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Investigation of electroosmotic fluid velocity in roughened capillaries

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**INVESTIGATION OF ELECTROOSMOTIC FLUID VELOCITY IN ROUGHENED
CAPILLARIES**

A Thesis

Presented to

The Faculty of the Department of Chemical and Materials Engineering

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Srikant Surya Mantha

December 2006

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ABSTRACT

INVESTIGATION OF ELECTROOSMOTIC FLUID VELOCITY IN ROUGHENED CAPILLARIES

by Srikant Surya Mantha

Control of electroosmotic fluid velocity is necessary to optimize analytical separations using capillary electrophoresis (CE) and capillary electrochromatography (CEC). Electric field strength and buffer concentration are common ways to control electroosmotic fluid velocity. Etching and surface derivitization are necessary in open tubular approaches to CEC in order to achieve a second degree of separation through solute bonded phase interactions. Etched capillaries can have a surface area of up to 1,000 times the surface area of unetched capillaries. This work explores the effect of surface roughness on electroosmotic mobility through a series of experiments. The experimental results are compared to numerical simulations obtained using COMSOL Multiphysics (Burlington, MA). Additionally, this work identifies the technical challenges that must be solved in order to miniaturize open-tubular separation techniques using planar microfluidic chips.

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LIST OF SYMBOLS

Symbol	Description	Units
A_{cs}	capillary cross-sectional area	m^2
E	electric field strength	$\frac{V}{m}$
f	Darcy friction factor	–
F	body force	N
F_E	electromotive forces	N
F_z	frictional force	N
F_z	body forces, z-component	N
I	electrical current	mA
L_d	capillary length, inlet to detector	m
L_f	migration length, final	m
L_i	migration length, initial	m
L_t	capillary length, total	m
n	concentration, bulk ionic	$\frac{ions}{m^3}$
p	fluid pressure	$\frac{kg}{m \cdot s^2}$
P_{wet}	capillary perimeter, wetted	m
$\langle Q_z \rangle$	fluid volumetric flow rate, average	$\frac{\mu L}{h}$
r	radial direction	m
r_i	ion radius, hydrodynamic	m

LIST OF SYMBOLS (Continued)

Symbol	Description	Units
R	capillary radius	m
R_h	capillary radius, hydraulic	m
T	temperature, absolute	K
t	time	s
t_{inj}	time, injection	s
t_{mig}	time, migration	s
t_{tr}	time, transfer	s
$\langle v \rangle$	fluid velocity, average	$\frac{m}{s}$
v_{eo}	fluid velocity, electroosmotic	$\frac{m}{s}$
v_{ep}	fluid velocity, electrophoretic	$\frac{m}{s}$
v_m	fluid velocity, migration	$\frac{m}{s}$
v_r	fluid velocity, radial component	$\frac{m}{s}$
v_z	fluid velocity, longitudinal component	$\frac{m}{s}$
v_θ	fluid velocity, rotational component	$\frac{m}{s}$
V_a	volume, analyte	m^3

LIST OF SYMBOLS (Continued)

Symbol	Description	Units
ε	fluid dielectric constant	$\frac{C}{V \cdot m}$
ε_a	absolute surface roughness	m
ε_r	relative surface roughness	–
λ_b	fluid bulk conductivity	$\frac{1}{\Omega}$
λ_D	Debye length	m
μ	viscosity, fluid	$\frac{kg}{m \cdot s}$
μ_a	viscosity, analyte	$\frac{kg}{m \cdot s}$
μ_{eo}	mobility, electroosmotic	$\frac{V}{m \cdot s}$
μ_{ep}	mobility, electrophoretic	$\frac{V}{m \cdot s}$
ρ	density, fluid	$\frac{g}{cm^3}$
ρ_a	density, analyte	$\frac{g}{cm^3}$
ρ_e	density, electric charge	$\frac{C}{m^3}$
ϕ	electric potential	V
ϕ_{wall}	wall electric potential	V
τ	viscous stress tensor	$\frac{kg}{m \cdot s^2}$
ζ	potential, zeta	V

1 INTRODUCTION

1.1 Capillary Electrophoresis

Capillary electrophoresis (CE) refers to a family of high-efficiency analytical techniques in which molecular species in solution are separated from one another in fused-silica capillaries under the application of an electric field. The separated species are commonly identified based on their UV absorbance. The basic components of a modern CE instrument include a fused-silica capillary tube with an optical viewing window, a controllable high-voltage power supply, two electrodes, two buffer reservoirs, a sample vial, and an ultraviolet (UV) absorbance detector [1]. The ends of the capillary are placed in the buffer reservoirs and the optical viewing window is aligned with the UV absorbance detector. The positively charged anode of the high-voltage power supply is usually connected to the buffer reservoir furthest from the detector and the negatively charged cathode is usually connected to the buffer reservoir closest to the detector. Figure 1 illustrates a typical CE configuration [2].

Figure 1. Schematic Diagram of a CE System.

Initially, the capillary is filled with a buffer electrolyte solution. During the sample injection step, the buffer reservoir at the anode is temporarily replaced by the sample vial. Pressure is applied to the sample vial to inject a small amount of the sample into the capillary.

During the separation process, the sample vial at the anode is replaced with the buffer reservoir and an electric potential is applied across the capillary. The buffer electrolyte and sample are transported through the capillary by two

different electrokinetic mechanisms, electrophoresis and electroosmosis. Electrophoresis refers to the movement of each molecular species within the buffer electrolyte due to attraction to the anode or cathode and depends on the polarity (i.e. positive or negative), the charge-to-mass ratio of the species, and applied electric potential. Electroosmosis refers to the bulk transport of the buffer electrolyte itself and depends on factors such as the buffer concentration, buffer pH, buffer viscosity, temperature, surface roughness, and the charge on the capillary walls. Since the basis of separation is charge, neutral uncharged species that are present in the sample will not be separated from one another. In these cases, additional separation techniques, such as capillary electrochromatography, which combine the benefits of capillary electrophoresis and high-pressure liquid chromatography, must be used.

When each of the separated species reaches the optical viewing window of the capillary, the UV absorbance is recorded and plotted. Analysis of the plot of UV absorbance versus time provides information on the identity of each of the separated species. Many commercial CE systems include auto-samplers that allow on-instrument storage of different samples, buffer electrolyte solutions, and reagents, thereby enabling the operator to program the instrument to perform analyses automatically for long periods without human intervention.

1.2 Capillary Electrochromatography

Capillary electrochromatography (CEC) is a hybrid analytical separation technique that combines beneficial characteristics of high-pressure liquid chromatography (HPLC) and CE. Similarities to CE include instrumentation and transport of analytes by electroosmosis and electrophoresis. Similarity to HPLC includes the use of solute-bonded phase interactions [3]. The separation column is usually packed with a stationary phase of porous microparticles, which in some cases can be chemically modified. Molecules that interact strongly with the stationary phase will be retained in the separation column longer than molecules that interact weakly with the stationary phase, thus providing a second degree of separation compared to CE alone.

In its native form, CE is incapable of separating neutral species. On the other hand, HPLC and CEC are capable of separating neutral species based on size using a column packed with a stationary phase. HPLC theory indicates that separation power increases with decreasing particle diameter. However, decreasing the particle diameter, and consequently increasing the packing density, generates a prohibitively large backpressure. High packing density can be employed in CEC without concern over backpressure limitations because the species are transported electrokinetically [4]. In addition, the flat fluid velocity profile of the electrically driven format improves separation results compared to the parabolic fluid velocity profile of the pressure-driven format by reducing

sample dispersion, which causes difficulties in analyzing the UV absorbance because of a phenomenon called band broadening.

Although traditional packed-capillary approaches to CEC are advantageous because of enhanced separation capability compared to CE and HPLC, a number of problems have been reported with packing micrometer-sized particles into micrometer-sized capillaries. These problems include difficulty retaining the packing material in the capillary [5, 6, 7, 8, 9], formation of bubbles around the packing material resulting in an unstable baseline, and difficulty analyzing basic compounds due to insufficient charged surface silanol groups to generate adequate electroosmotic flow [3].

Open tubular CEC (OTCEC) is a type of CEC that does not require packing material and does not possess the problems typically encountered in packed-capillary approaches. Instead, the mobile phase interacts with chemically modified capillary walls, which act as a stationary phase. Instead of separating species based on size differences, OTCEC separates them based on different degrees of interaction with the chemically modified capillary walls. Typical chemical modification techniques include esterification, chlorination, and organosilation [10].

Since separation by OTCEC results from interactions with a stationary phase bonded to the capillary walls, an intrinsic limitation of these systems is the low surface area available for modification. The loading capacity of the system decreases as the surface area decreases, which means that only small amounts

of sample can be injected at one time, consequently decreasing efficiency and requiring detection schemes with increased sensitivity [11]. Recently, a two-step surface derivitization process has been developed to improve the bonding characteristics of the stationary phase onto the capillary walls. Before surface derivitization, the walls of the capillary are etched to roughen the surface and increase the surface area by a factor of up to 1,000. The increased surface area increases the loading capacity of the capillary for solute-bonded phase interactions. Subsequently, the surface is alkylated which involves silinization of the surface with triethoxysilane (TES) followed by hydrosilation [3].

1.3 Research Focus

The ability to control, or even suppress, electroosmotic flow is of critical importance in some modes of CE and CEC. A significant amount of research has been published related to the dependence of electroosmotic fluid velocity on factors such as buffer pH, buffer concentration, temperature, microchannel geometry, and applied electric potential. However, relatively few research efforts have investigated the dependence of electroosmotic fluid velocity with respect to surface roughness. This could be due, in part, to the fact that purposefully etching capillary walls for OTCEC applications is a relatively new concept.

The surface roughness generated by the etching processes typically used in OTCEC, by certain manufacturing techniques or by adsorption of proteins or other biological molecules to the capillary surfaces, plays an important role in

fluid flow behavior [12]. The increased surface area of the etched microchannels, which in some cases can be 1,000 times the unetched surface area, could result in significantly reduced flow rates due to additional frictional forces acting upon the fluid.

This thesis will investigate the dependence of electroosmotic fluid velocity on surface roughness using unmodified capillaries and capillaries that have been etched using the two-step surface derivitization process described by Pullen, et al. [3, 10]. The effect of surface roughness coupled with other factors such as buffer pH, buffer concentration, and applied electric potential on electroosmotic fluid velocity were investigated through experimental work and the experimental results will be verified through multiphysics numerical simulations. The results provide insight related to the importance of surface roughness when developing methods for control of electroosmotic flow in OTCEC applications.

2 THEORETICAL BACKGROUND

The three main steps involved in separating molecular species using CE and CEC are injection, separation, and detection. During the injection step, pressure-driven transport mechanisms move a small volume of analyte from the sample vial into the separation column. During the separation step, electrokinetic transport mechanisms move the analyte and buffer electrolyte through the separation column. A UV detector located near the end of the separation column measures the UV absorbance of each of the separated species.

This chapter provides an overview of the governing equations and boundary conditions for pressure-driven flow during the injection step and electrokinetic flow during the separation step. All of the derived equations are in relation to channels of circular cross-section and are applicable for fluid flow in capillaries and other similarly shaped microchannels. Analytical solutions to the governing differential equations are generally not possible without making reasonable assumptions and approximations regarding fluid properties, flow geometry, and boundary conditions. These assumptions and approximations are also presented in this chapter.

Microscale flow is governed by the same equations that govern macroscopic flow. However, molecular interactions influence flow dynamics more predominantly in microscale flow compared to macroscale flow. If the flux is held constant as a microchannel is scaled down, the shear stresses and the acceleration are proportional to L_s^{-3} , where L_s is the channel length scale.

Similarly, the pressure gradient is proportional to L_s^{-4} . Due to these proportionality factors, fluid properties can change dramatically in microchannels. For example, a fluid that is Newtonian in macroscopic flow may be non-Newtonian as the channel size is scaled-down while holding the flux constant because of the dramatic increase in shear stress [13].

2.1 Injection

The two fundamental conservation laws that adequately describe pressure-driven sample transport during the injection step are the conservation of mass equation and the conservation of linear momentum equations. The simultaneous solution of these differential equations is useful for estimating the pressure and time required to inject the desired volume of analyte into the separation column. The following derivation leads to an expression for the volume of injected analyte in terms of capillary dimensions, analyte viscosity, injection pressure, and injection time.

Equation 1 shows the conservation of mass equation in cylindrical coordinates for an incompressible fluid, which is an appropriate starting point for describing analyte transport in fused-silica capillaries [14].

$$\frac{\partial \rho_a}{\partial t} + \frac{1}{r} \frac{\partial}{\partial r}(\rho_a r v_r) + \frac{1}{r} \frac{\partial}{\partial \theta}(\rho_a v_\theta) + \frac{\partial}{\partial z}(\rho_a v_z) = 0 \quad \text{Equation 1}$$

where ρ is the analyte density, t is time, r is the radial direction, and, v_r , v_θ , and v_z are the r , θ , and z -components of the fluid velocity, respectively.

If the system is at steady state, the time derivative term $(\partial p_a / \partial t)$ on the left hand side of Equation 1 can be neglected. Additionally, since the bulk fluid motion is in the axial direction, all velocity components except for v_z can also be neglected. Thus, Equation 2 shows the simplified continuity equation.

$$\frac{\partial v_z}{\partial z} = 0 \quad \text{Equation 2}$$

The conservation of linear momentum equations equate the rate of change of linear momentum to the influx of momentum, out flux of momentum, and the momentum due to forces acting on the system. Equation 3, Equation 4, and Equation 5 show the conservation of linear momentum relationships in cylindrical coordinates for the r , θ and z -axes, respectively [14].

$$\rho \left(\frac{\partial v_r}{\partial t} + v_r \frac{\partial v_r}{\partial r} + \frac{v_\theta}{r} \frac{\partial v_r}{\partial \theta} - \frac{v_\theta^2}{r} + v_z \frac{\partial v_r}{\partial z} \right) = -\frac{\partial p}{\partial r} - \left[\frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rr}) + \frac{1}{r} \frac{\partial \tau_{r\theta}}{\partial \theta} - \frac{\tau_{\theta\theta}}{r} + \frac{\partial \tau_{rz}}{\partial z} \right] + F_r \quad \text{Equation 3}$$

$$\rho \left(\frac{\partial v_\theta}{\partial t} + v_r \frac{\partial v_\theta}{\partial r} + \frac{v_\theta}{r} \frac{\partial v_\theta}{\partial \theta} - \frac{v_r v_\theta}{r} + v_z \frac{\partial v_\theta}{\partial z} \right) = -\frac{1}{r} \frac{\partial p}{\partial \theta} - \left[\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 \tau_{r\theta}) + \frac{1}{r} \frac{\partial \tau_{\theta\theta}}{\partial \theta} - \frac{\tau_{\theta\theta}}{r} + \frac{\partial \tau_{\theta z}}{\partial z} \right] + F_\theta \quad \text{Equation 4}$$

$$\rho \left(\frac{\partial v_z}{\partial t} + v_r \frac{\partial v_z}{\partial r} + \frac{v_\theta}{r} \frac{\partial v_z}{\partial \theta} - \frac{v_\theta^2}{r} + v_z \frac{\partial v_z}{\partial z} \right) = -\frac{\partial p}{\partial z} - \left[\frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rz}) + \frac{1}{r} \frac{\partial \tau_{\theta z}}{\partial \theta} - \frac{\tau_{\theta\theta}}{r} + \frac{\partial \tau_{zz}}{\partial z} \right] + F_z \quad \text{Equation 5}$$

where p is the pressure, τ is the viscous stress tensor, and F_r , F_θ , and F_z represent the sum of the body forces acting upon the analyte along the radial, axial, and longitudinal axes, respectively. Since bulk fluid motion is in the axial direction, Equation 5 and the simplified continuity equation given by

Equation 2 are sufficient for deriving an expression for the steady-state fluid velocity profile.

Equation 5 can be simplified by eliminating the time derivative term on the left hand side because the system is assumed to be at steady state. Next, the three terms on the left hand side containing non-axial velocity terms can also be eliminated since bulk fluid motion is in the axial direction. The last remaining term on the left hand side can be eliminated from the result obtained in Equation 2 from the simplified continuity equation. Equation 6 shows the simplified conservation of linear momentum equation.

$$-\frac{\partial p}{\partial z} = \frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rz}) + \frac{1}{r} \frac{\partial \tau_{\theta z}}{\partial \theta} - \frac{\tau_{\theta\theta}}{r} + \frac{\partial \tau_{zz}}{\partial z} + F_z \quad \text{Equation 6}$$

Since capillaries are symmetrical about their longitudinal axis, all derivatives with respect to θ can be neglected. Additionally, the body force term can be neglected for the injection step since electrokinetic phenomena are not present and since the effect of gravity may be neglected. Equation 7 shows the further simplified conservation of linear momentum equation.

$$-\frac{\partial p}{\partial z} = \frac{1}{r} \frac{\partial}{\partial r} (r \tau_{rz}) - \frac{\tau_{\theta\theta}}{r} + \frac{\partial \tau_{zz}}{\partial z} \quad \text{Equation 7}$$

If the analyte follows Newton's Law of Viscosity, then Equation 8, Equation 9, and Equation 10 are valid expressions for the components of the viscous stress tensor that appear in Equation 7 [15].

$$\tau_{rz} = -\mu_s \left[\frac{\partial v_z}{\partial r} + \frac{\partial v_r}{\partial z} \right] \quad \text{Equation 8}$$

$$\tau_{\theta\theta} = -\mu_s \left[2 \left(\frac{1}{r} \frac{\partial v_\theta}{\partial \theta} + \frac{v_r}{r} \right) - \frac{2}{3} \left(\frac{1}{r} \frac{\partial}{\partial r} (r v_r) + \frac{1}{r} \frac{\partial v_\theta}{\partial \theta} + \frac{\partial v_z}{\partial z} \right) \right] \quad \text{Equation 9}$$

$$\tau_{zz} = -\mu_a \left[2 \frac{\partial v_z}{\partial z} - \frac{2}{3} \left(\frac{1}{r} \frac{\partial}{\partial r} (r v_r) + \frac{1}{r} \frac{\partial v_\theta}{\partial \theta} + \frac{\partial v_z}{\partial z} \right) \right] \quad \text{Equation 10}$$

Where μ_a is the viscosity of the analyte.

Equation 8, Equation 9, and Equation 10 may be simplified by using the same assumptions that were used to simplify the continuity and conservation of linear momentum equations. After simplification, it is clear that $\tau_{\theta\theta}$ and τ_{zz} vanish and τ_{rz} is reduced to the expression shown in Equation 11.

$$\tau_{rz} = -\mu_s \left[\frac{\partial v_z}{\partial r} \right] \quad \text{Equation 11}$$

Elimination of any terms containing $\tau_{\theta\theta}$ and τ_{zz} in Equation 7 and substituting the result for τ_{rz} obtained in Equation 11, yields Equation 12.

$$\frac{r}{\mu_s} \frac{\partial p}{\partial z} = \left[\frac{\partial}{\partial r} \left(r \frac{\partial v_z}{\partial r} \right) \right] \quad \text{Equation 12}$$

Equation 12 can be further simplified by noting that pressure is a function of longitudinal position only and integrating both sides with respect to r . The result is shown in Equation 13.

$$\frac{r}{2\mu_s} \frac{\partial p}{\partial z} + C_1 = \frac{\partial v_z}{\partial r} \quad \text{Equation 13}$$

As shown in Equation 14, the constant of integration vanishes by application of the boundary condition that the shear stress at the center of the channel is finite or, alternatively stated, $\partial v_z / \partial r = 0$ at $r = 0$.

$$\frac{r}{2\mu_s} \frac{\partial p}{\partial z} = \frac{\partial v_z}{\partial r} \quad \text{Equation 14}$$

Integration of Equation 14 with respect to r and replacing $\partial p / \partial z$ with $-\Delta p / L_t$ yields Equation 15.

$$-\frac{r^2 \Delta p}{4\mu_s L_t} + C_2 = v_z \quad \text{Equation 15}$$

where Δp is the pressure-drop applied across the capillary for analyte injection and L_t is the total length of the capillary. The second constant of integration can be determined by application of the boundary condition that the fluid velocity at the capillary walls is zero, or alternatively stated, $v_z = 0$ at $r = R$. Equation 16 shows the resultant expression for C_2 .

$$C_2 = \frac{R^2 \Delta p}{4\mu_s L_t} \quad \text{Equation 16}$$

Equation 17 is obtained by substitution of Equation 16 into Equation 15 and rearranging terms.

$$v_z = \frac{R^2 \Delta p}{4\mu_s L_t} \left[1 - \left(\frac{r}{R} \right)^2 \right] \quad \text{Equation 17}$$

Since the theoretical fluid velocity profile given by Equation 17 is parabolic with respect to radial position, it is often useful to know the average velocity $\langle v_z \rangle$. The average velocity is one-half of the maximum velocity and the maximum velocity occurs at the center of the channel at $r = 0$. The expression for average velocity is shown in Equation 18.

$$\langle v_z \rangle = \frac{R^2 \Delta p}{8 \mu_s L_t} \quad \text{Equation 18}$$

Multiplication of Equation 18 by the cross-sectional area of the channel provides an expression for the average volumetric flow rate of the fluid. For a channel of circular cross-section such as a capillary, the result is shown in Equation 19.

$$\langle Q_z \rangle = \frac{\pi R^4 \Delta p}{8 \mu_s L_t} \quad \text{Equation 19}$$

where $\langle Q_z \rangle$ is the average volumetric flow rate.

Noting that the average volumetric flow rate is simply the volume of injected analyte divided by the time, Equation 19 can be rearranged to provide an expression for the volume of injected analyte. This expression is shown in Equation 20.

$$V_a = \frac{\pi R^4 t \Delta p}{8 \mu_s L_t} \quad \text{Equation 20}$$

where V_a is the volume of injected analyte.

A summary of the assumptions that facilitated the derivation of Equation 20 is provided below.

- The analyte is an incompressible fluid.
- The analyte is a Newtonian fluid.
- The analyte is moving in the axial direction only.
- The capillary is of circular cross-section.
- The capillary is longitudinally symmetrical.
- The system is at steady state.
- The effects of gravity are negligible.
- The only driving force is the pressure-gradient.

2.2 Separation

Once a plug of analyte has been injected, a high voltage is applied across the capillary to move the plug of analyte and the buffer electrolyte through the separation column. The electrokinetic flow that results consists of two fluid transport mechanisms, electroosmosis and electrophoresis [16].

Electroosmosis refers to the bulk movement of an aqueous solution (the buffer electrolyte) passed across a stationary solid surface (the walls of the capillary) due to an externally applied electric field. Electroosmosis requires the existence of a charged double-layer at the solid-liquid interface that results from an attraction between bound surface charges and ions in the passing fluid. In

capillaries, surface silanol groups become deprotonated and gain a negative charge. The negatively charged surface attracts positive ions from the passing fluid, which results in a charged double-layer [17].

Electrophoresis refers to the movement of charged colloidal particles (the sample) through an aqueous solution (the buffer electrolyte) due to an externally applied electric field. Neutral species in the sample solution are not transported by electrophoresis since they are not charged. Therefore, electroosmosis describes the flow of the buffer electrolyte and any uncharged species in the sample solution. Charged species in the sample solution are transported by *both* electrophoresis and electroosmosis and the velocity of these species is simply the linear superposition of the two velocity components as shown in Equation 21 [17].

$$v_z = v_{eo} + v_{ep} \quad \text{Equation 21}$$

Where v_z is the total velocity, v_{eo} is the component of the velocity due to electroosmosis, and v_{ep} is the component of the velocity due to electrophoresis.

2.2.1 Electroosmosis

When a buffer electrolyte is exposed to a capillary, the silanol groups on the capillary surface deprotonate. An electric double-layer (EDL) is formed from the charged capillary surface and the counter-ions in close proximity to the surface. The counter-ions reside in two distinct regions - the immobilized region immediately adjacent to the capillary surface (Stern Layer) and the mobile layer

that flows upon the application of an external electric field (Gouy-Chapman Layer). The potential at the shear-plane that exists in between the two counter-ion layers is called the electroosmotic zeta potential. Figure 2 illustrates this phenomenon [16] where ζ is the zeta potential, ϕ_{wall} is the wall potential, and λ_d is the Debye length. Because the Stern Layer is very thin and its thickness is negligible, the thickness of the EDL, the thickness of the Gouy-Chapman Layer, and λ_d , are all equivalent.

Figure 2. Schematic of a Simple EDL Model [16].

Similar to the case for sample injection, the fluid velocity profile for electroosmotic flow in a capillary can be determined from the continuity and conservation of linear momentum equations. However, two assumptions that were made in the pressure-driven case are not valid here. First, for electroosmotic flow it will be assumed that a pressure-gradient does not exist across the capillary and that the only driving force for fluid transport is the applied

electric potential. Second, the body forces, which were neglected in the pressure-driven case, cannot be neglected in this case because the electromotive forces acting upon the fluid are contained in this term. Equation 22 shows the differential equation that describes electroosmotic fluid transport.

$$\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial v_z}{\partial r} \right) = F_z \quad \text{Equation 22}$$

An expression for the body forces acting upon the fluid from the externally applied electric field can be derived by considering the relationship between the net excess charge density per unit volume and the electric potential. This relationship is known as the Poisson Equation and is shown in Equation 23 [18].

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{d\phi}{dr} \right) = -\frac{4\pi}{\varepsilon} \rho_e(r) \quad \text{Equation 23}$$

where ϕ is the electric potential, which due to symmetry is only a function of radial position, ε is the dielectric constant of the fluid, and $\rho_e(r)$ is a function representing the excess charge density per unit volume.

If the number of ions per unit volume can be described by a Boltzmann distribution, then Equation 24 is a valid expression for the excess charge density per unit volume.

$$\rho_e(r) = -2ne \sinh \left(\frac{e\phi}{kT} \right) \quad \text{Equation 24}$$

where n is the bulk ionic concentration of a univalent buffer electrolyte in ions per unit volume, e is the charge on an electron, k is the Boltzmann constant, and T is the absolute temperature.

Assuming that the EDL is thin compared to the capillary radius the approximation shown in Equation 25 can be applied, and the resultant expression for excess charge density per unit volume shown in Equation 26 is valid [16].

$$\sinh\left(\frac{e\phi}{kT}\right) \approx \frac{e\phi}{kT} \quad \text{Equation 25}$$

$$\rho_e(r) = -\frac{2ne^2\phi}{kT} \quad \text{Equation 26}$$

Substitution of Equation 26 into Equation 23 yields Equation 27, also known as the Poisson-Boltzmann Equation.

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{d\phi}{dr} \right) = \frac{8\pi ne^2\phi}{\epsilon kT} \quad \text{Equation 27}$$

Substitution of the definition for the Debye length given by Equation 28 provides Equation 29, which is a simplified form of Equation 27.

$$\lambda_D = \left(\frac{\epsilon kT}{8\pi ne^2} \right)^{1/2} \quad \text{Equation 28}$$

$$\frac{1}{r} \frac{d}{dr} \left(r \frac{d\phi}{dr} \right) = \frac{\phi}{\lambda_D^2} \quad \text{Equation 29}$$

The solution to Equation 29 can be found to be the zero-order Bessel function shown in Equation 30 by application of the boundary condition that the electric potential is finite at the center of the channel or, alternatively stated, $d\phi/dr = 0$ at $r = 0$.

$$\phi = C_3 I_0 \left(\frac{r}{\lambda_D} \right) \quad \text{Equation 30}$$

where the constant of integration, C_3 , can be evaluated using the boundary condition that the electric potential is the wall potential at the channel wall or, alternatively stated, $\phi = \phi_{wall}$ at $r = R$. The resultant expression for the electric potential is shown in Equation 31.

$$\phi = \phi_{wall} \frac{I_0\left(\frac{r}{\lambda_D}\right)}{I_0\left(\frac{R}{\lambda_D}\right)} \quad \text{Equation 31}$$

The expression for the excess charge density per unit volume is obtained by substitution of Equation 31 into Equation 26 and is shown in Equation 32.

$$\rho_e(r) = -\frac{\varepsilon}{4\pi\lambda_D^2} \phi_{wall} \frac{I_0\left(\frac{r}{\lambda_D}\right)}{I_0\left(\frac{R}{\lambda_D}\right)} \quad \text{Equation 32}$$

The longitudinal axis body force term in Equation 22 can be expressed in terms of the excess charge density per unit volume given by Equation 32 along with the fluid viscosity and the magnitude of the applied electric field. This expression is shown in Equation 33.

$$F_z = \frac{E_z}{\mu} \rho_e(r) = -\frac{\varepsilon E_z}{4\mu\pi\lambda_D^2} \phi_{wall} \frac{I_0\left(\frac{r}{\lambda_D}\right)}{I_0\left(\frac{R}{\lambda_D}\right)} \quad \text{Equation 33}$$

The differential equation that resulting from substitution of Equation 33 into Equation 22 is shown in Equation 34.

$$\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial v_z}{\partial r} \right) = - \frac{\varepsilon E_z}{4\mu\pi} \frac{1}{\lambda_D^2} \phi_{wall} \frac{I_0 \left(\frac{r}{\lambda_D} \right)}{I_0 \left(\frac{R}{\lambda_D} \right)} \quad \text{Equation 34}$$

Solution of Equation 34 using the same boundary conditions for axial velocity that were used in the pressure-driven case yields Equation 35.

$$v_{eo} = \frac{\varepsilon E_z \zeta}{4\mu\pi} \left[1 - \frac{I_0 \left(\frac{r}{\lambda_D} \right)}{I_0 \left(\frac{R}{\lambda_D} \right)} \right] \quad \text{Equation 35}$$

For the case where the EDL is thin compared to the capillary radius, the term in brackets becomes unity and the simplified expression for electroosmotic fluid velocity becomes the expression shown in Equation 36.

$$v_{eo} = \frac{\varepsilon \zeta}{4\mu\pi} E = \mu_{eo} E \quad \text{Equation 36}$$

All the terms on the right side of the equation with the exception of electric field strength are constant for a given capillary and buffer. These terms can be combined in to a term, μ_{eo} , which is the electroosmotic mobility. Therefore, electroosmotic fluid velocity is proportional to electric field strength and the proportionality constant is dependent on capillary material, geometry, buffer chemical composition, and concentration.

A summary of the assumptions that facilitated the derivation of Equation 36 is provided below. The assumptions are generally justifiable in the context of typical CE separations using standard fused-silica capillaries.

- The analyte and buffer electrolyte are incompressible fluids.
- The analyte and buffer electrolyte are Newtonian fluids.
- The analyte and buffer electrolyte are moving in the axial direction only.
- The capillary is of circular cross-section.
- The capillary is longitudinally symmetrical.
- The system is at steady state.
- The effects of gravity are negligible.
- The only driving force is the electric potential gradient.
- The bulk ionic concentration follows a Boltzmann distribution.
- The EDL and Debye length are small compared to the channel radius.

2.2.2 Electrophoresis

Electrophoresis is the phenomenon that describes the movement of charged species contained within the analyte through the buffer electrolyte under the application of an external electric field. The force of the electric field on the charged species is proportional to the effective charge and the electric field strength as shown in Equation 37 [19].

$$F_E = qE$$

Equation 37

where F_E is the force of the electric field on the charged species, q is the effective charge, and E is the magnitude of the electric field.

The force of the electric field is counter-balanced by the frictional forces caused by movement of the charged species through the electrolyte buffer. This frictional force is proportional to the friction coefficient and the fluid velocity as shown in Equation 38.

$$F_F = f v_{ep}$$

Equation 38

where F_F is the frictional force and f is the friction coefficient.

If the charged species in solution can be approximated as spherical particles with a hydrodynamic radius r_i , then the friction coefficient can be approximated by Equation 39.

$$f = 6\pi\mu r_i$$

Equation 39

where μ is the viscosity of the electrolyte buffer.

Equating the force relationships given by Equation 37 and Equation 38 and substituting the friction coefficient relationship given by Equation 39 results in Equation 40 for calculation of the electrophoretic fluid velocity.

$$v_{ep} = \frac{q}{6\pi\mu r_i} E = \mu_{ep} E$$

Equation 40

3 LITERATURE REVIEW

A significant amount of CE and CEC research has been published, however relatively few of these publications relate specifically to the effect of surface roughness on electroosmotic fluid velocity in capillaries. The relevant literature that is available can be broadly classified into the three categories shown below.

- Chemical Modification of Capillaries for OTCEC
- Electroosmotic Fluid Velocity Measurement Techniques
- Fluid Behavior in Capillaries

3.1 Chemical Modification of Capillaries for OTCEC

OTCEC is a good alternative to packed-capillary approaches for chromatography since high efficiency separations of neutral and charged species can be performed without the difficulties of packing micrometer-sized particles into micrometer-sized channels and retaining the packing material in the separation column. In addition, since analyte transport during the separation process occurs by electrokinetic flow mechanisms, high back-pressure and sample dispersion limitations that are present in HPLC are not present in OTCEC [20].

OTCEC separates charged species based on differences in electrophoretic mobility and it separates both neutral and charged species chromatographically based on differences in interaction with solutes bonded to

the capillary walls. The traditional mechanism for bonding solutes to the capillary walls has been adsorption. Adsorption of molecules to the surface of the capillary walls to interact with the analyte improves chromatographic separation of charged and neutral species. However, because the adsorbed molecules cover the glass surface, electrophoretic separation of charged species is hindered due to inaccessibility of the surface silanol groups [21]. Other problems include unstable baselines, differing migration times, especially if the layer of adsorbed molecules is not uniform, and reduction of capillary lifetime.

Another basic problem associated with direct adsorption of solutes onto the capillary walls is the relatively small surface area available in the narrow-bore capillaries typically used for OTCEC applications. The amount of solute that is present on the capillary walls will control the speed of the separation process. More solute molecules bonded to the capillary walls will allow more analyte molecules to interact with the solute, and consequently, will decrease separation time without sacrificing resolution.

Research efforts have been attempted to increase the surface area available for solute bonding. The techniques differ depending on the type of material used to fabricate the microchannel. In microchannels fabricated from glass, such as fused-silica capillaries or planar borosilicate glass microfluidic chips, chemical etching is typically used to roughen the microchannel surfaces and increase the available surface area. In microchannels fabricated from polymers such as polydimethylsiloxane PDMS, chemical etching is not possible.

As such, other techniques such as plasma oxidation surface treatments have been developed [22].

3.1.1 Etching Process for Capillaries

Pesek, et al. (2000) developed an etching process to increase the surface area available for chemical modification of the inner surfaces of capillaries. In their work, impurities were removed from the inner surfaces of the capillary with 12 M hydrochloric acid at 80°C for 12 hours and then the inner surfaces were etched with hydrogen fluoride gas between 300°C and 400°C for 3 to 4 hours. The surface morphology of the capillaries varied significantly depending on the etching time and temperature. Figure 3, Figure 4, and Figure 5 show scanning electron microscope (SEM) images obtained for etching conditions of (a) 300°C for 3 hours, (b) 300°C for 2 hours followed by 400°C for 2 hours, and (c) 300°C for 2 hours followed by 400°C for 1 hour, respectively. Once the surface area of the capillaries had been increased, the etched surfaces were chemically modified for solute bonding by silinization and hydrosilation [20].

Figure 3. SEM image of a 50- μm diameter capillary after etching with hydrogen fluoride gas for 300°C for 3 hours [20]. Reproduced by permission from author.

Figure 4. SEM image of a 50- μm diameter capillary after etching with hydrogen fluoride gas for 300°C for 2 hours followed by 400°C for 2 hours [20].

Reproduced by permission from author.

Figure 5. SEM image of a 50- μm diameter fused-silica capillary after etching with hydrogen fluoride gas for 300°C for 2 hours followed by 400°C for 1 hour [20].

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Shirke, et al. (2004) used the etching process developed by Pesek, et al. (2000) to etch the surfaces of capillaries and planar microfluidic chips [23, 24]. Figure 6 shows a fused silica capillary in the etched condition. Figure 7 and Figure 8 show a planar microfluidic chip in the unetched and etched conditions, respectively.

Figure 6. SEM image of a 75- μm diameter fused-silica capillary after etching with hydrogen fluoride gas at 350°C for 3 hours [24].

Figure 7. SEM image of a 50 μm wide by 20 μm deep D-shaped microchannel in a planar microfluidic chip in the unetched condition [24].

Figure 8. SEM image of a 50 μm wide by 20 μm deep D-shaped microchannel in a planar microfluidic chip after etching with hydrogen fluoride gas at 250°C for 3 hours [24].

Pesek, et al. (2000) also conducted experiments to determine the effect of pH on the electroosmotic fluid velocity in unetched and etched capillaries. They observed that a positive, cathodic, electroosmotic fluid velocity was observed for pH ranging from 2.0 to 6.0 in the unetched capillaries. However, a negative, anodic, electroosmotic fluid velocity was observed for pH ranging from 2.0 to approximately 4.5 in the etched capillaries. This negative electroosmotic fluid velocity was assumed to be due to treatment of the etched capillaries with ammonium hydrogen difluoride, which consists of a positively charged ammonium ion [20].

Pullen, et al. (2000) characterized the surface morphology of unetched, etched, hydrided, and alkylated capillaries using SEM and atomic force microscope (AFM) instruments. The hydrided capillary corresponded to the silinization process that took place after the etching process. The alkylated capillary corresponded to the hydrosilation process that took place after the silinization process. The root mean squared (rms) surface roughness for the native unetched, etched, hydrided, and alkylated capillaries was determined to be 0.8, 15.0, 33.0, and 16.0 nm, respectively [25].

3.2 Electroosmotic Fluid Velocity Measurements

Measurement of electroosmotic fluid velocity through a capillary can be accomplished by injecting a neutral marker into the buffer solution and recording the time required for detection of the neutral marker by a UV detector that is located downstream near the exit of the capillary. Most commercial CE instruments are capable of recording this time and printing it on the electropherogram output. Electroosmotic fluid velocity can then be calculated by dividing the length from the capillary inlet to the detection window by the measured migration time of the neutral marker as shown in Equation 41.

$$v_{eo} = \frac{L_d}{t_{mig}} \quad \text{Equation 41}$$

where L_d is the length from the capillary inlet to the detection window and t_{mig} is the time required for the neutral marker to travel from the capillary inlet to the detection window.

Even using a commercial CE instrument, one of the problems associated with a single detection window and single UV detector is that changes in the electroosmotic fluid velocity that occur before or after the neutral marker has passed the detection window are not recorded. Williams, et al. (1996) developed a method by which the electroosmotic fluid velocity can be calculated using three separate injections of a neutral marker into the capillary. The process is described below [26].

- The injection pressure is applied for a time t_{inj} to inject the first plug of the neutral marker into the capillary.
- The injection pressure is applied for a time t_{tr} to transport the first plug of the neutral marker some distance down the capillary.
- The first two steps are repeated with the second plug of the neutral marker.
- The run potential, V , is applied across the capillary to transport the first and second plugs of neutral marker further into the capillary.
- The first two steps are repeated with the third plug of the neutral marker and data acquisition is initiated to record the UV absorbance of the three neutral marker plugs as they pass the UV detector.

The velocity of all three neutral marker plugs as they migrate past the UV detector is governed by Equation 42.

$$v_m = \frac{L_d}{t_3 - 0.5t_{inj}} \quad \text{Equation 42}$$

where v_m is the migration velocity of the neutral markers, t_3 is the time required for the third plug of the neutral marker to pass the UV detector, and t_{inj} is the time required for injection of each plug of the neutral marker into the capillary.

The location of the second plug of the neutral marker at the start and end of electric potential application is determined by using Equation 43 and Equation 44.

$$L_i = (t_2 - t_1 - 0.5t_{inj})v_m \quad \text{Equation 43}$$

$$L_f = (t_3 - t_2 - 0.5t_{inj})v_m \quad \text{Equation 44}$$

where L_i is the position of the second plug of the neutral marker when the electric potential is applied, L_f is the position of the second plug of the neutral marker when the electric potential is removed, and t_1 and t_2 are the migration times for the first and second plugs of the neutral marker, respectively.

The electroosmotic fluid velocity can then be calculated based on Equation 45.

$$v_{eof} = \frac{L_f - L_i}{t_2} \quad \text{Equation 45}$$

In cases where using a commercial CE instrument is not feasible, a number of methods have been developed for determination of the electroosmotic fluid velocity. These methods include current monitoring techniques and flow visualization techniques.

3.2.1 Current Monitoring

Arulanandam, et al. (2000) developed a current monitoring method for measurement of electroosmotic fluid velocity in capillaries. In this method, one electrolyte solution is gradually replaced by a second electrolyte solution of slightly higher ionic concentration. As more of the second solution flows into the capillary and the first solution flows out of the capillary, the overall conductivity of the solution in the capillary will increase, and consequently, the electrical current will increase. Once the second solution completely replaces the first solution in the capillary, the current will reach a final steady-state value [27]. Figure 9 illustrates the relationship between current and time in the capillary [28].

Figure 9. Relationship between current and time when one electrolyte solution replaces a second electrolyte solution of slightly higher ionic concentration in a capillary [28].

The electroosmotic fluid velocity can be calculated by dividing the total capillary length by the time required for the current to change from the initial steady-state value to the final steady-state value as shown in Equation 46.

$$v_{eo} = \frac{L_t}{\Delta t} \quad \text{Equation 46}$$

Where L_t is the total length of the capillary and Δt is the time required for the current to change from the initial steady-state value to the final steady-state value.

The original study by Arulanandam, et al. (2000) was limited to electroosmotic fluid velocity measurements using one electrolyte solution gradually replacing a second electrolyte solution of the same chemical composition, but at a slightly higher ionic concentration. This limitation was imposed on the system because if the two solutions had possessed significantly different ionic concentrations, then the assumption of constant electric potential throughout the capillary would not be valid. Ren, et al. (2001) realized that in an industrial setting, it is more common to have one solution displacing another solution of different chemical composition and of significantly different ionic concentration. An example of such a situation would be a flushing process by which deionized water is used to flush electrolyte solution out of a capillary. They developed a theoretical model for the displacement process and compared the theoretical results to experimental results obtained for 10^{-2} M potassium chloride solution replacing 10^{-4} M potassium chloride solution and 10^{-2} M potassium

chloride solution replacing deionized water. The results of the theoretical model agreed with the experimental results [29].

Ren, et al. (2002) also realized that the method proposed by Arulanandam, et al. (2000) possessed a major drawback related to the accuracy of results. The starting and ending steady-state values of the current are difficult to determine because of noise and normal current fluctuations that exist throughout the separation process. They developed a way to improve the accuracy of the current monitoring technique by using the slope of the linear portion of the current versus time plot. The slope of the linear current-time relationship is given by Equation 47 [28].

$$slope = \frac{\Delta I}{\Delta t} = \frac{\Delta(EA_{cs}\lambda_b)}{\Delta t} = \langle v_z \rangle \frac{EA_{cs}(\lambda_{b2} - \lambda_{b1})}{L_t} \quad \text{Equation 47}$$

Where λ_{b2} is the bulk conductivity of the high concentration electrolyte and λ_{b1} is the bulk conductivity of the low concentration electrolyte. This equation indicates that if the slope of the linear current-time relationship and the bulk conductivities of the high concentration and low concentration electrolytes are known, then the average electroosmotic fluid velocity can be calculated without the inaccuracies associated with determining the time at which the initial steady-state ends and the time at which the final steady-state begins.

3.2.2 Flow Visualization Techniques

The electroosmotic fluid velocity measurement techniques that have been described thus far are referred to as indirect methods because the fluid velocity is

calculated based on factors such as UV absorbance and current. Another approach to measuring electroosmotic fluid velocity requires the direct observation of laser-excited fluorescent particles under a microscope as they travel through the capillary. The flow field is recorded using a CCD camera and the resultant particle velocity data is analyzed using a computational fluid dynamics (CFD) software package. S. Devasenathipathy, et al. (2002) used micro-PIV and micro-PTV techniques to measure electroosmotic fluid velocities in "D-shaped" microchannels fabricated from PDMS/glass with a width of 120 μm and a depth of 50 μm . The electroosmotic mobility of 10 mM borate solution (pH 9.2) in the untreated PDMS microchannels was determined to be -0.2 cm/Vs using the micro-PIV particle tracking technique [30]. This result is in good agreement with previous electroosmotic mobility measurements using the current monitoring technique [31].

Sinton, et al. (2002) also conducted a study to determine how the electroosmotic fluid velocity measured by the micro-PIV particle tracking technique compared with the current monitoring technique. They measured the electroosmotic velocity of two different electrolytes in a capillary with a 100- μm inner diameter. The first electrolyte used in the experiments was a buffer (pH 8.0) composed of 40 mM Tris base, 40 mM glacial acetic acid, and 1 mM EDTA in de-mineralized and distilled water. The electroosmotic fluid velocity was measured to be 0.374 mm/s using the current monitoring technique and 0.375 mm/s using the particle tracking technique. The second electrolyte used in the

experiments was a buffer (pH 8.3) composed of 89 mM Tris base, 89 mM boric acid, and 2 mM EDTA in de-mineralized and distilled water. The electroosmotic fluid velocity was measured to be 0.204 mm/s using the current monitoring technique and 0.213 mm/s using the particle tracking technique [32]. The results indicate that there is an excellent correlation between indirect and direct methods of electroosmotic fluid velocity measurement.

3.3 Factors that Affect Electroosmotic Fluid Velocity

The electroosmotic fluid velocity in microchannels can be calculated using Equation 48.

$$v_{eo} = \frac{\epsilon \zeta}{4 \mu \pi} E = \mu_{eo} E \quad \text{Equation 48}$$

If the dielectric constant, ϵ , is assumed to be constant for a particular fluid, the main factors affecting electroosmotic fluid velocity are the viscosity of the buffer electrolyte, μ , the zeta potential, ζ , and the applied electric field, E . Microchannel surface roughness could reduce electroosmotic fluid velocity due to increased frictional forces opposing the bulk fluid motion and microchannel geometry could be a factor. Each of these factors will be discussed in this section.

3.3.1 Surface Roughness

Several publications have investigated the effect of surface defects due to manufacturing techniques on electroosmotic fluid velocity [33, 34] and the effect

of microchannel heterogeneity due to different microchannel materials on electroosmotic fluid velocity [35, 36]. The results have shown that these surface conditions could produce regions of bulk flow circulation resulting in induced pressure gradients and reduced electroosmotic fluid velocity. However, to this date, no published work is available which investigates the effect of intentionally imposed surface roughness on electroosmotic fluid velocity in microchannels, such as that required for OTCEC applications.

Hu, et al. (2003) used a computer simulation to model the effect of uniform rectangular surface roughness elements on electroosmotic fluid velocity. The surface roughness elements were shown to induce a periodic pressure field in the microchannel, which made the electroosmotic fluid velocity lower in the central region than in the regions near the microchannel walls. They reported that the average electroosmotic fluid velocity was significantly reduced in rough microchannels as opposed to smooth microchannels. The size of the roughness elements and the distance between the roughness elements were the main factors affecting the electroosmotic fluid velocity [37].

These results indicate that microchannel surface roughness could significantly affect electroosmotic fluid flow, especially in applications such as OTCEC, in which intentional roughening of the capillary walls to increase surface area for solute bonding is necessary.

3.3.2 Microchannel Geometry

Ren, et al. (2002) investigated the effect of channel size on electroosmotic fluid velocity by using 100- μm and 200- μm diameter fused-silica capillaries. The working electrolytes were potassium chloride and LaCl_3 . Electroosmotic fluid velocity was measured using the current monitoring technique by which one buffer reservoir was filled with 1.0×10^{-3} M electrolyte solution and the other buffer reservoir was filled with 0.95×10^{-3} M electrolyte solution. Their results indicated that when the capillary radius is much larger than the Debye length, the electroosmotic fluid velocity is independent of capillary radius, which is in agreement with theoretical predictions [28].

Zhang, et al. (2000) investigated the effect of channel geometry on electroosmotic fluid velocity in microchannels of rectangular and circular cross-section. The results indicated that analyte diffusion was more prevalent in microchannels of rectangular cross-section, especially for aspect ratios greater than 4.0. Since analyte diffusion can cause the undesired result of band broadening during electrophoretic and chromatographic separation processes, they recommended the use of fused-silica capillaries for CE and CEC applications [38].

Sinton, et al. (2003) investigated the effect of microchannel shape and size on electroosmotic fluid velocity using applied electric field strengths between 5 and 20 kV/m. The electroosmotic fluid velocity was nearly identical for microchannels of square cross-section with diagonals measuring 50 and 100- μm and for

capillaries of circular cross-section with diameters measuring 20, 100, and 200- μm [39].

3.3.3 Viscosity and Temperature

Although fluid viscosity will change depending on the buffer solution that is used, the changes will be very small in most CE and CEC applications because most buffer solutions are aqueous and have a viscosity near that of water. However, viscosity is highly dependent on temperature, which is shown in Equation 49 for water [40].

$$\ln\left(\frac{\mu}{\mu_0}\right) = 1.704 - 5.306\left(\frac{273}{T}\right) + 7.003\left(\frac{273}{T}\right)^2 \quad \text{Equation 49}$$

Where μ is the fluid viscosity at the temperature of interest, μ_0 is the fluid viscosity at 0°C, and T is the absolute temperature in Kelvin.

Therefore, if temperature gradients exist within the capillary due to insufficient heat dissipation, viscosity gradients will also exist. These viscosity gradients will result in velocity gradients as well because regions of lower viscosity buffer solution will move faster than regions of higher viscosity buffer solution. This phenomenon is called Joule heating and can result in undesired band broadening.

Erickson, et al. (2003) investigated the effect of Joule heating in two different types of microchannels. One channel was fabricated from PDMS and the other channel was fabricated from PDMS/glass. At room temperature the

electroosmotic mobility for the PDMS and PDMS/glass channels were 6.2×10^{-4} cm²/Vs and 6.0×10^{-4} cm²/Vs, respectively. However, after application of electric potentials between 700 and 2100 V, a plot of volumetric flow rate versus applied electric potential showed a linear relationship for the PDMS/glass microchannel and a non-linear relationship for the PDMS microchannel [40]. The differences between the PDMS microchannel and the PDMS/glass microchannel can be attributed to the fact that PDMS has a lower thermal conductivity than glass. Therefore, PDMS surfaces cannot dissipate heat as fast as glass surfaces and Joule heating is observed at a lower level of electric field strength. Above approximately 1300 V, lower fluid viscosity and higher volumetric flow rates were observed in the PDMS microchannel compared to the PDMS/glass microchannel.

Nickolaj, et al. (2004) proposed that temperature gradients could also cause gradients in the electrical conductivity of the buffer solution, which would also result in velocity gradients in the microchannel. They determined that the electrical conductivity of 10 mM, 25 mM, and 100 mM tetraborate buffer solution increased linearly with temperature in a capillary with an inner diameter of 50- μ m [41].

The results are interesting because an increase in temperature will cause an increase in electrical conductivity and a decrease in viscosity of the buffer solution. An increase in electrical conductivity would result in a lower electroosmotic fluid velocity, and a simultaneous decrease in viscosity would

result in a higher electroosmotic fluid velocity. The two effects would not offset each other completely because viscosity is more sensitive to changes in temperature compared to changes in electrical conductivity.

3.3.4 Zeta Potential

The zeta potential is the electric potential at the shear plane between the stationary layer and mobile layer in the electrical double layer caused by a redistribution of charges that occurs when an electrolyte is exposed to a surface such as glass. Although, microchannel surface roughness could affect the uniformity of the zeta potential, the zeta potential is highly dependent on the charge on the microchannel walls. Kirby, et al. (2004) investigated the effect of buffer concentration and pH on the zeta potential. The zeta potential was calculated from electroosmotic mobility measurements at various electric field strengths [42]. The results of the study indicated that regardless of ion valency, an inverse relationship exists between the negative logarithm of ionic concentration and the zeta potential.

3.4 Literature Summary

In this review of available literature, it was shown that capillaries often need to be etched to increase the surface area available for solute bonding when used in OTCEC applications. Computer simulations have shown that increased surface area significantly decreases electroosmotic fluid velocity. However, very little experimental data exists to verify this outcome. A review of some of the

other factors that could affect electroosmotic fluid velocity were presented and they include, buffer ionic concentration, buffer pH, and temperature. Other factors such as microchannel shape, microchannel size, and viscosity variations between different buffer electrolytes were also shown to be of importance, but to a lesser extent.

4 RESEARCH HYPOTHESIS AND OBJECTIVES

4.1 Research Hypothesis

Successful analysis of samples using CE and CEC requires optimization of electroosmotic flow. If the electroosmotic fluid velocity is too high, species may pass through the capillary before they have had a chance to separate. If the electroosmotic fluid velocity is too low, cationic species within the sample may become adsorbed to the negatively charged capillary surfaces. Additionally, modes of CE that require interactions with a stationary phase such as CEC often require reduction in the electroosmotic fluid velocity to allow sufficient time for the sample to interact with the stationary phase. The main methods of controlling electroosmotic fluid velocity involve careful selection of electric field strength (or applied electric potential) and buffer concentration or pH [43].

Changing the electric field strength or applied electric potential is the easiest way to control the electroosmotic fluid velocity. The electroosmotic fluid velocity varies linearly with electric field strength. However, time efficiency may decrease when the electric field strength is lowered and Joule heating may occur when the electric field strength is raised. Joule heating occurs when the heat generated by the electrical current passing through the fluid cannot be dissipated through the capillary walls at a sufficiently high rate. Viscosity variations caused by gradients in temperature cause the fluid flow rate to vary along the radial axis, which leads to sample dispersion and difficulty in analysis of UV absorbance data

in the electropherogram output. Typical applied electric potentials range from 500 V to 20 kV.

Changing the buffer concentration is another way to control the electroosmotic fluid velocity. As the buffer concentration is increased the electric double layer becomes compressed, the zeta potential decreases, and the electroosmotic fluid velocity is lowered. Typical buffer concentrations range from 10 to 50 mM [43].

The goal of this thesis was to investigate the effect of capillary surface roughness on the electroosmotic fluid velocity by experiment and multiphysics numerical simulation. Surface modifications to the capillary walls are a necessity in CEC and often lead to significant increases in surface roughness. Control of the chemical modification process to achieve predetermined levels of surface roughness could be a useful way to control the electroosmotic fluid velocity in CEC applications. Electroosmotic mobility was obtained by measuring the migration time of a neutral solute under various electric potentials and buffer concentrations.

HYPOTHESIS #1

Surface roughness will lower the electroosmotic mobility in OTCEC applications.

HYPOTHESIS #2

Electroosmotic fluid velocity will decrease with a decrease in applied electric potential, an increase in buffer concentration, and an increase in surface roughness. All three factors are independent from one another.

4.2 Research Objectives

The main objective of this thesis was to investigate the effect of surface roughness on electroosmotic fluid velocity through chemical modification of capillaries and by calculation of electroosmotic mobility for each of the modified capillaries by varying buffer concentration and applied electric potential.

OBJECTIVE #1

Chemically modify capillaries using chemical modification processes similar to those described by Pesek et al. [10]. Determine the electroosmotic mobility in each capillary by varying buffer concentration and applied electric potential.

OBJECTIVE #2

Model the electroosmotic fluid velocity using COMSOL Multiphysics (Burlington, MA), a multiphysics simulation package.

OBJECTIVE #3

Identify challenges and future work that would be involved to transfer the surface modification technologies used in OTCEC to a more efficient planar format.

5.1 Design of Experiment

A three-factor three-level full factorial experimental design was used. The levels of surface roughness that were investigated were 0.8, 15.0, and 33.0 nm, which corresponded to the roughness of native unetched capillaries, capillaries etched with hydrogen fluoride gas, and capillaries etched with hydrogen fluoride gas followed by treatment with triethoxysilane (TES), respectively. The chemical modification process was based on a process previously published by Pesek et al. [10]. The levels of buffer electrolyte concentration that were investigated were 12.5, 25.0, and 50.0 mM borate solutions because of their wide use. The levels of applied electric potential that were investigated were 10, 15, and 20 kV. Electric potentials of 25 and 30 kV were also investigated, but only to confirm that the effects of Joule heating were apparent at these high electric potential levels.

A summary of the factors and levels that were investigated is shown in Table 1 and the full factorial experimental design is shown in Table 2.

Table 1. Summary of factors and levels that were investigated.

Factor	Level 1	Level 2	Level 3
Surface Roughness	0.8 nm	15.0 nm	33.0 nm
Ionic Concentration	12.5 mM	25.0 mM	50.0 mM
Electric Potential	10 kV	15 kV	20 kV

Table 2. Full Factorial Experimental Design.

Experiment	Surface Roughness (nm)	Ionic Concentration (mM)	Applied Electric Potential (kV)
1	0.8	12.5	10
2	0.8	12.5	15
3	0.8	12.5	20
4	0.8	25.0	10
5	0.8	25.0	15
6	0.8	25.0	20
7	0.8	50.0	10
8	0.8	50.0	15
9	0.8	50.0	20
10	15.0	12.5	10
11	15.0	12.5	15
12	15.0	12.5	20
13	15.0	25.0	10
14	15.0	25.0	15
15	15.0	25.0	20
16	15.0	50.0	10
17	15.0	50.0	15
18	15.0	50.0	20
19	33.0	12.5	10
20	33.0	12.5	15
21	33.0	12.5	20
22	33.0	25.0	10
23	33.0	25.0	15
24	33.0	25.0	20
25	33.0	50.0	10
26	33.0	50.0	15
27	33.0	50.0	20

5.2 Experimental Apparatus

Bare capillaries of circular cross-section with an inner diameter of 75 μm and an outer diameter of 360 μm (Polymicro Technologies LLC, Phoenix, AZ) were used for the experiments. Chemical modification of the capillary surfaces required filling the capillaries with various reagents. This was accomplished using the simple apparatus shown in Figure 10.

Figure 10. Schematic of apparatus used to fill the fused-silica capillaries with reagents.

A reagent was placed inside a small vial and the small vial was placed inside a larger vial. A fitting was placed on top of the larger vial to seal the system. The fitting contained two openings, one for the inlet of nitrogen gas and one for the outlet of reagent. The nitrogen tank was connected to the top of the larger vial using 1/16 in. inner diameter PEEK tubing (Upchurch Scientific, Oak

Harbor, WA). The capillaries extended into the reagent and through the outlet opening in the larger vial. After the tank was opened, nitrogen gas flowed into the larger vial and pushed the reagent through the capillary since this was the only exit from the system. The capillary was determined to be filled with reagent when droplets of excess reagent were formed at the exit of the capillary. The nitrogen gas supply was switched off to stop the fluid flow and conserve the necessary reagents.

Electroosmotic fluid velocity was calculated by measuring the time required for a neutral marker to migrate with the buffer electrolyte solution through the capillary using a commercial CE instrument (Agilent Technologies, Palo Alto, CA) coupled to a computer with ChemStation data acquisition software (Agilent Technologies, Palo Alto, CA). CE instrument features included an autosampler tray, pressure-driven and electrokinetic injection capability, a deuterium lamp coupled to a UV diode-array detector, and separate temperature controls for the capillary and the autosampler trays.

5.3 Experimental Methods

5.3.1 Etching and Chemical Modification Process

Three capillaries were required for the experiments. One capillary was retained without chemical modification, one capillary underwent an etching process to roughen the capillary walls, and one capillary underwent both an etching process and a chemical modification process by hydrosilation [10]. Each of the capillaries possessed significantly different surface roughness. The

minimum length of each capillary necessary to conduct electroosmotic fluid velocity measurements in the CE instrument was approximately 70 cm.

One of the capillaries was retained in the unmodified condition (surface roughness of approximately 0.8 nm). Both of the other capillaries underwent an etching process. The first step of the etching process involved cleaning the capillary surfaces with hydrochloric acid. To do this, the capillaries were filled with 12M concentrated hydrochloric acid and heated for approximately 12 hours at 80°C.

The second step of the etching process involved cleaning the capillaries to remove excess hydrochloric acid. The capillaries were rinsed with deionized ultrafiltered water until the exiting fluid was no longer acidic as determined with litmus paper (approximately 10 minutes). Then, the capillaries were rinsed with methanol (CH_3OH) for 30 minutes to assist drying and ensure uniform distribution of the etchant on the capillary surfaces. The excess methanol was removed from the capillaries using pressurized nitrogen gas (N_2).

The third step of the etching process involved etching the capillaries with hydrogen fluoride (HF) gas. The capillaries were filled with 5-percent weight/volume saturated ammonium hydrogen difluoride (NH_4HF_2) in methanol and allowed to stand for 1 hour at room temperature. To ensure complete saturation, the solution was stirred prior to use. During this time, ammonium hydrogen difluoride salt was deposited onto the surface of the capillaries leaving the methanol in the liquid state. After 1 hour had passed, the methanol liquid

was removed from the capillaries using pressurized nitrogen gas. The ends of the capillaries were sealed using an acetylene blowtorch and were heated in a modified gas chromatograph (GC) oven at 300°C for 3 hours. It was essential that this high temperature step be conducted with pressurized nitrogen gas flowing through the oven in order to prevent damage to the protective polyimide coating of the outside of the capillaries. During this process, the ammonium hydrogen difluoride salt formed hydrogen fluoride gas, which further etched the walls of the capillaries. The capillaries were allowed to cool and were unsealed under a chemical fume hood to allow the complete exhaust of the highly caustic hydrogen fluoride gas.

One of the two capillaries that underwent the etching process was set aside (surface roughness was approximately 15.0 nm). The remaining capillary underwent further chemical modification by hydrosilation. The capillary was rinsed with methanol for 30 minutes, filled with 0.1 M sodium hydroxide (NaOH), and allowed to stand for approximately 12 hours. The capillary was then rinsed with deionized water for 10 minutes, 0.1 M hydrochloric acid for 10 minutes, and deionized water for 10 minutes. The capillary was rinsed with acetone (CH_3COCH_3) and dried with pressurized nitrogen gas at 100°C for approximately 12 hours. To complete the silinization process, the capillary was filled with triethoxysilane (TES) in dioxane ($\text{C}_4\text{H}_8\text{O}_2$) solution with a 2.3 M hydrochloric acid catalyst and heated at 90°C for 90 minutes.

Table 3 summarizes the etching and chemical modification processes and Table 4 summarizes the different surface conditions that were investigated.

Table 3. Summary of Microchannel Chemical Modification Process.

Process	Step	Action	Reagent	Temperature	Time
Clean	1-A	Fill/Stand	12 M HCl	80°C	12 hours
Clean	2-A	Rinse	DI H ₂ O	25°C	10 minutes
	2-B	Rinse	CH ₃ OH	25°C	30 minutes
	2-C	Flush	N ₂	25°C	5 minutes
Second Etch	3-A	Fill/Stand	5% (w/v) NH ₄ HF ₂ in CH ₃ OH	25°C	1 hour
	3-B	Flush	N ₂	25°C	5 minutes
	3-C	Heat	---	300°C	3 hours
Silinization	4-A	Rinse	CH ₃ OH	25°C	30 minutes
	4-B	Fill/Stand	0.1 M NaOH	25°C	12 hours
	4-C	Rinse	DI H ₂ O	25°C	10 minutes
	4-D	Rinse	0.1 M HCl	25°C	10 minutes
	4-E	Rinse	DI H ₂ O	25°C	10 minutes
	4-F	Rinse	CH ₃ COCH ₃	25°C	5 minutes
	4-G	Flush	N ₂	100°C	12 hours
	4-H	Fill/Stand	TES/C ₄ H ₈ O ₂ /2.3 M HCl	90°C	90 minutes

Table 4. Summary of Chemical Modification Conditions to be Investigated.

Channel Designation	Channel Type	Chemical Modification Conditions (Step 3-C Temperature/Time)
S	Capillary	No Chemical Modification (0.8 nm Surface Roughness)
T	Capillary	Etching Process Only (15.0 nm Surface Roughness)
U	Capillary	Etching and Derivatization Processes (33 nm Surface Roughness)

5.3.2 Electroosmotic Fluid Velocity Measurements

After the capillaries were prepared as described in the previous section, electroosmotic fluid velocity measurements were conducted using a commercial CE instrument. Various ionic concentrations of buffer electrolytes solution were tested by loading the CE instrument with 50 mM borate buffer solution, 25 mM borate buffer solution, and 12.5 mM borate buffer solution. A 1 percent (w/v) DMSO neutral marker was used as the sample. When the neutral marker was injected into the buffer solution, it was transported through the capillary by electroosmosis only. Electrophoresis was not present because DMSO was electrically neutral. Different applied electric potentials were tested by programming the CE instrument using the ChemStation software interface. The same software interface recorded the migration time of the neutral marker under the various experimental conditions. Each experimental condition was repeated five times to ensure the results were statistically viable.

The CE instrument was programmed to follow the procedure described below for proper conditioning of the capillaries in between experiments.

1. Pre-Condition Capillary

- a. Flush capillaries with 0.1 M NaOH for 5 minutes.
- b. Flush capillaries with deionized ultrafiltered water for 5 minutes.
- c. Flush capillaries with the buffer solution to be used in the next run for 5 minutes.

2. Inject sample using a pressure of 50 mbar for 3 seconds.

3. Apply the desired potential difference across the capillaries and run until the DMSO peak is observed on the electropherogram output.

4. Post-Condition

- a. Flush capillaries with the buffer solution that was used in the last run for 5 minutes.
- b. Flush capillaries with deionized ultrafiltered water for 5 minutes.
- c. Flush capillaries with 0.1 M NaOH for 5 minutes.

5.3.3 COMSOL Modeling

The electroosmotic fluid velocity in the unetched capillary was simulated using a pre-loaded simulation for electroosmotic fluid velocity in a planar microchip with a "T" crossing. The geometry of the microchip "T" crossing was replaced with a two dimensional representation of the unetched and etched capillaries and the boundary conditions were modified accordingly. The following procedure was used to run the simulation:

- Step 1 Launched COMSOL (version 3.2b).
- Step 2 Clicked on the "Model Navigator" tab and selected "MEMS Module."
- Step 3 Opened "Electroosmotic Biochip" under "Microfluidics Models."
- Step 4 Selected "Draw Mode" from the "Draw Menu."
- Step 5 Deleted the existing geometry and replaced with geometry of unetched capillary.
- Step 6 Selected "Stokes Flow" from the "Multiphysics" menu.

- Step 7 Selected "Boundary Settings..." from the "Physics" menu and reset the boundary conditions based on the new geometry. The boundary conditions were set as follows: capillary inlet – neutral, capillary outlet – outflow / pressure, capillary walls – electroosmotic velocity. The electric field strength and zeta potential along the capillary walls were entered on the "Electroosmotic Velocity" tab.
- Step 8 Selected "Conductive Media DC" from the "Multiphysics" menu.
- Step 9 Selected "Subdomain Settings" from the "Multiphysics" menu. For subdomain 1, entered the value for electrical conductivity of the buffer solution being investigated. The electrical conductivity of 2 mM and 100 mM borate solutions has been reported in the literature as 0.04 and 1.6 $\mu\text{S/cm}$, respectively [44]. A linear relationship was assumed and the electrical conductivity of 50 mM, 25 mM, and 12.5 mM was determined to be 0.8, 0.4, and 0.2 $\mu\text{S/cm}$, respectively.
- Step 10 Select "Boundary Settings..." from the "Physics Menu" and reset the boundary conditions based on the new geometry. The boundary conditions were set as follows: capillary inlet and outlet – electric potential of zero Volts, capillary walls – electrical insulation.

6 RESULTS AND DISCUSSION

6.1 Experimental Results

The measured response variable in each of the experiments was the time required for the neutral DMSO marker to travel from the capillary inlet to the UV detection window. The migration time was measured using Hewlett Packard ChemStation software that interfaces with the CE instrument. Each experimental condition was repeated five times and the average of the five migration times was calculated. The average electroosmotic velocity was calculated by dividing the capillary length from the inlet to the UV detection window by the average migration time. Table 5 and Table 6 provide a summary of the results. The error was reported as plus or minus the 95% confidence limit for the data set.

Results were obtained only on the unetched (0.8 nm surface roughness) and etched (15 nm surface roughness) capillaries. Capillaries that underwent the etching and surface derivitization processes (33 nm surface roughness) became blocked by crystallized reagents and were not suitable for the experiments.

Table 5. Results for 0.8 nm Surface Roughness Capillary.

Surface Roughness (nm)	Ionic Concentration (mM)	Applied Electric Potential (kV)	Average Migration Time (min)	Average Electroosmotic Velocity (cm/min)
0.8	12.5	0	-	0.00 ± 0.00
0.8	12.5	10	14.789	4.16 ± 0.24
0.8	12.5	15	9.279	6.63 ± 0.07
0.8	12.5	20	6.831	9.00 ± 0.25
0.8	12.5	25	5.215	11.79 ± 0.02
0.8	12.5	30	3.947	15.58 ± 0.54
0.8	25.0	0	-	0.00 ± 0.00
0.8	25.0	10	14.513	4.24 ± 0.05
0.8	25.0	15	10.173	6.05 ± 0.06
0.8	25.0	20	7.620	8.07 ± 0.13
0.8	25.0	25	5.618	10.95 ± 0.26
0.8	25.0	30	4.417	13.92 ± 1.10
0.8	50.0	0	-	0.00 ± 0.00
0.8	50.0	10	19.057	3.23 ± 0.03
0.8	50.0	15	11.714	5.25 ± 0.03
0.8	50.0	20	7.929	7.76 ± 0.37
0.8	50.0	25	5.386	11.42 ± 0.05
0.8	50.0	30	4.074	15.09 ± 0.60

Table 6. Results for 15.0 nm Surface Roughness Capillary.

Surface Roughness (nm)	Ionic Concentration (mM)	Applied Electric Potential (kV)	Average Migration Time (min)	Average Electroosmotic Velocity (cm/min)
15.0	12.5	0	-	0.00 ± 0.00
15.0	12.5	10	10.571	5.39 ± 0.10
15.0	12.5	15	6.910	8.25 ± 0.02
15.0	12.5	20	4.979	11.45 ± 0.03
15.0	12.5	25	3.881	14.69 ± 0.02
15.0	12.5	30	3.397	16.78 ± 0.98
15.0	25.0	0	-	0.00 ± 0.00
15.0	25.0	10	12.831	4.44 ± 0.07
15.0	25.0	15	8.103	7.04 ± 0.15
15.0	25.0	20	5.975	9.54 ± 0.39
15.0	25.0	25	5.047	11.29 ± 0.45
15.0	25.0	30	3.355	17.00 ± 0.10
15.0	50.0	0	-	0.00 ± 0.00
15.0	50.0	10	15.803	3.61 ± 0.00
15.0	50.0	15	9.848	5.79 ± 0.04
15.0	50.0	20	6.633	8.59 ± 0.02
15.0	50.0	25	4.912	11.60 ± 0.12
15.0	50.0	30	4.655	12.24 ± 0.43

6.2 Discussion

6.2.1 Electroosmotic Mobility

Electric field strength was calculated by dividing the applied electric potential by the total capillary length from inlet to outlet. The total length of the unetched capillary was approximately 70 cm and the total length of the etched capillary was approximately 65 cm. Total capillary length was used instead of the capillary length from the inlet to the detection window because the CE instrument applied the electric potential across the entire capillary.

A plot of electroosmotic velocity versus electric field strength was generated for each concentration of buffer electrolyte solution. Data from both the etched and unetched capillaries were plotted on the same graph to facilitate comparison. The plots are shown in Figure 11, Figure 12, and Figure 13 for 50.0 mM, 25.0 mM, and 12.5 mM borate buffer, respectively.

Figure 11. Plot of electroosmotic fluid velocity versus electric field strength for 50.0 mM borate buffer electrolyte concentration.

Figure 12. Plot of electroosmotic fluid velocity versus electric field strength for 25.0 mM borate buffer electrolyte concentration.

Figure 13. Plot of electroosmotic fluid velocity versus electric field strength for 12.5 mM borate buffer electrolyte concentration.

A plot of electric field strength versus electroosmotic velocity must be linear in the absence of Joule heating. A second-order polynomial fit to each of the data sets suggests nonlinearity, particularly at electric field strengths greater than approximately 350 V/cm, which corresponds to an applied electric potential of approximately 20 kV. Joule heating was more pronounced during experiments that utilized 50 mM borate solution compared to experiments that utilized lower buffer concentrations, probably because the lower electroosmotic velocity

expected under these conditions further hindered heat dissipation. The data from Figure 11, Figure 12, and Figure 13 was re-plotted using only data from applied electric potentials less than 20 kV and the results are shown in Figure 14, Figure 15, and Figure 16.

Figure 14. Linear region of plot of electroosmotic fluid velocity versus electric field strength for 50.0 mM borate buffer electrolyte concentration.

Figure 15. Linear region of plot of electroosmotic fluid velocity versus electric field strength for 25.0 mM borate buffer electrolyte concentration.

Figure 16. Linear region of plot of electroosmotic fluid velocity versus electric field strength for 12.5 mM borate buffer electrolyte concentration.

Eliminating the data points affected by Joule heating caused the plots of electric field strength versus electroosmotic fluid velocity to exhibit the linear relationship that is predicted by theory and is in concert with published results.

Per Equation 36, the slope of the electroosmotic velocity versus electric field strength plot is the electroosmotic mobility. The electroosmotic mobility is a direct indication of how different capillary conditions affected the movement of

the neutral DMSO marker. A summary of electroosmotic mobility for each of the conditions investigated is included in Table 7.

Table 7. Summary of Electroosmotic Mobility for Each Experimental Condition.

Buffer Concentration (mM)	Electroosmotic Mobility ($\text{cm}^2/\text{V-min}$) $\times 10^4$	
	Unetched (0.8 nm Roughness)	Etched (15.0 nm Roughness)
50.0	4.3	4.4
25.0	4.7	5.1
12.5	5.2	6.1

The electroosmotic mobility was nearly identical between the unetched and etched capillaries at the 50.0 mM buffer concentration. However, as the buffer concentration was lowered to 25.0 mM and then 12.5 mM, the electroosmotic mobility between the unetched and etched capillaries began to diverge. Of particular interest was the fact that the etched capillaries had a higher electroosmotic mobility compared to the unetched capillaries for all the buffer concentrations investigated. This result does not agree with the results of similar experiments that employed non-electrokinetic flow mechanisms. In these cases, the additional frictional drag caused by the roughened walls and increased surface area lowered electroosmotic fluid velocity in the etched capillaries compared to the unetched capillaries.

Some explanations of the discrepancy in the results can be explained by unexpected variations in capillary geometry. The inner diameter of the capillary could have varied along the longitudinal axis of each capillary because of variations in the manufacturer's fabrication process. Additionally, the chemical

etching process could have increased the inner diameter of the etched capillary compared to the unetched capillary. Variations in capillary inner diameter were not accounted for in the electroosmotic mobility calculations because the capillaries were not physically measured. Additionally, the etched capillary should have had a surface roughness of approximately 15 nm compared to 0.8 nm for the unetched capillary. One potential reason for the discrepancy in the results could be that the etched capillary was not conditioned properly and consequently, the surface roughness might not have actually been 15 nm.

Another reason the etched capillary could have had a lower electroosmotic mobility is an unexpected interaction of the buffer electrolyte with residual chemicals from the etching and cleaning steps. In both the unetched and etched capillaries, the electroosmotic mobility decreased with increasing buffer concentration, which is in good agreement with theory and previously published results. However, the percentage difference between electroosmotic mobility in unetched and etched capillaries follows roughly an inverse linear relationship with buffer electrolyte concentration, which could indicate the buffer solution has an affinity to the capillary walls. The interaction between the buffer and the capillary walls would be stronger at higher buffer concentrations, which would lower the electroosmotic mobility.

Lastly, the subtle differences that were observed between the etched and unetched capillaries could be due to noise in the measurement systems. Since the electroosmotic fluid velocity is dominated by the magnitude of electric field

strength, it may not have been possible to distinguish electroosmotic mobility differences between 0.8 and 15.0 nm surface roughness capillaries. This could be verified by performing a sensitivity analysis in which roughness extremes are evaluated.

The data collected in this thesis agreed with theoretical predictions and previously published results regarding the effect of Joule heating, changes in applied electric potential, and changes in buffer concentration. Further work needs to be performed to explain the unexpected observation of higher electroosmotic mobility for etched capillaries versus unetched capillaries.

6.2.2 Electroosmotic Velocity Model

The electroosmotic fluid velocity in unetched capillaries was modeled in COMSOL Multiphysics (version 3.2b), a widely used multiphysics modeling software package, to validate a component of the experimental results obtained in this thesis. COMSOL numerically solves systems of differential equations using a method called the finite element method (FEM). Although FEM is not the ideal numerical method to use for turbulent flow (which has a fluid velocity profile similar to electrokinetic flow), it is well suited for solving electrokinetic microfluidic flow in which fluid flow is typically in the low Reynolds number laminar flow domain [45, 46]. FEM has been successfully used to model microfluidic problems such as an electroosmotic mixer [47], an electroosmotic valve in a DNA microchip [48], an electroosmotic pump [49], microfluidic fluid flow

in a biosensor [50], and species detection in electrokinetic focusing [51]. The results of the flow simulation for a smooth capillary are presented in Table 8 and Table 9.

Table 8. COMSOL Electroosmotic Velocity Simulation in a Smooth Capillary.

Surface Roughness (nm)	Ionic Concentration (mM)	Applied Electric Potential (kV)	Average Electroosmotic Velocity (cm/min)
0.0	12.5	0	0.0
0.0	12.5	10	6.6
0.0	12.5	15	10.2
0.0	12.5	20	13.2
0.0	12.5	25	16.8
0.0	12.5	30	19.8
0.0	25.0	0	0.0
0.0	25.0	10	5.4
0.0	25.0	15	8.4
0.0	25.0	20	11.4
0.0	25.0	25	14.4
0.0	25.0	30	18.0
0.0	50.0	0	0.0
0.0	50.0	10	4.8
0.0	50.0	15	7.2
0.0	50.0	20	9.0
0.0	50.0	25	12.0
0.0	50.0	30	13.8

Table 9. COMSOL Electroosmotic Mobility in a Smooth Capillary.

Buffer Concentration (mM)	Electroosmotic Mobility (cm ² /V-min) X 10 ⁴	
	Experimental (0.8 nm Roughness)	Simulation (Smooth)
50.0	4.3	5.4
25.0	4.7	6.8
12.5	5.2	7.8

The electroosmotic mobilities obtained through the COMSOL simulation were based on an assumed zeta potential of -100 mV. The experimental results

were obtained using a borate buffer at pH 9. Vallano, et al. (2001) characterized the pH dependence of zeta potential through experimental measurements of electroosmotic mobility. Their results indicate that a pH 9 buffer solution flowing through a fused-silica capillary has a zeta potential of approximately -40 mV [52]. Table 10 shows the electroosmotic mobilities from the COMSOL simulation corrected for the observed zeta potential of approximately -40 mV.

Table 10. COMSOL Electroosmotic Mobility in a Smooth Capillary with Corrected Zeta Potential.

Buffer Concentration (mM)	Electroosmotic Mobility ($\text{cm}^2/\text{V}\cdot\text{min}$) $\times 10^4$	
	Experimental (0.8 nm Roughness)	Simulation (Smooth)
50.0	4.3	2.1
25.0	4.7	2.7
12.5	5.2	3.1

The COMSOL simulation showed a similar trend to the experimental results in terms of electroosmotic mobility versus buffer concentration. In both cases, the electroosmotic fluid velocity decreased with increasing buffer concentration, which is in agreement with theory and previously published results. However, the absolute values of electroosmotic mobility between the experimental data and the COMSOL simulation did not agree. This could be due to the two dimensional and smooth capillary approximations that were utilized in the COMSOL simulation. Even the unetched capillary had a small amount of surface roughness. The manufacturer of the capillary reported the surface roughness to be approximately 0.8 nm. Additionally, the disagreement could

further support the theory of chemical interaction between the borate buffer and residues on the capillary walls because the COMSOL simulation agreed with experimental results more closely at high buffer concentrations. The percentage difference between the COMSOL simulation and experimental results increased with increasing buffer concentration and consequently, with increasing electrolyte residence time within the capillary.

The COMSOL simulation was repeated using a two dimensional model of a roughened capillary. The initial model geometry was the same as the model for the 75 cm long by 70 μm wide two-dimensional representation of the smooth capillary, but with 15 nm high by 15 nm wide surface roughness elements placed along the top and bottom surfaces. The spacing between surface roughness elements was also 15 nm. However, the model was too complex to solve because the computer hardware could not accommodate the boundary conditions around each of the many surface roughness elements. The computer was able to solve the model only after reducing the length of the two dimensional model from 75 cm long to 5 μm long. The results were very similar to the results from simulation of the smooth capillary. The flow resistance induced by the surface roughness elements may not have been apparent because of the very short capillary length that was modeled.

Alternatively, the results could also indicate that the difference between electroosmotic mobility between a smooth capillary and a roughened capillary is insignificant, which could occur if the thickness of the immobile layer of the EDL

was greater than the magnitude of the surface roughness. In this case, the surface roughness would be encapsulated by the immobile region and would not affect the electroosmotic fluid velocity. Wang, et al. (2006) presented a method to calculate the EDL thickness based on permittivity of the buffer solution, temperature, and ionic concentration of the buffer solution [53]. Using this calculation, the thickness of the EDL was found to be 2.7 nm, 1.9 nm, and 1.4 nm for the 12.5 mM, 25.0 mM, and 50.0 mM buffer concentrations, respectively. In all cases, the thickness of the EDL was much less than the 15.0 nm surface roughness of the etched capillaries, indicating the surface roughness was not encapsulated by the immobile layer of the EDL.

6.3 Conclusions

The main hypothesis of this thesis work was that capillary surface roughness would reduce electroosmotic mobility, and consequently electroosmotic velocity. Experimental data obtained using etched and unetched capillaries do not support the hypothesis. The experimental data showed that surface roughness actually increased electroosmotic mobility. Possible reasons for the discrepancy include (a) chemical etching process did not produce the desired surface roughness of 15 nm, (b) capillary I.D. varied between the etched and unetched capillaries, (c) capillary I.D. varied with longitudinal position, (d) residues from cleaning processes coated capillary walls and reduced EOF in the unetched capillary, and (e) a high enough magnitude of surface roughness was not evaluated to obtain differentiation between unetched and etched capillaries.

Future work is warranted to reveal the true cause of the perceived discrepancy. Nonetheless, this work has shown that the chemical etching process and capillary surface roughness does have an effect on electroosmotic mobility, which is an important first step to understanding the detailed relationship between all of the factors involved in fluid flow through chemically modified capillaries.

6.4 Future Work

A clear need exists to perform additional experimental work to investigate buffer interaction with chemically modified capillary walls based on the data presented in the previous sections. This could be accomplished by repeating the experiments and simulations presented in this work and by taking actual surface roughness measurements using AFM. Additionally, although the OTCEC approach to chemical analysis is very efficient, the length of the fused-silica capillaries that is required to perform separations is still quite long and consequently, the amount of analyte and buffer electrolyte solution that is required to perform an analysis is quite large. An extension to the OTCEC approach that is showing signs of promise is planar capillary electrochromatography (PCEC) in which a planar microfluidic chip is used to perform the separation. The channels that are etched in the planar microfluidic chip are very small in comparison to fused-silica capillaries and can be adaptable

to more complex separation processes. A typical microfluidic chip is shown in Figure 17.

Figure 17. Example of a Planar Microfluidic Chip [54].

In the example shown above, two microfluidic D-shaped channels form an intersection and at the extremities are circular nanoports for bonding external fluidic connections. The long channel is the separation channel in which the buffer electrolyte solution is initially loaded. The short channel is the injection channel in which the analyte is loaded after the separation channel is already filled with buffer electrolyte solution. When an electric potential is applied across the separation channel, the buffer electrolyte solution begins moving by electrophoresis and with it travels the small plug of analyte that was located at the intersection of the injection and separation channels.

Chemically modified planar microchannels would be advantageous so that OTCEC separations could be performed in the more efficient planar microfluidic chips. In order to make this happen, the following challenges must first be overcome: (a) develop a fixture to chemically modify the channels of the

microfluidic chip that can withstand high temperatures, (b) develop a method for bonding external fluidic connections to the inlet and outlet ports of the microfluidic chip, (c) develop a way for the microfluidic chip to interface with traditional CE equipment and software, which includes both supporting the microfluidic chip in the CE instrument and aligning the microchannel with the UV detector.

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