may be a gas, a liquid, or a supercritical fluid. This mobile phase is then forced through an immiscible stationary phase placed in a column or coated on a solid surface. The constituents of the sample distribute themselves between the mobile and stationary phases to varying degrees and are thus separated. Liquid chromatography (LC), where the mobile phase is a liquid, encompasses four basic methods: (1) *partition chromatography*; (2) *adsorption, or liquid-solid chromatography*; (3) *ion chromatography*; and (4) *size exclusion, or gel, chromatography*.

Liquid chromatography was first developed by Tswett and was carried out in glass columns with diameters of 1 to 5 cm and lengths of 50 to 500 cm. In order to ensure reasonable flow rates the diameter of the solid stationary phase particles was in the 150 to 200 μm range. Nevertheless flow rates were low and separation times were long, and application of neither pressure nor vacuum was effective in increasing

separation efficiency. Decreasing the diameter of the particles of the solid stationary phase brought about major increases in column efficiencies. The development of technology for producing packings with particle diameters as small as 3 to 10 μm led to the technique of *high-performance liquid chromatography* (HPLC).

Silica gel still is the mainstay base material for most HPLC packings. Silica gel is rigid, provides excellent efficiency, is reasonably priced, and can be converted to bonded phase materials by virtue of its reactive surface silanol (Si-OH) groups and its relatively high surface area. Though it can be used in nonaqueous and aqueous environments, its silanols are acidic, and if they are not removed or covered, they can cause tailing or irreversible adsorption of basic compounds.

1.2 Electrophoresis

Electrophoresis is the movement of electrically charged particles or molecules in a conductive liquid medium, usually aqueous, under the influence of an electric field. Tiselius [1] developed the first electrophoretic apparatus in the 1930s and in subsequent years, powerful and widespread macro scale electrophoretic separations were performed. These macro scale separations were, however, slow, labor-intensive and obviously prone to poor reproducibility. Commercial apparatus for performing analytical electrophoresis on a micro scale in capillary columns changed this situation dramatically.

Electrophoresis can be performed in free solution or in a solution containing a nonconductive medium, such as a gel. In the latter case of electrophoresis, an electric

field is applied across the nonconductive medium after it is immersed in a conductive aqueous buffer solution. The purpose of the medium is to decrease zone spreading caused by Joule heating by dissipating heat with its high area-to-volume ratio. In some cases, the medium also serves as a molecular sieve for the separation of analytes based on their molecular size. Media that have been used include paper, cellulose acetate membranes, and agarose or polyacrylamide gels.

Though the use of a medium decreases zone spreading caused by convection, presence of channels in the material or interactions, such as adsorption, of the analytes with it can cause zone spreading. Gel electrophoresis is relatively slow and labor intensive compared to capillary electrophoresis.

1.2.1 Capillary electrophoresis (CE)

Capillary electrophoresis is sometimes referred to as *capillary zone electrophoresis* (CZE), *free solution capillary electrophoresis* (FSCE), *high-performance capillary electrophoresis* (HPCE), or just *capillary electrophoresis* (CE). In this technique aqueous buffer, called the electrolyte or run buffer, is filled in a glass tube and the ends of this glass tube are connected to containers filled with the same buffer. The buffer containers also have electrodes that are connected to a power supply. If a sample containing a mixture of neutral and electrically charged molecules is placed in the anodic end of the tube and an electric field is applied across the liquid, the ions in the sample will migrate through the tube at different rates and in different directions. The rates and directions of migration will depend on the sizes of

the ions and the magnitudes and signs of their charges. Neutral molecules will not be influenced by the electric field. Under the influence of an electric field, the buffer and neutral molecules move through the tube by electroosmosis. When the sample is placed in the anodic end of the tube, the electroosmotic flow of the buffer moves toward the cathode and carries the analytes with it. In the absence of any other influences, the analytes must, theoretically, travel through the conductive medium as zones that do not diffuse or spread out.

The passage of electric current through a conductive solution causes Joule heating, which heats the solution and leads to convective diffusion. The analytes in the warmer, center of the tube move faster than those near the cooler wall leading to zone spreading. It is found that in free solution ion mobility increases by about $2\%/^{\circ}\text{C}$ [2] and the analytes may not be well separated since the zones will spread out due to convective diffusion. In addition to causing zone spreading, high temperatures may also cause thermal degradation of some analytes such as proteins. Thus, in order to keep the zones narrow in electrophoresis, it is necessary to decrease the amount of heat generated and to dissipate that heat. The most widely used method to minimize convective diffusion is to decrease the tube diameter.

Hjerten performed the first successful electrophoretic analysis in free solution in 1958 wherein he used a quartz capillary of 1 to 3 mm internal diameter (i.d.) [3]. In 1967, he recognized that carrying out electrophoresis in narrow diameter tubes reduced thermal effects [4]. In 1969, Virtanen and Kivalo used glass capillaries of 0.2 to 0.5 mm i.d. and 50 to 100 mm length for their studies [5]. The measurement of

the potential gradient in the separated zones provided a means of detection and they also discussed the separation efficiency in detail. In 1970, Neuhoff and co-workers used polyacrylamide gel filled glass tubes of 0.45 mm i.d. and 3 cm length to analyze minute quantities of proteins [6]. In 1975, O'Farrell used flat bed and gel techniques for a two-dimensional method and achieved very high resolution [7].

In 1979, Castimpoalas used column techniques that are represented by disc electrophoresis in polyacrylamide gel rods for characterization of proteins [8]. The main drawback of this technique was the laborious multi-stage handling of gel beds and insufficient long-term reproducibility of the results. In order to overcome the problem of irreproducibility, the polyacrylamide gel rods were replaced with capillaries. Again in 1979, Mikkers et al. [9] demonstrated the use of small-diameter, 200 μm i.d., Teflon capillaries in capillary electrophoresis to decrease diffusion due to convection and to eliminate the problems associated with electrophoresis in a medium. In 1981, Jorgenson and Lukacs [10] used still smaller diameter capillaries of 75 μm i.d., made of Pyrex glass and reported that since the small-diameter capillaries dissipated heat very well, high voltages, 30 kV, could be used. They reported efficiencies in excess of 400 000 theoretical plates using capillaries only 50-100 cm long; the high voltages gave shorter separation times and high efficiencies. This compares favorably to separations by HPLC in which efficiencies of 20 000 theoretical plates are considered high. Jorgenson and Lukacs in 1981 [11], Stevens and Cortes in 1983 [12], and Knox and Grant in 1991 [13] studied separations based on electroosmotic flow and demonstrated that it was a good alternative to pressure

driven flow observed in HPLC. This was because both charged and uncharged analytes could be separated based on differential migration through the capillary. In 1983, Jorgenson and Lukacs reported [14] that glass capillaries could not be used with UV detection wavelengths shorter than about 280 nm, and that fused silica capillaries were better because they are transparent at shorter wavelengths.

Electroosmotic flow has a relatively flat profile compared to laminar or pumped flow found in HPLC. Due to frictional drag, the electroosmotic flow at the wall is slower than the flow through the rest of the tube. This decrease in flow subtracts very little from the overall flat profile, as the area near the wall is quite small. The flat flow profile causes all the analyte molecules to experience the same velocity component caused by electroosmotic flow regardless of their cross-sectional position in the capillary, and they elute as narrow bands. In HPLC, the analytes move through the column (tube) under the influence of pumped flow, wherein the analytes in the center of the column move faster than those nearer the wall. This uneven flow profile results in relatively broad peaks.

1.3 Electrochromatography

Berraz introduced the term electrochromatography in 1943 [15] to describe a form of paper electrophoresis that had been demonstrated by Strain in 1939 for separating dyes using an alumina column [16]. In 1949, both Tiselius and Synge suggested that electroosmosis could be used to move solvent through a porous gel structure, thus avoiding the use of hydrostatic pressures [17, 18]. In 1954, Mould and

Synge used this approach to separate polysaccharides in the presence of collodion membrane strips [19]. However, it was only in 1974 that the concept of electroosmosis as a pumping mechanism to drive the mobile phase through a separation column became more widely known when Pretorius used the technique to move the mobile phase through a 1 mm packed column [20]. The work of Jorgenson and others led to the use of electroosmotic flow as an attractive alternative to pressure-driven flows for separation of analytes [11, 12, 13].

2 Development of capillary electrochromatography

Advances in capillary LC combined with those in CE have led to the development of the new hybrid electroseparation technique of capillary electrochromatography (CEC). In this technique, separation is based primarily on the differential interactions (e.g., partitioning) of the analytes between the solid stationary and liquid mobile phases. The transport process in this technique, for moving the analyte and solvent zones through the capillary, is based on electroosmosis as opposed to pressure gradients found in HPLC. The technique is, in essence, a hybrid of CE (wherein an electric field is applied across the capillary generating a solvent flow caused by electroosmosis) and HPLC (wherein the separation of the analytes in a sample mixture is based on differences in distribution ratios between the two phases). CEC can be performed in open tubes where the stationary phase is fixed on the walls of the open tube, or in capillaries packed with conventional stationary phase materials such as 3-10 micron sized octadecyl silica particles. The mobile phase

moves through the capillary by electroosmosis and thus drives the analytes through the column.

Charged analytes in a sample mixture can be influenced by the applied electric field, causing the differential migration of such compounds, as in CE. Thus, both charged and neutral analytes can be separated depending on their differential migration through the capillary based on either the particular solute's interaction with the two phases or a combination of such interactions and the inherent electrophoretic mobilities of the molecules.

2.1 Capillary Electrophoresis System

2.1.1 System Overview

The main components of a capillary electrophoresis system are:

- a capillary tube, usually made of quartz (bore 50-200 μm , length 200-1000 mm);
- a high-voltage power supply capable of delivering up to 50 kV at 100 μA ;
- two electrolyte reservoirs into which the ends of the capillary dip, one connected to the high-voltage supply and the other grounded;
- an injection system (often on-column), usually at the high-voltage end of the capillary;
- a detector to quantitate the analyte (often on-column) within a small segment of the capillary (< 1mm). The most common methods used are UV and fluorescence detection;

- a means of pressurizing either or both the inlet and outlet ends of the capillary;
- a data output and handling device, such as an integrator or computer;
- thermostating of the capillary by either forced air or liquid.

An example of such instrumentation is shown in Figure 1. The capillary inlet is placed into a sample vial, the sample is introduced, the capillary inlet is then placed back into the source vial, and an electric field is applied between the source and destination vials. The fused silica capillary is transparent to ultraviolet (UV) as well as visible (Vis) light and thus the capillary itself can be used as the detector cell when either an UV/Vis or fluorescence detection method is used. The analytes migrate through the capillary at different rates and are detected by the detector while in the capillary (this is called on-column detection) at different times. The detector output is sent to an integrator or computer; the output from the integrator or computer is called an electropherogram and is a plot of detector response versus time. In chromatography, the time it takes a compound to elute through the column to the detector is called its retention time. In an electropherogram, the separated compounds appear as peaks with different migration times.

2.1.2 Sample Injection

The term is borrowed from chromatography where the sample is, in some cases, injected directly into a column or into a flowing mobile phase. For all practical purposes, it is not an injection of the sample into the capillary but it is the

Figure 1: Schematic of CEC instrumentation.

introduction of the sample into the capillary. Usually samples are loaded into vials with volumes of a few microliters to a few milliliters and only a few nanoliters are injected into the capillary. Samples are introduced into the capillary most commonly by electrokinetic or hydrodynamic injections. Hydrodynamic injection is done by pressure or siphoning. Pressure injection is done by either pressurizing the sample vial or by applying a vacuum to the destination vial. In siphon or gravity injection raising the vial causes the sample to flow into the capillary. Electrokinetic injection consists of applying an electric field to the vial causing the sample components to migrate into the capillary. Most commercial instruments have autosamplers into which many vials can be loaded and the samples can then be automatically injected into the capillary using one or more of the techniques described above.

2.1.3 Source and Destination Vials

The source vial is sometimes referred to as the inlet reservoir or inlet vial and the destination vial is called the exit reservoir or exit vial. Usually, the source vial, the capillary, and the destination vial are filled with the same buffer. The composition of the electrolyte buffer is one of the most important variables in capillary electrophoresis and small changes in buffer pH or concentration may cause significant changes in the rates of analyte migration and thereby variations in migration times. Thus, it is important that the source vial, the capillary and the destination vial be thoroughly rinsed when the buffer is changed. The analytes migrate through the capillary and exit into the destination vial where they can

accumulate. This accumulation of the solute molecules can result in a change in buffer composition in the destination vial relative to that in the source vial and the capillary. Thus, after repeated analyses, the buffer in the source vial may have a different composition from that in the destination vial, which can lead to nonreproducible migration times. Therefore, it is important to periodically rinse and refill the vials and the capillary even when the same buffer is used for each analysis for a prolonged time. The source and destination vials must be kept at the same level to prevent siphoning of buffer from one vial to another, which may cause changes in migration times.

2.1.4 Power Supply

The purpose of the power supply is to provide an electric field across the capillary and most instruments can be operated in either the constant voltage, or constant current, or constant power mode with the ability to reverse the polarity.

2.2 Advantages of CEC

The instrumentation used in CEC is essentially the same as in CE. It has all the advantages of a miniaturized separation technique such as high efficiency, increased mass sensitivity, low cost of operation, low sample-volume requirements, and low solvent consumption. It involves electrically driven flow of the mobile phase and not pressure-driven flow as in HPLC; thus stationary phases on support materials with small particle diameters can be used. It improves selectivity of separation of the

analytes through an electrically driven flat flow profile in contrast to the pressure-driven laminar or parabolic flow of HPLC; thus band broadening is decreased.

2.3 Electrochemical phenomena

2.3.1 The electrical double layer

In addition to the electrochemical phenomena of electroosmosis and electrophoresis, CEC also involves chromatographic partitioning between the stationary and mobile phases. The primary electrochemical phenomena of electroosmosis and electrophoresis result from the presence of an electrical double layer at the interface between a solid and an electrolyte. The surface of silica in contact with an electrolyte at a pH value > 3 contains chemically bound Si-O^- groups and is electrically charged. The excess of positive ions within the electrolyte balances the negative charges of the ions on the silica surface. The electrostatic interaction between the negative ions on the silica surface and the positive ions in the electrolyte solution is very strong and thus a very thin layer of excess positive ions is formed. This layer of excess positive ions can further be separated into two layers: a fixed layer and a mobile layer. Close to the silica surface is a layer of ions that are adsorbed on the surface and are essentially fixed, these ions are not involved in the electrokinetic phenomena and constitute the fixed Stern or Helmholtz layer. The remaining ions exchange continuously with those in the rest of the solution and constitute the diffuse or Gouy-Chapman layer. The charge density of the excess ions, σ , falls exponentially with distance, z , from the surface as

$$\sigma = \sigma_0 \exp(-z/\delta)$$

where δ is the so-called “thickness” of the double layer (often denoted by $1/K$), and is given as

$$\delta = [(\epsilon_0 \epsilon_r RT)/(2cF^2)]^{1/2}$$

where ϵ_0 is the permittivity of a vacuum, ϵ_r is the dielectric constant of the eluent, c is the buffer ionic strength and T is the temperature. The electrical potential at the boundary between the Helmholtz layer and the diffuse part of the double layer is called the “zeta potential”, ζ .

2.3.2 Electroosmosis

When a potential difference is applied to the silica surface the ions in the diffuse layer experience a force parallel to the surface. There is a slight excess of positive ions in this layer and thus the mobile phase in immediate contact with the silica surface experiences a net force towards the cathode. This is resisted by the viscosity, η , of the mobile phase and results in the development of shear within the electrical double layer. The magnitude of this shear force is proportional to the excess charge density at any point in the layer. The mobile phase at the solution-side boundary of the double layer moves at a constant velocity, u_{eo} , given as

$$u_{eo} = (\epsilon_0 \epsilon_r \zeta / \eta) E$$

This movement of the mobile phase is called electroosmosis. As the electric field E is an experimental variable, electroosmotic movement is conveniently characterized by the electroosmotic mobility, μ_{eo} , given as

$$\mu_{eo} = (\epsilon_0 \epsilon_r \zeta / \eta)$$

In case of a narrow open tube, the liquid within the tube moves as a plug and thus the velocity of the liquid is constant over almost the entire cross-section of the tube. The process of electroosmosis is illustrated in Figure 2.

The parameters that affect electroosmotic flow (EOF) are:

- applied voltage – an increase in the applied voltage increases EOF.
- buffer pH – an increase in the buffer pH increases EOF.
- buffer concentration or ionic strength – an increase in the buffer concentration or ionic strength decreases EOF when the capillary temperature is controlled.
- temperature – an increase in the temperature increases EOF.
- organic solvent – addition of an organic solvent may increase or decrease EOF depending on how it affects the viscosity, dielectric constant and zeta potential.
- chemical modification of the capillary wall – EOF can be greatly decreased or eliminated by coating or covalently bonding a chemical moiety to the capillary wall. While it may be desirable to eliminate EOF altogether in some cases, it is desirable to have enough EOF to assure that all solutes elute in a reasonable time.

2.3.3 Electrophoresis

Consider a spherical analyte of radius ' a ' that moves in its surrounding electrolyte by a process called electrophoresis according to the following equations:

Figure 2: The silanoate groups on the inner wall of the capillary attract the cations forming a fixed layer, while the mobile layer of cations is pulled towards the cathode resulting in electroosmotic flow.

electrophoretic velocity, $u_{ep} = (\epsilon_o \epsilon_r \zeta / \eta) f(a/\delta) E$

electrophoretic mobility, $\mu_{ep} = (\epsilon_o \epsilon_r \zeta / \eta) f(a/\delta)$

$f(a/\delta) = 1$ for $a/\delta \gg 1$ and $2/3$ for $a/\delta \ll 1$. Thus when the radius of the analyte is large in comparison to δ – the thickness of the double layer, the analyte moves at the same velocity as if it were subject to electroosmosis. But when the radius of the analyte is small in comparison to δ , as in the case of an ion, the analyte moves at a velocity $2/3$ that for electroosmosis with the same zeta potential.

2.4 Measurement of electroosmotic flow

An uncharged compound, a neutral marker, is chosen which moves through the capillary under the influence of only electroosmotic flow. The main criteria in choosing a neutral marker are that it be uncharged at the pH of the buffers used, be detectable by whatever type of detector is used, be pure, have no interaction with the walls of the capillary, and be soluble in the buffer. A variety of neutral markers such as benzene, dimethyl sulfoxide, mesityl oxide, pyridine etc. can be used, the most common among them being mesityl oxide or dimethyl sulfoxide (DMSO). The marker compound is injected by itself or added to a sample and the migration time of this substance is measured. The length of the capillary from the inlet to the detector is known and the electroosmotic velocity, v_{EOF} , in distance/time (cm/s) is calculated as

$$v_{EOF} = l/t_{nm}$$

where l is the effective length of the capillary, in cm, from its inlet to the detector, and t_{nm} is the migration time, in seconds, of the neutral marker. As described earlier, electroosmotic mobility, μ_{EOF} is given as

$$\mu_{\text{EOF}} = v_{\text{EOF}}/E$$

The units of electroosmotic mobility are $\text{cm}^2/\text{V}\cdot\text{s}$ and typical magnitudes of μ_{EOF} are $10^{-5} - 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$.

2.5 Reversing electroosmotic flow

In “normal” capillary electrophoresis, the detector side of the capillary, the cathode, has a negative charge and attracts the positively charged cations in the electrical double layer. Thus, the EOF is toward the cathode and the order of elution of the analytes is cations, neutrals, then anions. The direction of EOF can be reversed such that it is toward the anode and the order of elution of the analytes is anions, neutrals, then cations. This is done by reversing the polarity of the applied electric field such that the detector side of the capillary is the anode, which by virtue of its positive charge attracts the negatively charged anions in the electrical double layer.

3 Format of capillaries

3.1 Bare capillaries for capillary electrophoresis

The bare silica capillary is the simplest and still, quite often, the most reliable column for various applications. The most desirable diameters for bare capillaries are between 50 and 100 μm as Joule heat dissipation is efficient even at high voltages

resulting in little additional band broadening for most analytes. The diameter of the capillary chosen depends on the detection limit considerations with a larger column being chosen when higher sensitivity is desired. The presence of residual silanols on the surface of the unmodified silica capillary is of primary concern in its use. As described earlier, the Si-OH groups lead to the EOF, which causes the migration of analytes within the capillary. In case of cationic analytes moving toward the cathode, the EOF will contribute to movement in this direction; thus decreasing the overall migration time and consequently the resolution. In case of anionic analytes moving toward the anode, the EOF will retard the movement in this direction, thus increasing the overall migration time and consequently the resolution. Thus for cationic analytes the EOF must be decreased in order to improve resolution, so a bare unmodified capillary may not be the best choice for a separation column. In addition, the silanols on the surface of the bare capillary are potential sites for adsorption of some basic analytes and this could lead to poor recovery of the analyte and irreproducibility of the migration times with a subsequent decrease in the EOF.

3.2 Coated capillaries

Advances in column technology have led to a wide variety of coated capillaries which are either commercially available or have been reported in the literature. The purpose of coating technology is to produce a column that is devoid of the problems associated with bare capillaries such as high EOF and/or adsorption of the analytes to the wall. A successful coating must be stable over many injections in

order to have reproducible quantitative analyses. CE is frequently used to separate biomolecules (mostly proteins which are hydrophobic) and in order to minimize wall interactions a hydrophilic coating is desirable. Polyacrylamide is one of the most popular and successful of the bonded materials used to achieve good separations; its long-term stability is, however, its main drawback. Initial studies on coated capillaries were based first on 3-methacryloxypropyltriethoxysilane and later 7-oct-1-enyltrimethoxysilane as linkers between the surface and the polymerized, and in some cases cross-linked, acrylamide [21, 22]. But, at high pH, the amido bonds in polyacrylamide can hydrolyze and this can lead to degradation of the attached polymer and a subsequent loss in column performance. In order to overcome the effect of hydrolysis, steric protection of the amido bond was sought by the use of N-substituted acrylamide monomers such as poly(acroylaminoethoxyethanol) [23], a glycoside-bearing acrylamide polymer such as poly-(N-(acroylaminoethoxy)ethyl- β -D-glucopyranose (AEG) [24]. The polymer in the AEG capillary was linked to the capillary surface via a silicon-carbon bond made through a silanization/hydrosilation process that contributed to the coating's long lifetime in addition to the improved stability of the amido bond. The problems of bare capillaries can be addressed and the instability of bonded coatings can be overcome by the process of a combination of an attached moiety in contact with a surfactant [25]. This method involves first the attachment of an octadecylsilane to the silica surface followed by the physical adsorption of a surfactant on this hydrophobic surface. As the hydrophobic part of the surfactant will be attracted to the bonded octadecyl moiety, the polar group of the

adsorbed molecule will be in contact with the buffer creating a hydrophilic surface.

As the hydrophilic surface is subject to degradation during the electrophoresis process through desorption, a certain amount of the surfactant must be present in the buffer.

Although the coating produces excellent results in any single run, the irregularity of this desorption process creates problems in trying to maintain a uniform surface for long-term reproducibility of data. Though the surface of polymer-coated columns is usually well shielded from the analytes as they move through the column, most polymer procedures involve a time-consuming and/or experimentally difficult multi-step process. A single step production of polymer coatings has been reported wherein the polymer, a surface derivatizing agent, and a cross-linking agent have been mixed in a solvent which is then placed in a capillary and heat-treated [26]. A cryptand species which is immobilized on the capillary surface through polymer coating is used to generate a charged surface which then serves to control adsorption and EOF [27]. Colon and co-workers have reported an amino-silica glass coating process for the manipulation of EOF [28].

Some other aspects of column technology have also been evaluated such as the effects of column coiling [29], tapered geometries [30], and concentric configurations [31]. A fundamental consideration of whether the fused silica capillary is the only suitable material for CE columns has also been made. A number of other formulations such as polypropylene [32] and polyacrylamide [33] have been evaluated as possible substitutes for silica. Different types of polyethers [34] and diol

[35] moieties have also been used as effective hydrophilic coatings that function in a manner similar to polyacrylamide.

3.3 Packed capillaries

The packed capillary is similar to an HPLC column wherein the stationary phase is chemically bonded to a support particle that is packed into a fused silica capillary. The mechanism of solvent movement in CEC is by electroosmosis in contrast to high pressure in HPLC. The absence of high pressure permits the use of very small particle sizes in the column and results in higher efficiencies than are possible by HPLC. Silanols on the capillary surface promote solvent movement in the capillary that carries the analytes, especially neutral compounds, through the column. Electroosmotic flow is desirable and thus endcapping of the bonded material by reaction with silane is not needed. Three approaches for packing capillary columns are described here. The first approach, similar to the method used for packing HPLC columns, utilizes high pressure through a pneumatic amplification pump [36]. A 10% slurry of the packing material is placed in a reservoir (such as an HPLC column) and packed into the capillary at a pressure of about 600 bar. A precolumn filter placed at the end of the capillary retains the slurry material inside the column during the packing process. After the capillary is filled, on-line sintering with a heating coil is used to generate frits from the packing material. An appropriate choice of temperature and heating cycle are used to fuse the reversed-phase particles together without plugging the capillary. The lower frit is made first and the area

below it is flushed out before the top frit can be fabricated in the same manner. The column is conditioned first by solvent purging and then by application of a low voltage before regular operation is begun.

The second approach utilizes a drawn packed capillary made from a glass tube [13]. A glass tube with a 1 or 2 mm bore that is closed at one end is used to hold well-dried silica. A second high temperature treatment is used to remove any additional traces of water from the packing material. The glass tube is then placed in a drawing machine and the tube is drawn to the desired diameter. This packing process causes embedding of the silica particles in the glass wall giving the packed bed good stability. The drawback to this process is that only bare silica can be packed under these high temperature conditions. In order to make a chemically modified stationary phase the bonding process must be done *in situ* after packing. A solution containing the organosilane reagent is pumped through the column and the capillary is placed in a tubular heater to facilitate bonding. The column is conditioned by flushing with an appropriate solvent for several hours before regular operation is begun.

The third approach for packing capillary columns is called electrokinetic packing [37, 38]. An efficient heater such as a thermal wire stripper is used to make an inlet frit at one end of the capillary by sintering silica particles. A 10% w/w slurry of the packing material is suspended in a well-sonicated buffer containing 4 mM phosphate in methanol. The slurry of the packing material is electrokinetically moved into the column by subjecting it to an appropriate voltage for about 1 hour. A second sintering process is used to fabricate the second frit on the column.

3.4 Open tubular capillaries

This format overcomes some of the fundamental problems associated with using packed columns. The process of fabrication of the frits to hold the packing material in place is normally done *in situ* and thus the frits in each column may not be identical. Beside the addition of an extra step to the column production process, there is the likelihood of the formation of bubbles during the chromatographic run. Both the frits and the packed particles serve as nucleation sites for the formation of bubbles from dissolved gas in the electrolyte under the conditions of high voltage used during the run. This necessitates degassing of the buffer and proper conditioning of the system before the commencement of electrochromatographic measurements.

Various approaches have been developed in order to address the problems of bubble formation and/or the need for the elimination of frits. One of the approaches is to bond the appropriate moiety to the wall of the capillary and to use the solute (analyte)/bonded phase interactions to achieve separation of analytes in a manner similar to GC. In this format, the analyte molecule must travel a considerable distance from the liquid electrolyte at the center of the capillary in order to reach the bonded organic moiety on the wall of the capillary. Diffusion coefficients in liquids are lower by several orders of magnitude than in gases especially for large analyte molecules. This limits the number of solute (analyte)/bonded phase interactions throughout the length of the capillary and these insufficient interactions might not have a significant effect on the differential migration rate, especially for neutral analytes. Another format involves a microchip wherein an octadecyl (C-18) moiety

bonded to the channel operates in the CEC mode [39]. A final approach is to increase the interactions of the solute (analyte) and bonded phase by increasing the surface area of the inner wall of the capillary. This can be achieved by etching the silica surface of the capillary with an agent such as hydrofluoric acid (HF), or conveniently a reagent that is easier to handle such as ammonium hydrogen difluoride (NH_4HF_2) [40]. This process of etching causes an increase in the internal surface area of the capillary of up to 1000-fold and also produces radial extensions from the capillary surface of several microns in length. As a result, the distance the solute (analyte) must travel in order to interact with the stationary phase is decreased. After etching, the required organic moiety is attached to the surface of the capillary by standard bonding chemistry. This type of etched chemically bonded capillary appears to be the most suitable one for mixed mode separations, i.e., analysis of charged analytes wherein the separation mechanism is based on a combination of differences in electrophoretic mobility and solute (analyte)/bonded phase interactions.

4 Goals of the research

Polyacrylamide gels have been traditionally used as sieving media for the separation of electrically charged analytes such as biomolecules (proteins and polynucleotides). These gels have the following drawbacks:

- they are not effective for the separation of uncharged analytes that cannot migrate under an applied electric field, and

- bubble formation is a serious problem in capillary gel electrophoresis using cross-linked polyacrylamide gels and it can interrupt the flow of current.

One study reported in the literature involved polyacrylamide gels of various compositions using acrylamide (AA), N, N' – methylenebisacrylamide (BIS) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) through linear and cross-linking procedures [41]. The conclusion of this study was that dissociation of the AMPS that was built in the polymer made the generation of EOF possible for electrokinetic injection and separation of neutral and ionic compounds. The separation mechanism, however, was dependent on the restricted migration of analytes through the pores in the polymer network.

Another study reported in the literature involved the preparation of hydrophobic stationary phases in an *in-situ* polymerization process [42]. A mixture of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and N-isopropylacrylamide (IPAA_m), N, N' – methylenebisacrylamide (BIS), ammonium persulfate (APS) and N, N, N', N' –tetramethylethylenediamine (TEMED) is reacted in such a way that N-isopropyl groups are incorporated into the polyacrylamide matrix during the polymerization process. The resulting stationary phases gave high column efficiencies, sufficient retention and good column stability.

However, in both the above studies, adsorption of some components was likely to occur at the top of the column, in which case, column performance had to be restored by cutting off a few millimeters from the top of the column. The problem of bubble formation present in traditional gel electrophoresis columns was also inherent

in the preparation and use of the two columns discussed above implying no significant advantage over the traditional method. However, during the course of the studies the bubbles were found to become smaller and finally disappear on the application of a high voltage on the column. Moreover, the gel appeared to have somewhat liquefied inside the capillary possibly due to the Joule heating effect, but nevertheless it behaved as a solid.

This research was aimed at overcoming the drawbacks of capillary electrophoresis using polymer gels incorporating AMPS, as described earlier. Various capillaries were studied in order to elucidate the effects of bonding one organic moiety such as C-18 or AMPS and endcapping with another such as AMPS or C-18 respectively. Figures 3 and 4 show the structures of these organic moieties. The investigations carried out involved unmodified and chemically bonded stationary phases on unetched and etched capillaries. The capillaries studied were:

- bare capillary,
- unetched capillary modified with a C-18 moiety,
- etched capillary modified with a C-18 moiety,
- etched capillary modified with a C-18 moiety and endcapped with AMPS,
- etched capillary modified with an AMPS moiety,
- etched capillary modified with an AMPS moiety and endcapped with C-18.

The studies involving the modified capillaries used the open tubular (OT) approach to CEC wherein all but one of the above capillaries were etched, then modified by silanization with triethoxysilane (TES) followed by hydrosilation where

Figure 3: Structure of 1-octadecene (C-18).

Figure 4: Structure of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS).

the appropriate moiety was attached to the inner surface of the capillary in the presence of a catalyst. The effects of pH on the separation of various analytes such as antibiotics, lysozymes, etc. were studied. The performance of some of the capillaries was estimated by measuring electroosmotic flow (EOF) under conditions of varying pH.

Chapter II

EXPERIMENTAL

1 Materials

List of chemicals used in this study along with their chemical abstract service (CAS) registry numbers.

Name	Source	CAS Registry Number
β -alanine	Sigma	[107-95-9]
γ -aminobutyric acid (GABA)	Sigma	[56-12-2]
2-(N-morpholino)ethane sulfonic acid	Sigma	Not available
Acetic acid	Aldrich Chemicals	[64-19-7]
Acetone	Fischer Scientific	[67-64-1]
Ammonium hydrogen difluoride	Aldrich Chemicals	[1341-49-7]
Chloroplatinic acid hexahydrate	Aldrich Chemicals	[16941-12-1]
Citric acid	Sigma	[77-92-9]
Dichloromethane	Fischer Scientific	[75-09-2]

Diethylether	Fischer Scientific	[60-29-7]
Dimethyl sulfoxide	Cambridge Labs	[67-68-5]
Dioxane	Aldrich Chemicals	[1764-74-4]
Ethanol	Shield Chemicals	[64-17-5]
Hydrochloric acid	Fischer Scientific	[7647-01-0]
Lactic acid	Sigma	[50-21-5]
Methanol	General Chemicals	[67-56-1]
Phosphoric acid	Fischer Scientific	[7664-38-2]
Sodium hydroxide	Fischer Scientific	[1370-73-2]
Tetrahydrofuran (THF)	J. T. Baker	[109-99-8]
Toluene	Fischer Scientific	[108-88-33]
Triethoxysilane (TES)	Aldrich Chemicals	[998-30-1]
Tris [hydroxymethyl] aminomethane	Sigma	[77-86-1]
1-octadecene	Aldrich	[112-88-9]
2-acrylamido-2-methyl-1- propanesulfonic acid (AMPS)	Aldrich	[15214-89-8]
Caffeine	Aldrich	[58-08-2]
Theophylline	Aldrich	[317-34-0]
Serotonin hydrochloride	Aldrich	[153-98-0]

Tryptamine	Aldrich	[61-54-1]
Histidine	Aldrich	[4998-57-6]
2-propanol	Aldrich	[67-63-0]
Boric acid	Aldrich	[10043-35-3]
Lysozyme from chicken egg white	Aldrich	[12650-88-3]

2 Instrumentation

2.1 Capillaries

The capillary tubing used was 375 μm o.d. X 50 μm i.d. (Polymicro Technologies, Phoenix, AZ). The capillary was made of quartz (fused silica) and thus there were silanol groups on the interior wall of the capillary. The capillary had an external coating of polyimide that made it very flexible, strong and not easily broken.

2.2 Gas chromatography oven

The etching and modification of the capillaries were carried out in a Hewlett-Packard Model 5890 gas chromatographic oven. The GC apparatus was modified to permit the placement of several capillaries at both the inlet and the outlet.

Temperatures in the range of 300 $^{\circ}\text{C}$ to 400 $^{\circ}\text{C}$ were used for the etching process.

Thick-walled glass tubes were used as reservoirs for the etching and modification reagents and these glass tubes were placed into plastic containers for safety reasons.

The reservoirs were held in metal containers in the GC apparatus. The particular

reagent was placed into a vial that was either plastic or glass depending on the corrosivity of the reagent; this vial was then placed into the thick-walled glass tube. The glass tubes had swagelok fittings so that nitrogen gas could be used to force the reagents from the capillary inlet that was immersed in the reagent tube to the capillary outlet placed in a collection vial.

2.3 Capillary electrochromatography

2.3.1 Analysis

Different instruments were used to perform CEC separations in the various capillaries:

- bare capillary - a home-made instrument with a Spectra 200 programmable wavelength detector and a ChromJet (Spectra-Physics Analytical) integrator was used.
- unetched capillary modified with C-18, etched capillary modified with C-18 & AMPS – a Spectra PHORESIS 1000[™] system was used. The basic components of the instrument included an automated system for introducing samples and run buffers, a reversible high-voltage power supply and a high-sensitivity on-line detector. The particular capillary was wound in a cassette for easy handling and installation. The cassette was equipped with a detector window that meshed with the fiber-optic assembly for automatic alignment. The electrodes of the cassette were removable and could be installed on different cassettes, provided that proper cleaning procedures were followed.

The instrument had both integrator recorder outputs and an RS-232 interface for output to a computer.

- etched capillary modified with C-18, etched capillary modified with AMPS, and etched capillary modified with AMPS & C-18 – an Applied Biosystems Model 270A (Foster City, CA) Capillary Electrophoresis System was used. Measurements of EOF for the etched capillary modified with C-18 & AMPS and the etched capillary modified with AMPS were carried out in the same systems used for their respective separation studies.

3 Methodology

3.1 Etching of the capillaries

The procedure described earlier was used to connect two 50 μm bare capillaries in the GC oven. The inlet plastic vial was filled with about 1 mL of concentrated HCl. A nitrogen gas pressure of 40 psi was maintained to fill the capillaries with this concentrated HCl. The capillaries were heated overnight at 80 °C. The capillaries were washed by successively flushing them with deionized water, acetone and diethyl ether. Again a nitrogen gas pressure of 40 psi was used to dry the capillaries for an hour.

A 5% (w/v) saturated solution of ammonium hydrogen difluoride was prepared in methanol and was used to etch the capillaries. The inlet plastic vial was filled with about 1 mL of this etching solution and a nitrogen gas pressure of 40 psi was used to fill the capillaries with the etching solution. The capillaries remained

filled with the etching solution for about an hour and a nitrogen gas pressure of 40 psi for 5 minutes was used to remove the methanol from the capillary. The ends of the capillaries were sealed using rubber corks while the remaining sections of the capillaries were coiled neatly, wrapped in aluminum foil and placed inside the GC oven. The capillaries were first heated for 2 hours at 300 °C and then for 1 hour at 400 °C in the presence of nitrogen. Flushing them with methanol once using a syringe washed the capillaries and a nitrogen gas pressure of 40 psi for 2 hours was used to dry the capillaries. A light microscope was used to detect any blockage in the inner bore of the capillaries.

3.2 Formation of the hydride layer

A nitrogen gas pressure of 40 psi for 20 hours was used to treat the capillaries at room temperature with 0.1 M NaOH at a flow rate of 0.1-0.2 ml/h. The same nitrogen gas pressure was used to rinse the capillaries by flushing with deionized water for 1 hour and then with 0.1 M HCl for 4 hours. A nitrogen gas pressure of 40 psi was used to rinse the capillaries with deionized water for 2 hours and to dry them at 100 °C for 20 hours. This process was used to precondition the capillaries for the process of silanization.

In order to begin the process of silanization, the capillaries were washed by flushing them with dioxane for ten minutes. A 1 M TES solution in 8.4 mL of dioxane was freshly prepared by mixing in a nitrogen glove box. A plastic vial was used to hold the TES solution at the inlet end of the capillary. A nitrogen gas

pressure of 40 psi for 90 minutes was used to flush this solution through the capillaries at 90 °C. This step resulted in the formation of the hydride layer in the capillary. At the conclusion of this step, a nitrogen gas pressure of 40 psi was used to wash the capillaries for 2 hours at room temperature first with dioxane and then with acetone. Again a nitrogen gas pressure of 40 psi was used to wash the capillaries with dry toluene and dry them before attachment of the appropriate organic moiety.

3.3 Apparatus for the synthesis of the bonded material

The bare capillary, unetched capillary modified with C-18 and the etched capillary modified with C-18 were provided from another study. Three additional capillaries with stationary phases were synthesized for this study. One of the hydride-modified capillaries was further modified with a C-18 moiety and then endcapped with AMPS. A second hydride-modified capillary was further modified only with AMPS while a third such capillary was further modified with an AMPS moiety and then endcapped with C-18.

Prior to preparation of the material to be attached to the wall of the capillary, all glassware used was washed thoroughly, rinsed with deionized water and dried in an oven. A 50 mL three-neck round bottom flask was used to prepare the particular reagent solution. A thermometer was placed into one neck opening of such a flask and the opening was sealed with parafilm. A second neck opening was closed with a stopper and sealed with parafilm. A magnetic stirrer bar was placed inside the flask together with the reagents described in the methodology section below. A West

condenser was placed in the middle neck opening of the flask and cooling water was circulated through it. The flask was placed on a heating mantle that was then placed on a magnetic stirrer. The contents of the flask were refluxed for 1 hour.

3.3.1 Preparation of the C-18 mixture

The C-18 mixture was prepared as follows: 2 ml of pure 1-octadecene was mixed with 70 μ l of 10 mM Spiers catalyst (hexachloroplatinic acid in 2-propanol) and this mixture was heated at 60-70 °C for 1 hour. The experimental set up is described in Section 3.3. Spier's catalyst consists of 0.04 g of chloroplatinic acid hexahydrate dissolved in 100 mL of 2-propanol and stored in the freezer.

3.3.1.a Attachment of the C-18 moiety

Two capillaries, one hydride-modified and the other AMPS modified, were washed with dry toluene, dried and then flushed with the C-18 solution for 90 hours at 100 °C. At the end of this period the capillaries were washed with methanol for 12 hours and a nitrogen gas pressure of about 40 psi was used to dry them overnight at 100 °C.

3.3.2 Preparation of the AMPS mixture

The AMPS mixture was prepared as follows: 0.6 g of AMPS was dissolved in a 3:1 v/v ethanol/methanol solution i.e., 6 ml methanol and 2 ml ethanol. This solution was mixed with 70 μ l of 10 mM Spiers catalyst (hexachloroplatinic acid in

2-propanol) and heated for 1 hour at 60-70 °C in the apparatus described in Section 3.3.

3.3.2.a Attachment of the AMPS moiety

Two capillaries, one hydride modified and the other C-18 modified, were washed with dry toluene, dried and then flushed with the AMPS solution for 90 hours at 68 °C. At the end of this period the capillaries were washed with methanol for 12 hours and a nitrogen gas pressure of about 40 psi was used to dry them overnight at 100 °C.

3.4 Preparation of buffers

The buffers used in this study were: pH = 2.14, 30 mM phosphate (phosphate was from 85% H_3PO_4) and 19 mM Tris; pH = 3.00, 30 mM citric acid and 25 mM β -alanine; pH = 3.70, 60 mM β -alanine and 60 mM lactic acid; pH = 4.41, 30 mM acetic acid and 37.5 mM γ -amino butyric acid; pH = 6.00, 60 mM 2-(N-morpholino)ethanesulfonic acid and 45 mM histidine; pH = 7.40, 30 mM monobasic potassium phosphate (KH_2PO_4) and 19 mM Tris; pH = 8.14, 10 mM Tris and 15 mM boric acid. Deionized water was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA) and was filtered through a 0.20 μm Nylon 66 membrane filter (Altech Assoc., Deerfield, IL). The buffers were diluted with deionized water in the appropriate ratios to obtain the particular concentrations indicated on the electropherograms. For practical purposes, helium sparging which

removes approximately 80% of the air from solution is the best choice for degassing the buffers [43]. A suitable length of PTFE tubing was connected to the outlet of a regulator mounted on a high-purity helium cylinder. A sparging frit connected to the end of the tubing was submersed in the buffer and the regulator was adjusted to release a gentle stream of bubbles. Each buffer was degassed with helium for 10 minutes prior to use, in the manner described above, which was sufficient for a day's operation.

3.5 Electrochromatographic analysis

3.5.1 Making of a detection window on the capillary

The capillary was shortened to the appropriate length for the particular electrophoresis instrument with a capillary cutter. The appropriate position for the detection window was measured and marked on the capillary with a permanent marker. A glass block with tiny grooves was heated on a heating mantle in the fume hood until it was very hot; a few drops of concentrated sulfuric acid were added in the grooves. When the acid solution was hot the marked area for the window on the capillary was securely placed in one of the grooves for approximately two minutes. The polyimide coating was removed from the outer surface of the capillary exposing the quartz glass as a small white patch. The capillary was wiped with methanol and deionized water to prepare it for insertion in the electrophoresis instrument.

3.5.2 Preparation of the samples

Most of the drugs were water-soluble. Typically 1 mg of the sample was dissolved in 1 mL of deionized water (0.1%) and stored in vials in a refrigerator. Figures 5 and 6 show the structures of the tetracycline antibiotics studied. Figure 7 shows the structures of other compounds studied.

3.5.3 Effects of buffers and voltages on separations

Each of the samples was investigated for separation using most of the buffers prepared (Section 3.4) at the following voltages: 30 kV, 25 kV, 20 kV, 15 kV and 10 kV. The experimental set up is described in Chapter I, Section 2.1.1. Once in the instrument, the capillary was equilibrated with the running buffer for 10 minutes. After every run, the capillary was flushed with the running buffer for 2 minutes.

3.5.4 Measurements for electroosmotic flow (EOF)

The capillary was first flushed with the running buffer for 2 minutes. About 1 μ L of DMSO, the neutral marker, was dissolved in 1 mL of water (0.1%). This marker was injected into the capillary under a vacuum of 5" of Hg. The marker band was detected at 211 nm. The EOF for all buffer conditions used in the study was then calculated as discussed in Chapter I, Section 2.4.

Doxycycline

Meclocycline

Minocycline

Figure 5: Structures of the tetracycline antibiotics used in the study.

Figure 6: Structures of the tetracycline antibiotics used in the study (cont'd).

Oxytetracycline

Tetracycline

Caffeine

Theophylline

Serotonin

Tryptamine

Figure 7: Structures of some other drugs used in the study.

Chapter III

RESULTS AND DISCUSSION

1 Etching and modification of the capillaries

There are various chemical reactions used to modify silica surfaces [44]. A fused silica capillary consists of weakly acidic surface hydroxyl groups (silanols i.e., Si-OH) and when this surface is treated with the etching agent made up of a saturated solution of 5% ammonium hydrogen difluoride followed by heating at high temperatures, strong protonic sites are created on the silica surface by partial replacement of -OH groups by -F and the resulting surface exhibits high catalytic activity [45]. The electronegative fluorine atom causes an electron shift in the neighboring atoms, which results in the weakening of the hydroxyl bond in the silanol group and increase in the surface acidity. The acid groups produced are of the Bronsted type as seen in Figure 8 [45]. The capillary surface now consists of Si-OH and Si-F groups and has a high surface area.

In the above reaction, the fused silica capillaries first dissolve non-uniformly in the etching agent and then precipitate back on the capillary surface and are seen as radial extensions from the surface. The capillary surface area increases up to 1000-fold, thus opening up sites for attachment of the stationary phase material [46]. The process is allowed to continue first at 300 °C for 2 hours and then at 400 °C for 1 hour, which leads to the formation of a more uniform and better-defined surface. Various studies have proved the reproducibility of the surface morphology that results

from a given set of well-controlled etching conditions. The mechanical stability of these surface structures has been investigated by vibration of the capillaries for 30 minutes [41]. The studies found that the surfaces produced under the above conditions do not undergo any damage and can withstand mechanical stress from vibration.

The capillary surface is treated with TES, which removes the silanol groups that were exposed by the etching agent, and a monolayer of silica hydride is formed. This reaction process is called silanization.

A reaction process called hydrosilation brings about attachment of the stationary phase onto the silica hydride monolayer. This attachment requires the presence of a catalyst and a metal complex is often used. The catalyst usually consists of a solution of a halide, olefin, carbonyl, or phosphine complex of transition metals such as platinum, palladium etc. A 2-propanol solution of hexachloroplatinic acid called Spier's catalyst is the most commonly used compound. Usually 10^{-7} to 10^{-4} mol of platinum /mol of silicon hydride is sufficient for an effective hydrosilation reaction. The addition reaction becomes manifest after an "induction period" when a highly exothermic reaction occurs. In the case of simple liquid olefins such as C-18 no additional solvent is required. The reaction was conveniently carried out under dry conditions, at temperatures ranging from 60 °C to 70 °C [47]. Figure 9 shows the process described above. Figure 10 shows the structures of the bonded moieties on the silica surface.

Figure 8: The electronegative fluorine atom causes an electron shift in the neighboring atoms resulting in the weakening of hydroxyl bonds.

Figure 9: Diagram of the reactions on the surface of the capillary wall.

a. Bare capillary

b. Unetched or etched capillary modified with C-18

c. Etched capillary modified with AMPS

d. Etched capillary modified with C-18 and endcapped with AMPS or modified with AMPS and endcapped with C-18

Figure 10: Structures of the capillary surfaces used in the study.

2 Choice of test solutes

2.1 Caffeine and theophylline

Caffeine belongs to the group of medicines called central nervous system (CNS) stimulants. It is used to help restore mental alertness when unusual tiredness or weakness or drowsiness occurs. Caffeine is sometimes used in combination with an antihistamine to overcome the drowsiness caused by the antihistamine.

Theophylline, aminophylline and oxtriphylline are used to treat and/or to prevent the symptoms of bronchial asthma, chronic bronchitis, and emphysema. These medicines relieve cough, wheezing, shortness of breath, and troubled breathing. They work by opening up the bronchial tubes (air passages of the lungs) and increasing the flow of air through them.

2.2 Chicken and turkey lysozymes

Lysozymes dissolve certain bacteria by cleaving the polysaccharide component of their cell walls. A bacterial cell devoid of a cell wall usually bursts because of the high internal osmotic pressure.

2.3 Serotonin and tryptamine

Serotonin is 5-hydroxytryptamine; it increases the dilation of blood vessels and stimulates platelet aggregation (blood clotting).

2.4 Tetracycline antibiotics

Tetracyclines are used to treat infections and to help control acne.

Doxycycline and minocycline may also be used for other problems.

3 Interpretations of chromatographic data

3.1 Separation of caffeine and theophylline

The separations of a mixture of caffeine and theophylline at pH ~ 8.14 using the capillaries described above are shown in Figures 11(a) – 11(f). As seen in Figure 11(a), the result obtained on a bare 50 μm capillary is illustrated, where the two solutes are not baseline resolved and their migration times are small. The silica surface of a capillary in contact with an electrolyte at pH value > 3 contains chemically bound Si-O⁻ groups and is negatively charged. The pK_a for the cationic theophylline molecule is 2.5 and that for the neutral molecule is 8.8 [48]; a sharp change in electrophoretic mobility is observed between pH 7 and 9 [49]. At pH > 6.8, dissociation of the hydrogen atom in the imidazole ring of theophylline causes the molecule to acquire a negative charge [49]. There is no such proton available for dissociation in the imidazole ring of caffeine and thus it is uncharged at pH 8.14. Under the high pH conditions of pH 8.14, EOF in the bare capillary is high and separation of the solutes is only due to the differences in their electrophoretic mobilities; this separation pattern was difficult to reproduce using the same set of separation conditions. In Figure 11(b), chemical modification of the same i.d. capillary by a silanization/hydrosilation method using 1-octadecene as the organic

Figure 11(a): Separation of caffeine & theophylline on 50 μm bare capillary.
L = 50 cm, l = 32 cm, V = 25 kV, pH = 8.18 (1:10)
1 = theophylline, 2 = caffeine.

Figure 11(b): Separation of caffeine & theophylline on 50 μm unetched and C-18 modified capillary. $L = 34.4$ cm, $l = 26.4$ cm, $V = 10$ kV, $\text{pH} = 8.14$ (1:10)
1 = caffeine, 2 = theophylline.

**Figure 11(c): Separation of caffeine & theophylline on 50 μm etched and C-18 modified capillary. $L = 47\text{ cm}$, $l = 25\text{ cm}$, $V = 20\text{ kV}$, $\text{pH} = 8.18$ (1:10)
1 = caffeine, 2 = theophylline.**

Figure 11(d): Separation of caffeine & theophylline on 50 μm etched and C-18 & AMPS modified capillary. $L = 35\text{ cm}$, $l = 27\text{ cm}$, $V = 15\text{ kV}$, $\text{pH} = 8.18$ (1:10)
1 = caffeine, 2 = theophylline.

Figure 11(e): Separation of caffeine & theophylline on 50 μm etched and AMPS modified capillary. $L = 50\text{ cm}$, $l = 25\text{ cm}$, $V = 30\text{ kV}$, $\text{pH} = 8.14$ (1:10)
1 = caffeine, 2 = theophylline.

Figure 11(f): Separation of caffeine & theophylline on 50 μm etched and AMPS & C-18 modified capillary. $L = 49.6\text{ cm}$, $l = 25\text{ cm}$, $V = 30\text{ kV}$, $\text{pH} = 8.14$ (1:10)
1 = caffeine, 2 = theophylline.

moiety bonded to the inner wall, results in baseline resolution of the two sample components with reasonably symmetrical peaks. The chemical modification process decreases EOF in the capillary significantly by derivatizing most of the residual silanols on the column surface and leads to longer retention times for the solutes. The separation of the sample components is based on a combination of differences in electrophoretic mobilities and solute/bonded phase interactions and leads to resolution of the solutes. As seen in Figure 11(c), the result obtained on etching the inner surface of the capillary prior to chemical modification by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall is illustrated, where the two solutes are retained significantly longer. Etching of the silica surface of capillaries with ammonium hydrogen difluoride causes a large increase in surface area of up to 1000-fold. In addition, the chemical modification process of silanization/hydrosilation decreases EOF in the capillary significantly by derivatizing some of the residual silanols on the column surface. The enhanced solute/bonded phase interactions between each of the sample components and the etched and modified column surface are reflected in the longer retention times for the two solutes. Investigations with individual solutions of caffeine and theophylline, using the same set of separation conditions as those used for the two solute mixture, demonstrated similar retention times and peak shapes. In Figure 11(d), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall followed by a second hydrosilation method using AMPS as the organic

moiety, shows baseline resolution and relatively short retention times for the two sample components. The dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and an increase in EOF, which in turn causes shorter retention times for the two solutes. The second hydrosilation method serves as a process of endcapping the uncovered hydrides that remain after the first such reaction. This endcapping process results in lower interaction between each of the sample components and the column surface leading to better peak shapes for the solutes. As seen in Figure 11(e), the result obtained on chemical modification of the etched capillary with AMPS is illustrated, where the two solutes are baseline resolved with short retention times. In this case again, the dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and an increase in EOF, which in turn causes shorter retention times for the two solutes. Though the mechanism of separation of the sample components is based on a combination of differences in electrophoretic mobilities and solute/bonded phase interactions, the retention times are similar to those obtained with electrophoretic separation in the bare capillary. In Figure 11(f), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using AMPS as the organic moiety bonded to the inner wall followed by a second hydrosilation method using 1-octadecene as the organic moiety, shows further baseline resolution and slightly longer retention times for the sample components. The second hydrosilation method, which serves as a process of endcapping the remaining uncovered hydrides, leads to slightly longer retention times.

3.2 Separation of chicken and turkey lysozymes

The separations of a mixture of chicken and turkey lysozymes at pH 3.70 using the capillaries described above are shown in figures 12(a) – 12(f). In Figure 12(a), the result obtained on a bare 50 μm i.d. capillary is illustrated, which shows only a partial separation of these two proteins. The silica surface of a capillary in contact with an electrolyte at pH value > 3 contains chemically bound Si-O^- groups and is negatively charged. At pH 3.70, EOF in the bare capillary is sufficiently small to allow separation of the solutes due to the differences in their electrophoretic mobilities. As seen in Figure 12(b), chemical modification of the same i.d. capillary by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall does not lead to significant improvement in the separation, as to achieve baseline resolution of the two lysozymes. Moreover, the chemical modification process is expected to decrease EOF in the capillary by derivatizing some of the residual silanols on the column surface and lead to longer migration times for the solutes. This shows that, at pH 3.70, EOF is small compared to electrophoretic mobility and there is not much improvement in the separation. Any decrease in EOF that might cause longer retention times for the sample components is overcome with an increase in the applied potential prior to separation. This is observed on comparison of the retention times exhibited by the solutes in the bare capillary at 25 kV and in the unetched and chemically modified capillary at 15 and 20 kV. A comparative separation study of the effect of applied potential between

Figure 12(a): Separation of chicken & turkey lysozymes on 50 μm bare capillary. $L = 50\text{ cm}$, $l = 32\text{ cm}$, $V = 25\text{ kV}$, $\text{pH} = 3.70$ (1:10)
 1 = turkey lysozyme, 2 = chicken lysozyme.

Figure 12(b): Separation of chicken & turkey lysozymes on 50 μ m unetched and C-18 modified capillary. L = 34.4 cm, l = 26.4 cm, pH = 3.70 (1:10)

1 = turkey lysozyme, 2 = chicken lysozyme

A. V = 15 kV,

B. V = 20 kV.

**Figure 12(c): Separation of chicken & turkey lysozymes on 50 μm etched and C-18 modified capillary. $L = 47\text{ cm}$, $l = 25\text{ cm}$, $V = 15\text{ kV}$, $\text{pH} = 3.70$ (1:10)
1 = turkey lysozyme, 2 = chicken lysozyme.**

**Figure 12(d): Separation of chicken & turkey lysozymes on 50 μ m etched and C-18 & AMPS modified capillary. L = 35 cm, l = 27 cm, V = 15 kV, pH = 3.70 (1:10)
1 = turkey lysozyme, 2 = chicken lysozyme.**

Figure 12(e): Separation of chicken & turkey lysozymes on 50 μm etched and AMPS modified capillary. $L = 50\text{ cm}$, $l = 25\text{ cm}$, $V = 25\text{ kV}$, $\text{pH} = 3.70$ (1:10)
1 = turkey lysozyme, 2 = chicken lysozyme.

Figure 12(f): Separation of chicken & turkey lysozymes on 50 μm etched and AMPS & C-18 modified capillary. $L = 49.6$ cm, $l = 25$ cm, $V = 20$ kV, $\text{pH} = 3.70$ (1:10)
 1 = turkey lysozyme, 2 = chicken lysozyme.

10 kV and 30 kV, in 5 kV increments, showed better and optimum resolution of the two species at the lower applied potential of 15 kV than at 20 kV. In Figure 12(c), the result obtained on etching the inner surface of the capillary prior to chemical modification by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall is illustrated. In comparison to the earlier capillaries, there is further improvement in the separation of these two proteins with separation of each of the two principle sample components as well as one small impurity. Etching of the silica surface of capillaries with ammonium hydrogen difluoride causes a large increase in surface area of up to 1000-fold. The enhanced solute/bonded phase interactions between each of the sample components and the etched and modified column surface are reflected in the improved separation and longer retention times for the two solutes. As seen in Figure 12(d), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall followed by a second hydrosilation method using AMPS as the organic moiety shows only a partial separation of the two lysozymes. The dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and an increase in EOF. The decreased solute/bonded phase interactions between each of the sample components and the etched, modified and endcapped column surface are reflected in the partial separation and in the shorter retention times of the two solutes. In Figure 12(e), analysis of the sample mixture in the etched capillary modified with AMPS does not show complete separation of these two proteins. In this case again, the

dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and an increase in EOF. As seen in Figure 12(f), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using AMPS as the organic moiety bonded to the inner wall followed by a second hydrosilation method using 1-octadecene as the organic moiety, shows complete separation and baseline resolution of the two sample components. The second hydrosilation method serves as a process of endcapping the uncovered hydrides that remain after the first such reaction. This process of endcapping results in lower interaction between the sample components and the column surface leading to better peak shapes for the solutes. In this capillary, it is found that the elution of a small amount of impurity in the sample mixture occurs at the end of separation of the two principle solute components. This elution order of the impurity is in sharp contrast to that obtained in the etched capillary modified with 1-octadecene wherein elution of the sample impurity occurs between that of the two principle solute components. It can be surmised that of all the capillaries used for the separation of chicken and turkey lysozymes, the etched capillary modified first with AMPS and then with 1-octadecene appears to have the most favorable properties to achieve baseline resolution of these two proteins.

3.3 Separation of serotonin and tryptamine

The separations of a mixture of serotonin and tryptamine at pH 2.14 using the

capillaries described above are shown in figures 13(a) – 13(f). In Figure 13(a), the result obtained on a bare 50 μm i.d. capillary is illustrated, where the two solutes are baseline resolved. Under the low pH conditions of pH 2.14, serotonin and tryptamine are fully charged and EOF is fairly low; thus separation of the solutes is only due to the differences in their electrophoretic mobilities. As seen in Figure 13(b), chemical modification of the same i.d. capillary by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall, leads to almost insignificant peak tailing with improvement in the peak shape. But, the chemical modification process is expected to decrease EOF in the capillary by derivatizing some of the residual silanols on the column surface and lead to longer retention times for the solutes. The enhanced solute/bonded phase interactions between each of the sample components and the modified column surface are reflected in the improved peak quality. A comparative separation study of the effect of applied potential between 10 kV and 30 kV, in 5 kV increments, showed better resolution of the two species at the lower applied potential of 15 kV than at 20 kV. In Figure 13(c), the result obtained on etching the inner surface of the capillary prior to chemical modification is illustrated, where the two solutes are baseline resolved. Etching of the silica surface of capillaries with ammonium hydrogen difluoride causes a large increase in surface area of up to 1000-fold. In addition, the chemical modification process of silanization/hydrosilation decreases EOF in the capillary significantly by derivatizing some of the residual silanols on the column surface. The enhanced solute/bonded phase interactions between each of the sample components and the

Figure 13(a): Separation of serotonin & tryptamine on 50 μm bare capillary.
L = 50 cm, l = 32 cm, V = 20 kV, pH = 2.14 (1:10)
1 = tryptamine, 2 = serotonin.

Figure 13(b): Separation of serotonin & tryptamine on 50 μm unetched and C-18 modified capillary. $L = 34.4$ cm, $l = 26.4$ cm, $\text{pH} = 2.14$ (1:10)

1 = tryptamine, 2 = serotonin

A. $V = 15$ kV,

B. $V = 20$ kV.

**Figure 13(c): Separation of serotonin & tryptamine on 50 μm etched and C-18 modified capillary. $L = 47\text{ cm}$, $l = 25\text{ cm}$, $V = 15\text{ kV}$, $\text{pH} = 2.14$ (1:10)
1 = tryptamine, 2 = serotonin.**

Figure 13(d): Separation of serotonin & tryptamine on 50 μm etched and C-18 & AMPS modified capillary. $L = 35\text{ cm}$, $l = 27\text{ cm}$, $V = 15\text{ kV}$, $\text{pH} = 2.14$ (1:10)
1 = tryptamine, 2 = serotonin.

Figure 13(e): Separation of serotonin & tryptamine on 50 μm etched and AMPS modified capillary. $L = 50\text{ cm}$, $l = 25\text{ cm}$, $V = 20\text{ kV}$, $\text{pH} = 2.14$ (1:15)
1 = tryptamine, 2 = serotonin.

Figure 13(f): Separation of serotonin & tryptamine on 50 μm etched and AMPS & C-18 modified capillary. $L = 49.6$ cm, $l = 25$ cm, $V = 10$ kV, $\text{pH} = 2.14$ (1:20)
1 = tryptamine, 2 = serotonin.

etched and modified column surface are reflected in the longer retention times for the two solutes. Under separation conditions that are identical to those used in case of the unetched capillary modified with 1-octadecene, an increase in retention time is readily apparent. As seen in Figure 13(d), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall followed by a second hydrosilation method using AMPS as the organic moiety, shows short retention times for the two sample components. But, this decrease in retention time is accompanied by degradation in peak shape with only partial resolution of the sample components and some peak tailing for the compounds. In Figure 13(e), analysis of the sample mixture in the etched capillary modified with AMPS shows baseline resolution and good peak shape for the two sample components. In this case again, the dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and an increase in EOF, which in turn causes shorter retention times for the two solutes. As seen in Figure 13(f), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using AMPS as the organic moiety bonded to the inner wall followed by a second hydrosilation method using 1-octadecene as the organic moiety takes away the baseline resolution achieved earlier. The second hydrosilation method, which serves as a process of endcapping the remaining uncovered hydrides, results in slightly longer retention times and broader peaks for the solutes.

3.4 Separation of tetracycline antibiotics

The separations of a mixture of tetracycline antibiotics at pH 2.14 using the capillaries described above are shown in figures 14(a) – 14(f). As seen in Figure 14(a), the result obtained on a bare 50 μm i.d. capillary is illustrated, which clearly shows the separation of only one of the components from the rest of the sample mixture. Investigations with individual solutions of each antibiotic using the same set of separation conditions as those used for the sample mixture demonstrated similar migration times and peak shapes for each of the species. Minocycline has an additional nitrogen atom and a higher charge than the other compounds in the sample mixture. This explains its higher electrophoretic mobility than the rest of the compounds and its subsequent elution as the first peak in the electrochromatogram. Under the low pH conditions used for the analysis, it has the smallest degree of interaction with the capillary surface in contrast to the other solutes. In Figure 14(b), chemical modification of the same i.d. capillary by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall, results in shorter retention times for the solutes. But, the chemical modification process is expected to decrease EOF in the capillary by derivatizing some of the residual silanols on the column surface and lead to longer retention times for the solutes. In contrast to the earlier bare capillary, there is a slight decrease in retention times for the solutes, which is accompanied by incomplete separation of some of the sample components. A comparative separation study of the effect of applied potential between 10 kV and 30 kV, in 5 kV increments, did not show better peak shape or separation efficiency at the lower applied potential of 15 kV than at 20 kV. As seen in

Figure 14(a): Separation of tetracycline antibiotics on 50 μm bare capillary.
L = 50 cm, l = 32 cm, V = 20 kV, pH = 2.14 (1:10)
1 = minocycline, 2 = tetracycline, 3 = oxytetracycline, 4 = doxycycline,
5 = methacycline, 6 = meclocyline.

Figure 14(b): Separation of tetracycline antibiotics on 50 μm unetched and C-18 modified capillary. $L = 34.4$ cm, $l = 26.4$ cm, $\text{pH} = 2.14$ (1:10)

1 = minocycline, 2 = tetracycline, 3 = oxytetracycline,
4 = doxycycline, 5 = meclocycline, 6 = methacycline

A. $V = 15$ kV,

B. $V = 20$ kV.

Figure 14(c): Separation of tetracycline antibiotics on 50 μm etched and C-18 modified capillary. $L = 47\text{ cm}$, $l = 25\text{ cm}$, $V = 20\text{ kV}$, $\text{pH} = 2.14$ (1:10)
1 = minocycline, 2 = tetracycline, 3 = oxytetracycline, 4 = doxycycline,
5 = methacycline, 6 = meclocyline.

Figure 14(d): Separation of tetracycline antibiotics on 50 μm etched and C-18 & AMPS modified capillary. $L = 35\text{ cm}$, $l = 27\text{ cm}$, $V = 15\text{ kV}$, $\text{pH} = 2.14$ (1:10)
1 = minocycline, 2 = tetracycline, 3 = oxytetracycline, 4 = doxycycline, 5 = methacycline, 6 = meclocyline.

Figure 14(e): Separation of tetracycline antibiotics on 50 μm etched and AMPS modified capillary. $L = 50\text{ cm}$, $l = 25\text{ cm}$, $V = 10\text{ kV}$, $\text{pH} = 2.14$ (1:15)
1 = minocycline, 2 = tetracycline, 3 = oxytetracycline, 4 = doxycycline,
5 = methacycline, 6 = meclocyline.

Figure 14(f): Separation of tetracycline antibiotics on 50 μm etched and AMPS & C-18 modified capillary. $L = 49.6$ cm, $l = 25$ cm, $V = 15$ kV, $\text{pH} = 2.14$ (1:20)
1 = minocycline, 2 = tetracycline, 3 = oxytetracycline, 4 = doxycycline, 5 = methacycline, 6 = meclocycline.

Figure 14(c), the result obtained on etching the inner surface of the capillary prior to chemical modification by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall is illustrated, which shows separation of two additional sample components, but with no baseline resolution. In Figure 14(d), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using 1-octadecene as the organic moiety bonded to the inner wall followed by a second hydrosilation method using AMPS as the organic moiety, shows baseline resolution and longer retention times for the sample components. The second hydrosilation method serves as a process of endcapping the uncovered hydrides that remain after the first such reaction. This endcapping process results in lower interaction between each of the sample components and the silanols on the column surface leading to better peak shape for the solutes. As seen in Figure 14(e), the result obtained on chemical modification of the etched capillary with AMPS is illustrated, which shows separation of some of the sample components, but with no baseline resolution. Moreover, in contrast to the earlier capillary, the dissociation of AMPS built into the stationary phase that generates a negative charge on the column surface, is expected to increase EOF and lead to shorter retention times for the solutes. In Figure 14(f), chemical modification of the same i.d. capillary first by the silanization/hydrosilation method using AMPS as the organic moiety bonded to the inner wall followed by a second hydrosilation method using 1-octadecene as the organic moiety is illustrated. As seen in the figures, the number of solutes in the tetracycline sample mixture was precisely determined in relatively short time by analysis in the etched capillary modified first with 1-octadecene and endcapped with AMPS. Of

all the capillaries used for the separation of the tetracycline antibiotics, this capillary appears to have the most favorable properties to achieve near baseline resolution of these six compounds.

4 EOF measurements

Dimethylsulfoxide (DMSO) was used as a neutral marker and the effect of various pHs on EOF was measured. The observation and interpretation of data are discussed with respect to each capillary.

4.1 Effect of pH on EOF

An increase in the pH of the buffer causes more dissociation of the silanols on the capillary surface to negatively charged silanoate ions. A zeta potential develops on the surface of the capillary wall and is proportional to the surface charge. This results in an increase in the electroosmotic velocity. An increase in pH would thus lead to an increase in EOF. Figure 15 shows the effect of EOF as a function of pH for the etched capillary modified with C-18 and AMPS and Figure 16 shows the effect of EOF as a function of pH for the etched capillary modified with AMPS.

4.1.1 Etched capillary modified with C-18 and AMPS

In case of the etched capillary modified with C-18 and AMPS, an anodic EOF is observed at pH less than 4.41. With an increase in pH, an increase in EOF was

**Figure 15: Effect of EOF as a function of pH in etched capillary modified with C-18 & endcapped with AMPS.
(Marker is DMSO with detection at 211nm)**

**Figure 16: Effect of EOF as a function of pH in etched capillary modified with AMPS.
(Marker is DMSO with detection at 211 nm)**

observed as shown in Figure 15. The anodic EOF is probably due to the etching of the silica surface (containing the silanol groups) with ammonium hydrogen difluoride. A nitrogen containing species from the etching agent may be attached to the silica surface forming a continuum in the silica matrix. This entity would be affected by the pH of the buffer used. Since the nitrogen species has a lone pair of electrons, it would accept a hydrogen ion from the buffer. Therefore, at pH below 4.41, the nitrogen is probably protonated and the EOF is observed to be anodic. Above pH = 4.41, the EOF switches to the cathodic direction because the nitrogen as well as the silanols become deprotonated giving the surface a net negative charge. Although the anodic flow is small it retards the movement of solutes through the column thus enhancing analyte (solute)/bonded phase interactions and resolution.

4.1.2 Etched capillary modified with AMPS

In case of the etched capillary modified with AMPS, EOF was positive throughout. With an increase in pH, an increase in EOF was observed as shown in Figure 16. The etching of the silica surface (containing the silanol groups) with ammonium hydrogen difluoride should result in the attachment of a nitrogen containing species to the silica surface forming a continuum in the silica matrix. This entity should be affected by the pH of the buffer used and result in switching of the direction of the EOF at a particular pH. But, the dissociation of AMPS built into the stationary phase generates a negative charge on the column surface and produces a cathodic EOF. Thus, cathodic EOF is an evidence of the successful bonding of AMPS.

Chapter IV

CONCLUSION

The chemical modifications of the capillaries were carried out by the sequence of etching, silanization, and hydrosilation reactions. The etching process results in an increase in the internal surface area of the capillaries of up to 1000-fold. In addition, in some cases, chemical modification of the capillaries after etching led to selective interaction between the stationary phase and the solutes resulting in a more efficient separation. Surface coverage could not be estimated but it could be assumed that 1-octadecene being bulky, surface coverage would be less in terms of the number of 1-octadecene molecules per m^2 when compared to the AMPS bonded phase. In case of the etched capillary modified with 1-octadecene and endcapped with AMPS, EOF was anodic below $\text{pH} = 4.41$ and cathodic above it. In case of the etched capillary modified with AMPS, EOF was cathodic throughout.

Though the process of endcapping was found to be effective in preventing the adsorption of some basic compounds, the order of attachment of the organic moieties played a significant role in the overall effectiveness of the capillary as a valuable separation medium. For a tetracycline sample mixture, it was possible to separate all the solute components on the etched capillary modified with 1-octadecene and endcapped with AMPS. But, it was not possible to separate five of the six solutes in the sample mixture on the etched capillary modified with AMPS and endcapped with 1-octadecene.

Spectroscopic studies (such as IR) are needed in order to further confirm the success of etching and modification procedures of the capillaries. The mechanisms of separations mentioned earlier are only probable and future studies of the capillaries are needed. Such studies could involve computer modeling of the solutes in order to definitely explain the retention mechanisms during analyses of the various samples. Elucidation of the chemical features of the etched surface and of the surfaces obtained after each silanization/hydrosilation reaction might be beneficial. Such characterization data might provide an explanation for the range of separation patterns obtained among the various etched and chemically modified capillaries. Other solute samples that could be analyzed include hydrophobic drugs (such as dextromethorphan, methadone, etc.); amino acids and DNA adduct mixtures; and uncharged molecules such as ketones.

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