Electrokinetic Chromatography using Novel Unilamellar Vesicles for Unique Separations and Prediction of Intestinal Permeability

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This thesis is dedicated to Tom and Aidan, the most loving, thoughtful, and understanding husband and son anyone could ever want.
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Abstract
Electrokinetic Chromatography using Novel Unilamellar Vesicles for Unique Separations and Prediction of Intestinal Permeability
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Using synthetic unilamellar vesicles as the pseudostationary phase in electrokinetic chromatography has several advantages. These types of vesicles are easy to prepare, inexpensive, and are similar in structure to cell membranes. The research presented in Chapter 2 explores the effect of changing the counterion of the cationic surfactant component of the vesicles from bromide to chloride. In addition, the effect of adding acetonitrile and 2-amino-1-butanol is investigated for both types of vesicles. Linear free energy relationship (LFER) analysis is performed on the vesicles in order to quantify the intermolecular interactions that are responsible for retention. The results indicate that there are unique selectivity differences between using cetyltrimethylammonium bromide (CTAB)/sodium n-octyl sulfate (SOS) and cetyltrimethylammonium chloride (CTAC)/SOS vesicles.

Chapter 3 investigates the potential of using a partially fluorinated vesicle by combining CTAB with sodium perfluorooctanoate (FC7). The goal was to identify vesicles with smaller mean diameters in order to increase the sensitivity of the analysis. In addition, the effect of the fluorinated surfactant on selectivity was determined along with LFER analysis in order to compare to the CTAB/SOS vesicles. The resulting CTAB/FC7 vesicles were smaller than the CTAB/SOS vesicles and the CTAC/SOS vesicles. The
CTAB/FC₇ vesicles, however, were difficult to work with in terms of their reproducibility and unstable baseline at short wavelengths.

In Chapter 4 are presented the correlations of Caco-2 permeability and intestinal permeability with retention factor measured by CTAB/SOS vesicles and CTAC/SOS vesicles for 22 pharmaceutical compounds. The results were promising in terms of the neutral and cationic analytes. However, there was difficulty in correlating the retention factors of the anionic compounds to both the Caco-2 and the intestinal permeabilities due to problems in being able to accurately calculate retention factor. In addition, the logarithm of the octanol-water partition coefficient for each compound was also correlated to the logarithm of the retention factors.

This research has proven that two new unilamellar vesicle systems (CTAC/SOS and CTAB/FC₇) are viable as pseudostationary phases for use in electrokinetic chromatography. Furthermore, there were promising results in terms advancing VEKC as a high throughput method for screening intestinal permeability.
Chapter 1: Introduction to Capillary Electrophoresis

1.1 Historical Background of Capillary Electrophoresis (CE)

Capillary electrophoresis (CE) is a powerful analytical technique that was developed by Hjerten in 1967 [1]. Electrophoretic methods of analysis were in existence before that time, though. Hjerten’s research advisor, Tiselius, was a pioneer in the use of electrophoresis to investigate proteins and was awarded the Nobel Prize in 1948 for his work [2]. After Hjerten’s landmark discovery that using 300 µm tubes for electrophoresis would decrease thermal effects and that detection of the compounds could be done using UV absorbance, other researchers continued to develop the technique [2]. The next breakthrough discovery came when Jorgenson and Lukacs published results using capillaries with internal diameters of less than 100 µm [3, 4]. This led to enhanced interest in CE and a steady increase in publications per year for about two decades [5].

1.1.1 Components of CE Instrumentation

The basic components of CE include a polyimide-coated fused silica capillary, a high voltage power supply, two buffer reservoirs that can hold the capillary and the electrodes, and a detector [6]. See Figure 1.1 for a schematic of CE instrumental components. The inner diameter of the capillary can vary from 2 to 100 µm, while the range of the power supply is typically from 0 to 60 kV with most commercial instruments operating at a maximum of 30 kV. The detector could be a UV detector (single, variable wavelength can be collected), a photo diode array (PDA) detector (entire spectrum can be collected), a laser induced fluorescence detector, electrochemical detector, or a mass spectrometer.
Figure 1.1. Schematic of CE instrumental components [6].
1.1.2 Modes of CE and Fundamental Theory of CE

The modes of CE are the different types of CE that are commonly employed by analysts. The different modes are capillary zone electrophoresis (CZE) or free solution CE (FSCE), micellar electrokinetic chromatography (MEKC), capillary isoelectric focusing (CIEF), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), and capillary electrophoresis (CEC) [7]. The basic theory behind each of these modes of CE can be simplified to the theory behind CZE, which is the least complicated. To begin a CZE separation, a fused silica capillary is first activated with a concentrated solution of aqueous hydroxide. Then it is rinsed with water and filled with a buffer [8]. Next, sample is introduced to the inlet end of the capillary using either hydrodynamic or electrokinetic injection. The ends of the capillary and platinum electrodes are placed in vials filled with buffer. Then voltage is applied to the system. The charged analytes in the sample plug each move according to their individual electrophoretic mobilities [6]. The analytes eventually move past a detector where the data is collected and stored by a data acquisition system [6].

The CE separation is governed by the mobility of the analytes. The net mobility of an analyte is given by the following equation:

\[ \mu_{net} = \mu_{eo} + \mu_{ep} \tag{1.1} \]

where \( \mu_{eo} \) (electroosmotic mobility) and \( \mu_{ep} \) (electrophoretic mobility) are given by the following equations [9]:

\[ \mu_{eo} = \frac{\varepsilon \zeta}{4\pi \eta_{dl}} \tag{1.2} \]
where \( \varepsilon \) is the permittivity of the buffer, \( \zeta \) is the zeta potential, and \( \eta_{dl} \) is the viscosity in the electrical double layer.

\[
\mu_{ep} = \left( \frac{2}{3} \right) \varepsilon_0 \varepsilon_r \frac{\zeta_a}{\eta} \tag{1.3}
\]

where \( \varepsilon_0 \) is the permittivity of a vacuum, \( \varepsilon_r \) is the relative permittivity of the buffer (\( \varepsilon_r = \varepsilon_{buffer} / 4\pi \varepsilon_0 \)), \( \zeta_a \) is the zeta potential of the analyte, and \( \eta \) is the viscosity of the buffer. It should be pointed out the neutral molecules have no electrophoretic mobility. Normally, Equations 1.2 and 1.3 are not used to calculate \( \mu_{eo} \) and \( \mu_{ep} \), respectively. It is more practical to use the following equation for \( \mu_{eo} \):

\[
\mu_{eo} = \frac{L_{det} L_{tot}}{V t_0} \tag{1.4}
\]

where \( L_{det} \) is the length of the capillary to the detector, \( L_{tot} \) is the total length of the capillary, and \( V \) is the total applied voltage, and \( t_0 \) is the migration time of a neutral analyte.

The practical equation for \( \mu_{ep} \) is as follows:

\[
\mu_{ep} = \frac{\mu_{eo} (t_0 - t_R)}{t_R} \tag{1.5}
\]

where \( t_R \) is the migration time of the analyte.

For traditional CZE using a positive applied voltage at the capillary inlet, the migration order is cations, neutrals, and then anions. Cations and anions can be separated from each other, but neutral compounds elute in one peak. See Figure 1.2 for a typical CZE separation. Since neutrals have no electrophoretic mobility, they co-migrate with the same velocity as the EOF, reaching the detector window at the same time.
Figure 1.2. Typical CZE separation [6].
Electroosmotic flow (EOF) is the pumping mechanism behind CE. EOF is generated at the interface of the capillary wall and the buffer solution when voltage is applied. The resulting electric double layer is shown in Figure 1.3. Above pH 2 cations adsorb to the negatively charged ionized silanol groups that compose the wall of the capillary to form the inner Helmholtz plane (IHP) or Stern layer [10]. A diffuse layer of solvated cations and anions make up the outer Helmholtz plane (OHP) or Gouy-Chapman layer. In the presence of an electric field, the solvated cations migrate to the cathode and bring solvent with them [10]. The movement of the solvated cations is what creates EOF.

The motion of the solvated cations that generates EOF occurs in several steps. First, as the cations in the diffuse layer migrate toward the cathode, they bring with them the water molecules that are solvating them. Next, the water molecules on the cations drag other water molecules that are solvating the rest of the components in the capillary, including anions and neutrals, through friction. This frictional drag occurs all the way across the internal diameter of the capillary. The fluid filling the capillary begins to move almost all at the same velocity, which gives the flat flow profile characteristic of electrophoretic techniques.

EOF is affected by several factors, including buffer pH and concentration, temperature, viscosity, field strength, and organic modifiers [7]. Buffer pH affects the zeta potential in Eq. 1.2. EOF is faster at high pH compared to low pH due to the fully ionized silanols [7]. Since there are more SiO\(^{-}\) groups, more cations adsorb to the surface, creating a dense electrical double layer [7]. Figure 1.4 illustrates the effect of pH on EOF using
Figure 1.3. A. Dimensions of a typical capillary used for CE. B. Electrical double layer inside a capillary. [6].
Figure 1.4. Effect of pH on EOF using Pyrex, silica, and Teflon capillaries[11].
three different types of capillaries: Pyrex, silica, and Teflon. Silica capillaries have the biggest range of EOF over the pH range of 3 to 8 out of the three capillary types.

Buffer concentration or ionic strength influences EOF. With increasing ionic strength, EOF decreases. See Figure 1.5 for an example. The ionic strength controls the zeta potential, which is in the numerator of Eq. 1.2. Zeta potential is defined as the potential at the plane of shear and is proportional to the amount of charge on the surface of the silica multiplied by the counterion layer thickness [12] or electrical double layer. The amount of charge on the surface is reduced due to the presence of more counterions with increasing ionic strength [13]. In addition, higher ionic strength compresses the electrical double layer [13]. Since the two parameters that are proportional to zeta potential are reduced, the zeta potential, and hence, EOF are reduced.

EOF is also affected by temperature because temperature affects the viscosity of the buffer. Remember that viscosity is in the denominator of Eq. 1.2. When temperature increases, the viscosity decreases which increases EOF. With increased field strength, the EOF also increases. Adding organic solvents to the buffer affects the buffer viscosity and the zeta potential, depending on the identity of the organic solvent [7]. Short chain alcohols, such as methanol and ethanol, usually decrease the EOF by increasing the viscosity [7].

Controlling EOF is essential to obtaining reproducible results in CE. There are both chemical and physical methods for controlling EOF. Chemical methods rely on i)
Figure 1.5. Effect of ionic strength on EOF. A box = phosphate; circle = borate; triangle = carbonate [14].
surface-coating methods that bind polymers or nonionic surfactants to the surface silanols or ii) changing the chemical composition of the background electrolyte by varying the pH and ionic strength or adding organic solvents or surfactants [10]. A surfactant that is often used to reverse the direction of EOF is cetyltrimethylammonium bromide (CTAB). The physical methods for controlling EOF make use of a radial electric potential gradient to directly control the zeta potential and therefore EOF [10].

Temperature control in CE also plays a crucial part in obtaining reproducible, highly efficient peaks. Commercially available CE instruments offer either air cooled or liquid cooled thermostating systems. Joule heating is caused by the movement of ions relative to neutral solvent molecules in an electric field. To reduce Joule heating, one can either decrease the cross-sectional area of the capillary or lower the current [6]. The preferred method is to reduce the current by using a lower applied voltage or a separation buffer with a lower ionic strength [6]. Of these two options, using a lower applied voltage would increase the total run time whereas using a lower strength separation buffer would not. Also, using a lower separation strength buffer could potentially mean an increase in EOF and then a decrease in resolution.

In order to separate neutral compounds, Terabe et al. [15] performed electrophoretic separations with sodium dodecyl sulfates (SDS) micelles included in the background electrolyte (BGE). This is called Micellar Electrokinetic Chromatography (MEKC). Analytes partition into and out of the micelles during the separation. If the analyte is more hydrophobic, it will spend a greater amount of time associated with the micellar
phase than the BGE phase. The micelles act similarly to the stationary phase (SP) in high performance liquid chromatography (HPLC), but they are not fixed inside the capillary. Hence the term pseudostationary phase (PSP) is used to describe the micelles [16]. PSPs today are not limited to micelles alone. Other PSPs that have been used include vesicles [17], microemulsions [18], cyclodextrins [19], polymers [20], and surfactant coated carbon nanotubes [21]. With this in mind, the broad term electrokinetic chromatography (EKC) is used and then the PSP is specified as the first one or two letters as in vesicle EKC (VEKC) and microemulsion EKC (MEEKC).

There are three modes of EKC: normal, reversed, and restricted [22]. In normal mode, the electroosmotic velocity and the velocity of the PSP have the same direction, but the electroosmotic velocity is greater than the PSP velocity. In both reversed mode and restricted mode, the electroosmotic velocity and the velocity of the PSP have the opposite direction. In addition, the electroosmotic velocity is lower than the PSP velocity. Reversed mode and restricted mode have the potential for long analysis times [22]. Since the PSPs used in the research described here were negatively charged, the experiments were restricted to normal mode EKC.

In EKC, the PSP is typically charged and has an effective electrophoretic mobility [23]. The PSP will eventually move past the detection window. The usual elution order of the analytes in normal mode, when a positive voltage is applied to the inlet of the capillary, is the neutral EOF marker ($t_0$), cations, neutrals, anions, and then the marker for the PSP ($t_{psp}$). For a typical MEKC separation, see Figure 1.6. The ratio of $t_{psp}/t_0$ is called the
Figure 1.6. Typical MEKC separation [24].
migration window [25] or elution range. All of the neutral molecules must elute in the
time span between \( t_0 \) and \( t_{p_{ph}} \) in order to be separated in normal mode [23]. Proper
maintenance of pH is essential to maintaining the expected elution order. Figure 1.7
illustrates an example of the effect of pH on elution order. In Figure 1.7 A, the buffer pH
was 7.2 and the elution was phenol followed by methyl benzoate. However, the elution
order reversed when the buffer pH increased to 10.6. In Figure 1.7 B the methyl
benzoate elutes first followed by the phenol. Since the pH was now higher than the pK\(_a\)
of phenol (9.89) [26], phenol was converted from its neutral state to a predominantly
anionic state (via ionization of the hydroxyl group) and thus eluted after the neutral
methyl benzoate.

The retention factor \( (k) \) is the ratio of the number of moles of solute in the micellar phase
to the number of moles of solute in the aqueous buffer phase. Since it is not realistic to
count the moles of solute in either phase, an equivalent calculation using retention time
has been derived. In MEKC \( k \) is given by [27]:

\[
k = \frac{t_R - t_0}{t_0 \left(1 - \frac{t_r}{t_R}\right)}
\]

(1.6)

where \( t_{mc} \) is the migration time of the micelle. Eq. 1.6 is only valid for neutral solutes. \( k \)
can also be written in terms of mobility and is applicable for both neutral and charged
solutes [28]:

\[
k = \frac{t_R (1 + \mu_r) - t_0}{t_0 - \left(\frac{\mu_{eo} + \mu_{ep,ves}}{\mu_{eo}}\right)t_R}
\]

(1.7)
Figure 1.7. Elution order reversal for phenol (1) and methyl benzoate (2) using unmodified CTAC/SOS vesicles (A) and 0.5 % 2-amino-1-butanol modified CTAC/SOS vesicles (B). A 10 mM HEPES buffer at pH 7.2 was used for the unmodified vesicles and a 10 mM HEPES buffer at pH 10.6 was used for the 2-amino-1-butanol modified vesicles. Separation conditions: A new fused silica capillary of 31.2 cm total length, 20.0 cm length to the detector, 50 µm ID, and 363 µm OD at 25°C was used for each vesicle system. An applied voltage of 14 kV was used and injection was done hydrodynamically for 4.0 s at 13.8 mbar (0.2 psi). Detection was done using PDA at 214 nm.
where $\mu_{ep,ves}$, the electrophoretic mobility of the vesicle pseudostationary phase, is found by subtracting the electroosmotic mobility ($\mu_{eo}$) from the net mobility of the vesicle ($\mu_{ves}$), i.e., $\mu_{ves} - \mu_{eo}$. The relative electrophoretic mobility ($\mu_r$) is the ratio of $\mu_{ep,analyte}/\mu_{eo}$.

Chromatographic column efficiency in MEKC has to do with the random movement of the solute as it alternates between interacting with the stationary phase (micelles) and being carried along by the mobile phase (BGE). The random movement prevents all of the solute molecules from reaching the detector at the same time, which causes the signal to appear as a symmetrical peak with width instead of a delta function (spike). The amount of broadening of the signal peak is caused by the broadening of the injection plug band as it moves through the capillary [29]. Efficiency can be measured by two related terms, which are plate height ($H$) and number of theoretical plates ($N$). These are related by the following equation:

$$N = \frac{L}{H} \quad (1.8)$$

where $L$ is the length of the capillary. Given this simple equation, it should not be difficult to solve for either $H$ or $N$, provided the other two variables are known. However, $H$ and $N$ are theoretical concepts, not physical parameters that can be easily measured. In addition, the plate theory has been replaced by the rate theory, which still uses the terms $N$ and $H$ [29]. Today $H$ is defined as:

$$H = \frac{\sigma^2}{L} \quad (1.9)$$

where $\sigma^2$ is the variance (standard deviation squared) of the peak measurement. Mathematically, the variance of the peak can be determined and there are several
variations of the formula to calculate N, depending on how and where the peak width is measured. This group’s preferred method of calculating N is to use the Foley-Dorsey equation [30]:

\[
N = \frac{41.7 \left( \frac{t_R}{W_{0.1}} \right)^2}{\left( \frac{b_{0.1}}{a_{0.1}} \right) + 1.25}
\] (1.10)

where \( t_R \) is the migration time, \( W_{0.1} \) is the peak width at 10% peak height, and \( a_{0.1} \) and \( b_{0.1} \) are the respective widths of the first and second halves of the peak at 10% peak height.

Figure 1.8 shows a diagram of the specific measurements of the peak.

Compared to the efficiencies observed in HPLC, the efficiencies observed in EKC are far superior [22]. Conventional HPLC efficiencies are 15,000 compared to 200,000 in EKC [22]. Part of the reason for the drastic difference is the difference in the flow profiles of the two techniques. HPLC with hydrodynamic or pressure-driven flow has a parabolic profile while EOF has a flat plug-like profile. See Figure 1.9. Pressure-driven flow has a leading edge that contributes to the broader peaks that are observed in HPLC. With EOF, there is no leading edge so the analyte molecules reach the detector at virtually the same time.

Resolution (\( R_s \)) can be calculated using the following equation, which is valid for neutral solutes and charged enantiomers [28]:

\[
R_s = \sqrt{\frac{N}{4}} \left( \frac{\alpha - 1}{\alpha} \right) k \left( 1 + \frac{\mu_r - \frac{t_0}{t_{pp}}}{1 + \frac{t_0}{t_{pp}} k_{avg}} \right) \left( 1 + \frac{\mu_r}{1 + \frac{t_0}{t_{pp}} k_{avg}} \right)
\] (1.11)
Figure 1.8. Diagram of the specific measurements of a peak where $t_R$ is the migration time of the analyte, $A$ is the width at 10% of the first half of the peak, and $B$ is the width at 10% of the second half of the peak. The total width at 10% peak height ($W$) is given by $A+B$ [30].
Figure 1.9. Comparison of a.) Laminar or hydrodynamic flow profile to b.) electroosmotic flow profile [31].
where $\alpha = k_2/k_1$ is the chromatographic selectivity, $k_1$ and $k_2$ are the retention factors of the first and second analytes, respectively, $k_{\text{avg}}$ is the average retention factor, and $t_{\text{psp}}$ is the time that it takes the pseudostationary phase to migrate to the detector. All other symbols have been previously defined.

Peak capacity is the maximum number of analytes that can be separated in a single run [32]. In the normal mode of EKC, the migration window limits both the peak capacity and the maximum achievable resolution. The last term in Eq. 1.11 has the ratio $t_0/t_{\text{psp}}$. Even with a value of infinity for $t_{\text{psp}}$, the maximum value of resolution in normal mode EKC would be much less than conventional HPLC for the same values of efficiency, selectivity, and retention factor. Fortunately, the extremely high efficiencies that are obtained in EKC offset the limitation of the migration window [22].

### 1.1.2.1 Introduction to and Brief Review of Vesicle Electrokinetic Chromatography (VEKC)

Numerous papers have been published on the characterization of vesicles using EKC, however, for the purposes of this review, only papers where the vesicles were used as a pseudostationary phase have been included. What are vesicles and/or what are they comprised of? Vesicles are a type of surfactant aggregate that consist of one or more concentric bilayers surrounding an aqueous core. They can be composed of natural or synthetic surfactants. Figure 1.10 has the structures of the surfactants that were investigated in this thesis. Liposomes are vesicles that are composed of phospholipids. Vesicles are larger than micelles and can either be unilamellar or multilamellar, depending on the processing method [33].
Figure 1.10. Structures of the surfactant components of the vesicles in this thesis.
Figure 1.11 shows a comparison of a micelle versus unilamellar and multilamellar vesicles.

In Table 1.1 is a comprehensive list of all the vesicles and liposomes that have been used as pseudostationary phases for EKC. Vesicle electrokinetic chromatography (VEKC) was first reported by Zhang et al. in 1995 [34]. They used liposomes composed of L-α-phosphatidylcholine and sodium cholate to separate peptides and four drugs. This initial report was followed by two papers published in 1996. The first was published by Roberts et al. [35] and focused mainly on the characterization of liposomes using CE. They were able to calculate the concentration of the liposomes, as well as the uniformity of the size distribution. Additionally, at the end of their paper, they included the electropherograms of riboflavin with and without liposomes. When riboflavin was analyzed in the presence of liposomes, the migration time of riboflavin increased from 9.7 min. to 13.5 min. This proved that there was an interaction between riboflavin and the liposomes. The second report was published as a Letter to the Editor in *Analytical Sciences* by Nakamura et al. [36]. They described the use of liposomes formed from dimyristoyl L-α-phosphatidylcholine (DMPC) and dimyristoyl L-α-phosphatidyl-D,L-glycerol (DMPG) for the separation of i) naphthalene and biphenyl and ii) nitrobenzene and three positional isomers of dinitrobenzene.

1.1.2.1 Early VEKC

From the late 1990’s to the present, various groups have used vesicles as a pseudostationary phase. The interest in this area of research has prompted the publication
Figure 1.11. Structure and size of a micelle compared to a unilamellar vesicle and a multilamellar vesicle. SUV = small unilamellar vesicle; LUV = large unilamellar vesicle; MUV = multilamellar vesicle [33].
Table 1.1 Components of vesicles that have been used for VEKC.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Modifier</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-α-phosphatidylcholine (PC) and cholate</td>
<td>None</td>
<td>[34]</td>
</tr>
<tr>
<td>Dimyristoyl L-α-phosphatidylcholine (DMPC) and dimyristoyl L-α-phosphatidyl-D,L-glycerol (DMPG)</td>
<td>None</td>
<td>[36]</td>
</tr>
<tr>
<td>DMPG, dicetyl phosphate, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, and cholesterol</td>
<td>None</td>
<td>[35]</td>
</tr>
<tr>
<td>N-dodecyltrimethylammonium bromide and sodium dodecyl sulfate (SDS)</td>
<td>None</td>
<td>[17]</td>
</tr>
<tr>
<td>Bis (2-ethylhexyl) sodium sulfosuccinate (AOT)</td>
<td>None</td>
<td>[37]</td>
</tr>
<tr>
<td>Cetyltrimethylammonium bromide (CTAB) and dodecoxycarbonylvaline (DDCV)</td>
<td>None</td>
<td>[38]</td>
</tr>
<tr>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC), egg yolk phosphatidylglycerol (PG), cardiolipin, phosphatidic acid (PA), and bovine brain phosphatidylinerine (PS)</td>
<td>None</td>
<td>[39]</td>
</tr>
<tr>
<td>Dihexadecyl hydrogen phosphate</td>
<td>None</td>
<td>[40, 41]</td>
</tr>
<tr>
<td>CTAB and sodium n-octyl sulfate (SOS)</td>
<td>None</td>
<td>[42]</td>
</tr>
<tr>
<td>PC, PG, cardiolipin, PA, and PS</td>
<td>None</td>
<td>[43]</td>
</tr>
<tr>
<td>Dipalmitoyl-L-alpha-phosphatidylcholine, dipalmitoyl-L-alpha-phosphatidylycerol, and cholesterol</td>
<td>None</td>
<td>[44]</td>
</tr>
<tr>
<td>CTAB and SOS; AOT</td>
<td>None</td>
<td>[45]</td>
</tr>
<tr>
<td>CTAB and SOS</td>
<td>Glycitol, 1,3-butanediol, 2-amino-1-butanol, 1,2,6-hexanetriol and ACN</td>
<td>[46]</td>
</tr>
<tr>
<td>POPC and cholesterol</td>
<td>None</td>
<td>[47]</td>
</tr>
<tr>
<td>Dihexadecyltrimethylammonium bromide</td>
<td>None</td>
<td>[48]</td>
</tr>
<tr>
<td>POPC or POPC with different proportions of bovine brain PS</td>
<td>Cholesterol</td>
<td>[49]</td>
</tr>
<tr>
<td>Commercial cationic liposome formulations, Lipofectamine and Escort,</td>
<td>None</td>
<td>[50]</td>
</tr>
<tr>
<td>Sodium N-[4-dodecyloxybenzoyl]-L-valinate</td>
<td>None</td>
<td>[51]</td>
</tr>
<tr>
<td>CTAB and SOS; octyltrimethylammonium bromide (OTAB) and SDS; AOT, POPC and PS</td>
<td>None</td>
<td>[52, 53]</td>
</tr>
<tr>
<td>POPC</td>
<td>None</td>
<td>[54, 55]</td>
</tr>
<tr>
<td>(1R,2S)-(−)-N-dodecyl-N-methyl-ephedrinium bromide</td>
<td>None</td>
<td>[56]</td>
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Table 1.1 (continued)

<table>
<thead>
<tr>
<th>Component</th>
<th>Additives</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-palmitoyl-2-oleyl-sn-glycero-3-phosphatidylcholine and PS</td>
<td>None</td>
<td>[57]</td>
</tr>
<tr>
<td>PC; DPPC</td>
<td>Calcium, Magnesium, and Zinc</td>
<td>[58]</td>
</tr>
<tr>
<td>PC, PS, PG, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (PE), L-α-phosphatidylinositol (PI), and sphingomyelin, (SPH)</td>
<td>Cholesterol</td>
<td>[59]</td>
</tr>
<tr>
<td>PC and PG</td>
<td>Cholesterol</td>
<td>[60]</td>
</tr>
<tr>
<td>PC and PS</td>
<td>None</td>
<td>[61, 62]</td>
</tr>
<tr>
<td>Sodium N-(4-n-dodecylxybenzoyl)-l-valinate</td>
<td>None</td>
<td>[63]</td>
</tr>
<tr>
<td>POPC and PS</td>
<td></td>
<td>[64]</td>
</tr>
<tr>
<td>CTAB and SOS; Cetyltrimethylammonium chloride (CTAC) and SOS</td>
<td>2-amino-1-butanol; ACN</td>
<td>[65]</td>
</tr>
<tr>
<td>1-palmitoyl-2-oleyl-sn-glycero-3-phosphatidylcholine, PC, and PS</td>
<td>Cholesterol</td>
<td>[66]</td>
</tr>
<tr>
<td>Dipalmitoylphosphatidylcholine, SPH, and human red blood cell ghost lipids</td>
<td>Cholesterol</td>
<td>[67]</td>
</tr>
<tr>
<td>Sodium N-[4-n-dodecylxybenzoyl]-L-leucinate (SDLL) and sodium N-[4-n-dodecylxybenzoyl]-L-isoleucinate (SDLIL)</td>
<td>None</td>
<td>[68]</td>
</tr>
<tr>
<td>PC vesicles in the presence of 20 mol% PS, PA, PI, and PG</td>
<td>Cholesterol; Calcium chloride</td>
<td>[69]</td>
</tr>
<tr>
<td>PA, PG, PI, PS, and POPC</td>
<td>None</td>
<td>[70]</td>
</tr>
</tbody>
</table>
of two reviews [33, 71]. In the early stages of VEKC, Hong et al. [17] reported the first use of synthetic vesicles as a pseudostationary phase (PSP). Some of the major findings of this study were improvements in efficiency, selectivity, and elution range compared to using micelles as the PSP. Following this report using synthetic vesicles was one that included surfactant vesicles composed of CTAB and the chiral surfactant DDCV [38]. However, in spite of an increased elution range and selectivity improvements, no chiral separations were reported. Another report investigated vesicles formed from bis(2-ethylhexyl)sodium sulfosuccinate (AOT) which were used in the separation of antioxidants that are commonly found in food [37]. Another early use of liposomes in EKC was published by Wiedmer et al. [39]. This group used several different combinations of phospholipids for their study which investigated the effect of buffer, concentration, cardiolipin (CL) concentration, and polar head group for the separation of six corticosteroids. By increasing the amount of negative charge on the liposomes and by increasing the total lipid concentration, the separations improved [39].

### 1.1.2.1.2 Use of VEKC to Determine Partition Coefficients

There have been several reports using VEKC to determine various partitioning coefficients (K). In chromatography, K is ratio of the concentration of the analyte in the stationary phase to the concentration of the analyte in the mobile phase [29]. In EKC, the stationary phase is usually a surfactant aggregate and the mobile phase is the BGE. The earliest report from Agbodjan et al. investigated solute partitioning into dihexadecyl hydrogen phosphate (DHP) vesicles [40]. Here they used the Linear Solvation Energy Relationship (LSER) model to characterize the solute-vesicle interactions. The LSER
model is useful for determining the types of intermolecular interactions that contribute to partitioning. This is possible due to the relationship between retention factor \( k \) and the partition coefficient \( K \) as shown in the following equation [44]:

\[
k = K_{lw} \left( \frac{V_l}{V_{aq}} \right) = K_{lw} \ast \bar{v}([P] - CAC) \quad (1.12)
\]

where \( K_{lw} \) is the liposome-water partition coefficient, \( V_l \) is the volume of the liposomes, \( V_{aq} \) is the volume of the aqueous phase, \( \bar{v} \) is the partial specific molar [sic] volume of the phospholipid, \([P]\) is the phospholipid concentration, and \( CAC \) is the critical aggregation concentration. From the LSER model, they learned that solute size and hydrogen bond acceptor strength contributed the most to partitioning.

Other researchers have attempted to correlate retention factors with octanol-water partition coefficients \( (K_{ow}) \) [42, 45] with the goal of developing a rapid alternative to the shake-flask method. Vesicle-water partition coefficients were determined for 41 uncharged solutes using DHP vesicles at both 36 °C and 71 °C [41]. Liposome-water partition coefficients were studied by Burns et al. [72] using liposomes composed of dipalmitoyl-L-\( \alpha \)-phosphatidylcholine, dipalmitoyl-L-\( \alpha \)-phosphatidylglycerol (sodium salt), and cholesterol. Similarly, partitioning into cationic vesicles of dihexadecyldimethylammonium bromide was investigated [48]. LSER analysis was used to supplement the study. In addition, LSER analysis has been reported for vesicle analysis by other researchers [44, 52]. The main conclusion from the LSER analysis has been that the factors that contribute the most to retention are the size and hydrogen bond basicity of the solute.
1.1.2.1.3 Traditional Separations using VEKC

There are a limited number of traditional separations using VEKC. Hong et al. investigated the separation of substituted benzenes and geometrical isomers of nitrotoluene [17]. One example used POPC/PS liposomes to separate benzene derivatives, steroids, and phenols while adjusting the pH and the salt concentration [43]. Another example used 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC) for the analysis of basic proteins [54, 55] and peptides [54]. They obtained nearly baseline separation of cytochrome c, lysozyme, ribonuclease A, and α-chymotrypsinogen using 60 µM POPC suspension in 20 mM phosphate buffer, pH 7.4, containing 68 mM NaCl.

1.1.2.1.4 Chiral Analysis using VEKC

Chiral separations using VEKC were first reported in 2003 by Mohanty and Dey [51]. In this work, Mohanty and Dey utilized vesicles composed of sodium N-[4-dodecyloxybenzoyl]-L-valinate (SDLV) for the separation of (±)-1,1’-bi-2-naphthol and (±)-1,1’-binapthyl-2,2’-diylhydrogenphosphate. They continued to explore the possibility of using VEKC to conduct chiral analyses of nonsteroidal anti-inflammatory drugs [56]. Additional work was conducted to determine the interaction of the chiral SDLV vesicles with five sterically hindered atropisomeric compounds, such as (±)binaphthyl diamine, and benzoin (BZN) [63]. Two more types of chiral vesicles formed from sodium N-[4-n-dodecyloxybenzoyl]-L-leucinate (SDLL) and sodium N-[4-n-dodecyloxybenzoyl]-L-isoleucinate (SDLIL) were also described to test the role of the surfactant headgroup for the chiral separations of atropisomeric compounds [68].
1.1.2.1.5  Modifiers in VEKC

In terms of modifying the environment of the vesicles, two different methods have been employed i) the addition of cholesterol and ii) the addition of organic modifiers. Wiedmer et al. included cholesterol in their liposome solutions in order to better model real biological membranes [47]. Depending on the identity of the buffer that was used, the cholesterol-containing liposomes were larger (AMPSO; DIPSO; HEPES) or smaller (MOPS; Tricine) than the liposomes without cholesterol. Also, as the concentration of cholesterol increased, the interaction between the liposomes and estradiol decreased. Pascoe and Foley were the first to use organic modifiers with VEKC to investigate potential enhancements to the separations [46]. They used CTAB/SOS vesicles with five different organic modifiers and included LSER analysis to describe the interactions that were responsible for retention. A subsequent project investigating organic modifiers is reported in Chapter 2 of this thesis [65]. The research in Chapter 2 also considers the effect of changing the cationic counterion from bromide to chloride. One of the significant findings of this work was the improvement in efficiency observed for hexanophenone and heptanophenone using vesicles modified with 10% ACN.

1.1.2.1.6  Liposome Capillary Coatings

Several papers have been published on the use of liposomes for coating capillaries for EKC. The liposomes form a bilayer coating on the surface of the capillary as a stationary phase [49]. Hence, separation of neutral compounds is possible and has been demonstrated [49]. Some of the parameters that have been investigated regarding liposome coatings have been the addition of calcium [57], the effect of pH [61], and the
effect of piperazine buffers [66]. Calcium improved the stability of the coatings. In an attempt to increase the rigidity of the membrane, other divalent metals such as magnesium and zinc were investigated [58], along with the effect of temperature. Just as cholesterol was added to the free liposomes, it was also added to those for capillary coatings [67]. The presence of cholesterol improved the stability of the DPPC coating at 45 °C, but had no effect on the DPPC coating stability at 25 °C. In addition, red blood cell ghost lipids were used as a capillary coating [67]. Unfortunately, the stability of the red blood cell ghost lipid coating changed with time over the course of 18 runs of five neutral steroids. The stability of the phospholipid coatings was examined via asymmetrical flow field-flow fractionation and CE [69]. Most recently, the importance of the phospholipid polar head group for coatings on silica has been examined [70]. Liposomes with 1-palmitoyl-2-oleyl-sn-glycero-3-phosphatidylcholine (POPC) and one of the following: phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI), or phosphatidylserine (PS), as well as 3 mM of CaCl$_2$ were included in the study. Calcium was shown to interact the most strongly with POPC membranes that contained PA.

1.1.2.1.7 Drug-vesicle Partitioning and Drug Delivery via Vesicles

Finally, there has been interest in studying drug-vesicle interactions using VEKC. Manetto et al. studied the interaction of POPC with salicylic acid, acetylsalicylic acid, ketoprofen, phenytoin and propranolol [73]. Their results indicated that acidic and neutral compounds could be analyzed together, but basic compounds needed to be analyzed separately. Carrozzino and Khaledi also examined the interactions between
drugs and lipids, but with regard to buffer type, concentration, ionic strength, and liposome composition [59]. Of these, ionic strength and increased charge on the liposomes were more influential than buffer type and concentration. They continued their studies by investigating the effect of pH on the drug-liposome interactions [60]. The study concluded that with increasing pH, the retention of basic drugs decreases sigmoidally when they interact with negatively charged liposomes [60]. Yet another example of drug membrane permeability correlated with VEKC data has been given by Oernskov et al. who correlated retention factor data to two parameters that estimate intestinal absorption: the distribution coefficient in octanol/water for charged compounds at a given pH (Log D) and Caco-2 permeability [64]. Pascoe et al. also correlated retention factor data from VEKC to intestinal permeability [53]. The correlations were strong and proved that intestinal permeability could be modeled using VEKC. Very recently, Wang et al. attempted to correlate retention factors from liposome EKC to the fraction of the drug absorbed in humans (Fa) {Wang, 2007 #189}. Their results showed a sigmoidal relationship between the logarithm of the retention factor and Fa. Related to the study of drug-liposome interactions is the delivery of drugs via liposomes. There is one example of such work by McKeon and Khaledi who used laser-induced fluorescence to monitor the delivery of antisense oligonucleotide [50]. In this work, they were able to calculate the binding constants for the oligonucleotide with two commercially available liposomes, Lipofectamine and Escort.
1.2 **Background of Linear Free Energy Relationship (LFER) Analysis**

LFER analysis was developed in order to quantify the intermolecular interactions that are responsible for retention. Presented here is a brief introduction to LFER. For a comprehensive discussion of LFER, please see the 2006 review by Vitha and Carr [74]. Although LFER was first reported in 1921 [75], it was Hammett’s research [76, 77] that brought attention to the subject. Kamlet et al. introduced LFER analysis using the solvatochromic model in 1983 [78], which is described by the following equation:

\[
\alpha \beta \pi = \log V + s \pi + b \beta + a \alpha \tag{1.13}
\]

where \( k \) is the retention factor, \( \log k_0 \) is a regression constant, and the coefficients \( m, s, b, \) and \( a \) are the cohesiveness, dipolarity, hydrogen-bond donor ability, and hydrogen-bond acceptor ability of the applied surfactant system. \( V \) is the molar volume of the solute, \( \pi \) is a measure of the solute dipolarity/polarizability, \( \beta \) is the hydrogen-bond acceptor ability, and \( \alpha \) is the hydrogen bond donor ability of the solute [79]. The solvatochromic model was later modified by Abraham [80] who added a sixth parameter and called his model the solvation model. One advantage of the solvation model over the solvatochromic model is the use of free-energy related solute properties over spectroscopic energies [81]. Also, one can easily determine more solute descriptors, whereas there is no set method of determining other solvatochromic “solute” parameters [81]. The following equation is used to describe most of the contributing factors to solute-solvent interactions:

\[
\log k = c + vV + eE + sS + aA + bB \tag{1.14}
\]

where \( k \) is the retention factor, \( V, E, S, A, \) and \( B \) are the solute descriptors, \( c \) is the y-intercept, and \( v, e, s, a, \) and \( b \) are the system coefficients. Among the solute descriptors,
\( V \) is the McGowan’s characteristic volume, which can be calculated \([82]\). Provided the structure of the compound is known, there is a simple formula for calculating McGowan's characteristic volume by taking the sum of the atomic volumes minus the value of each bond (6.56 cm\(^3\)mol\(^{-1}\)), where all bonds are treated equally no matter if they are single, double, or triple bonds \([83]\). \( E \) represents excess molar refraction and can be calculated using the refractive index of the solute at 20°C for the sodium D-line \([81]\). Next \( S \) represents dipolarity/polarizability, which is determined from liquid-liquid distribution constants and chromatographic measurements \([83]\). \( A \) and \( B \) represent hydrogen bond acidity and hydrogen bond basicity, respectively. Both \( A \) and \( B \) are determined in a similar fashion to \( S \). The system coefficient \( v \) is the difference in the ease of cavity formation for the solute between the buffer and the aggregate phase, \( e \) is the difference in capacity of the buffer and the aggregate phase to interact with the solutes’ \( n- \) or \( \pi- \) electrons, \( s \) is the difference in dipolarity/polarizability between the aggregate phase and the bulk aqueous phase, \( a \) is the difference in hydrogen bond basicity, and \( b \) is the difference in hydrogen bond acidity. The system coefficients are calculated using multiple linear regression on a highly overdetermined data set, i.e., a data set in which the number of retention factor measurements (of different solutes) greatly exceeds the number of system coefficients and y-intercept in Eq. 1.14.

Once the system coefficients are obtained, then they are evaluated based upon their magnitude, chemical meaning, and signs \([74]\). The magnitude of the coefficient corresponds to the difference between the interactions of the two phases under study and the amount each interaction contributes to retention \([74]\). The chemical meaning of the coefficients is that they are “complementary to the solute parameter which they modify”
For example, the coefficient $b$ represents differences in solvent hydrogen bond acidity and modifies $B$ which is the solute’s hydrogen bond basicity descriptor [74]. Depending on how the solute property is defined, the sign of the coefficient signifies “which phase has a greater interaction ability for each specific interaction being modeled” [74]. In the LFER results presented in this work, the solute property is retention so the differences are between retention by the pseudostationary phase (vesicles) versus the BGE. Negative values indicate that the BGE has more interactions of a specific type than the vesicle phase. The number of solutes required for the LFER analysis is between 20 and 40 [83]. However, the solutes must comprise a wide range of interactions. This requirement can be tested through the use of cross-correlation analysis. If the correlation coefficient is $\geq 0.8$ ($r^2 > 0.64$), then the group of solutes chosen must be reconsidered [83].

Abraham based his solvation parameter model on a cavity model of solution [83]. Figure 1.12 shows the steps of solvation. Initially, an appropriately sized cavity is formed in the solvent for the solute [83]. This process requires an amount of energy dependent upon the intermolecular forces holding the solvent together and the size of the solute [83]. Next, the solvent molecules gather around the solute to reestablish equilibrium, a process that requires minimal energy [83]. Lastly, the solute enters the cavity and begins to interact with the solvent molecules surrounding it [83]. For a neutral analyte, the forces could be dispersion, induction, orientation, and hydrogen bonding [83]. The free energy of transfer that occurs in electrokinetic chromatography is equal to “the difference in
Figure 1.12. Steps of solvation [74].
cavity formation and solute-solvent interactions in the electrolyte solution and pseudostationary phase” [83].

Once the LFER coefficients have been obtained, they are usually compared to other sets of coefficients. There have been a few different methods for LFER coefficient comparison [84-86]. The most recent approach normalizes each coefficient by dividing by a normalization factor (ω) [87]. Taking the coefficient e as an example:

\[
e_u = \frac{e}{\omega}
\]

where the subscript u indicates the normalized coefficient and ω is given by:

\[
\omega = \sqrt{e^2 + s^2 + a^2 + b^2 + v^2}
\]

Then, the normalized coefficients are used to calculate a distance (d) using the following equation [88]:

\[
d = \sqrt{(e_u - e_j)^2 + (s_u - s_j)^2 + (a_u - a_j)^2 + (b_u - b_j)^2 + (v_u - v_j)^2}
\]

If the value of d is < 0.25, then the two systems are considered similar [88]. One of the most significant aspects of these comparisons is that all types of separation systems may be compared to each other. The LFER coefficients do not have to be taken from EKC data, but they could be from HPLC or GC, as well.

Overall, LFER analysis is a powerful tool for investigating the intermolecular interactions that contribute to retention/partitioning. Much invaluable information can be obtained by using LFER analysis. This easy method has applicability to several different systems, such as MEKC, HPLC, GC, and SFC, which makes it truly impressive [74].
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Chapter 2: Effect of Surfactant Counterion and Organic Modifier on the Properties of Surfactant Vesicles in Electrokinetic Chromatography

2.1 Introduction

Drug delivery devices, cell membrane models, and pseudostationary phases (PSPs) in EKC are some of the most beneficial applications of vesicles. Targeted drug delivery is made possible by vesicles [1, 2]. Also, vesicles are a less expensive, lower maintenance alternative to using \textit{in-vivo} cell cultures, such as Caco-2, to investigate cell membrane permeability [3, 4]. Finally, compared to micelles, vesicles offer unique separation mechanisms and increased elution ranges as PSPs in EKC.

Nakamura et al. reported the first separation using L-\(\alpha\)-phosphatidylcholine/L-\(\alpha\)-phosphatidyl-D,L-glycerol liposomes in CE in 1996 [5] and coined the phrase “liposome electrokinetic chromatography.” Also in 1996, Roberts et al. [6] showed that the electropherogram of riboflavin was altered in the presence of liposomes. Then Hong et al. described the first use of synthetic DTAB/SDS vesicles in EKC [7]. They reported improvements in efficiency, selectivity, and elution range over conventional SDS micelles. Following those events, papers detailing other applications of vesicles in EKC other than pure separations began to emerge including the determination of octanol-water partition coefficients [8, 9], and vesicle-water partition coefficients [10]. Separations using vesicles have also been reported [11-14] with one of the most recent being enantiomeric separations using chiral vesicles reported by Mohanty and Dey [15, 16].
There have been numerous reports on the effect of counterions on micelles in EKC [17-23], but no reports on the effect of counterions on vesicles in EKC. Results of a comparison of counterion effects on vesicles not within the context of EKC have revealed that tosylate counterions yield larger diameter vesicles compared to bromide counterions with a cetyltrimethylammonium surfactant/dodecylbenzene sulfonic acid vesicle system [24].

Organic modifier effects in CE have long been studied by a variety of researchers and have been reviewed [25, 26]. Among the motivations that Huie cites for groups using organic modifiers in the BGE are increased solubility of hydrophobic compounds, improved selectivity, ability to use higher applied voltages resulting in decreased run times, and making the CE to MS interface more compatible. Additionally, organic solvents have been used in field-amplified sample stacking to lower the conductivity of the BGE [27]. Another advantage is decreased retention, which has been demonstrated by Ahmed and Lloyd [28]. Decreased retention also results in decreased run times, and also in improved resolution for compounds whose retention is greater than the optimum value. Organic modifiers have been divided into Class I and Class II materials [29]. Class I modifiers are incorporated into the surfactant aggregate and Class II modifiers affect the solvent-aggregate interactions. Examples of Class I modifiers include long-chain amides and alcohols. Examples of Class II modifiers include short-chain alcohols, formamide, acetonitrile (ACN), guanidinium salts, and urea.
While there have been several reports regarding the effect of organic modifiers on micelles [30-34], there have not been nearly as many reports regarding the effect of organic modifiers on polymeric PSPs and vesicles. The effect of adding methanol and acetonitrile to anionic siloxane surfactants was investigated by Peterson et al. [35]. Their results showed that the siloxanes’ electrophoretic mobilities remained high in the presence of the organic solvents, the methylene selectivities were also high, and the elution range was large. In addition, they demonstrated the nearly complete baseline separation of 14 PAHs in the presence of 40% ACN. In 2002, Pascoe and Foley [36] described the effect of Class I and Class II modifiers on CTAB/SOS vesicles. Their findings included the fact that (i) increasing the percentage of ACN increased the elution range; and (ii) 2-amino-1-butanol (AB) had the least dramatic effect on methylene selectivity. Therefore, these two modifiers were chosen for the present study.

The experiments described here include the first report of CTAC/SOS vesicles as a PSP in EKC. Both unmodified CTAC/SOS and CTAB/SOS vesicles are compared to AB modified vesicles and ACN modified vesicles of the same types. Electrophoretic and chromatographic parameters are provided for all six vesicle systems studied. Despite the mixed results concerning the effect of organic modifier on efficiency in MEKC [19, 37-39], significant increases in efficiency were observed for the more hydrophobic alkylphenones in the presence of 10% ACN vs. unmodified vesicles for both CTAB/SOS and CTAC/SOS. LFER analysis has also been included to aid in the quantification of specific molecular interactions.
2.2 Materials and Methods

2.2.1 Chemicals and Reagents

SOS was purchased from Lancaster Synthesis Inc. (Windham, NH, USA). CTAB, CTAC, HEPES, 2-amino-1-butanol, and all of the solutes used in LFER analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and HPLC grade acetonitrile were purchased from Fisher (Fairlawn, NJ, USA). Water from a Barnstead E-pure Water system (Dubuque, IA, USA) was used to prepare the buffer.

2.2.2 Instrumentation

All separations were performed on a Beckman Coulter P/ACE™ MDQ Series Capillary Electrophoresis System (Fullerton, CA, USA) equipped with a diode array detector. Wavelengths of 214 and 246 nm were collected. The temperature was set at 25°C for both the capillary cartridge and the compartment that holds both the sample and buffer solutions. An applied voltage of 14 kV was used for all separations so that Joule heating would not exceed 1.5 W/m; in addition, a linear voltage ramp (0.17 min.) from 0 to 14 kV was employed during the application of the applied voltage to prevent accidental sample loss due to thermal expansion caused by Joule heating. The samples were injected for 2.0 seconds at 13.8 mbar (0.2 psi). The data were collected and processed using Beckman Coulter 32 Karat™ Software Version 5.0 (Fullerton, CA, USA).

The size of the vesicles was measured using a Horiba LB 500 Dynamic Light Scattering Particle Size Analyzer (Irvine, CA, USA) while the temperature was maintained at 25°C.
A new 31.2 cm x 50 µm ID fused silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used for each vesicle system investigated. Prior to first use, each capillary was conditioned for 5 min with HPLC-grade water, 10 min with 1.0 M sodium hydroxide, 5 min with 0.1 M sodium hydroxide, 3 min with HPLC-grade water, and 15 min with background electrolyte (BGE), all at an applied pressure of 2070 mbar (30 psi). Between injections the capillary was rinsed with BGE for 2 min at 2070 mbar.

2.2.3 Vesicle Preparation

All of the vesicle systems were prepared in a 1:3.66 mole ratio of CTAB:SOS or CTAC:SOS for a total surfactant concentration of 69 mM. The mole ratio and total surfactant concentration was optimized in earlier work [40, 41]. First CTAB was dissolved in a beaker in a small amount of water and an appropriate amount of stock HEPES buffer adjusted to pH 7.2 by 1.0 M LiOH. The solution was stirred and heated on a low setting until all of the CTAB dissolved. Next the heat was turned off and the SOS was stirred into the still warm CTAB solution with more water until it dissolved. The resulting solution appeared bluish, was transferred to a volumetric flask, and was vortex mixed for approximately 2 minutes. The vesicles were allowed to equilibrate for a minimum of 12 hours before use. The same procedure was used to prepare CTAC/SOS vesicles. For the Class I modified vesicles, 0.5 % (v/v) of AB was added to a volumetric flask and then the surfactant solution (CTAB or CTAC and SOS) was added to the flask. For the Class II modified vesicles, 10 % (v/v) ACN was added similarly. The concentrations of AB and ACN were chosen based upon previous work by Pascoe and Foley [36]. However, organic modifier was added into the beaker initially [36], whereas it was added to the volumetric flask in the present study. Prior to use, the vesicles were
filtered using a 0.45 µm nylon membrane filter (13 mm diameter) from Whatman (Clifton, NJ, USA).

2.2.4 Sample Preparation

Stock solutions ranging from 3-6 mg/mL were prepared for all solutes by dissolution of the appropriate amount in HPLC grade methanol. The stock solutions were then diluted with 10 mM aqueous HEPES buffer at pH 7.2 (7 parts stock + 3 parts buffer). Since the conductivity of the final sample was somewhat lower than that of the BGE, a slight amount of field-amplified zone sharpening occurred for the sample zone.

2.2.5 Calculations

All of the migration times were corrected for the 0.17 min voltage-ramp that was used according to the following equation:

\[ t_{VRC} = t_r - \frac{t_{ramp}}{2} \]  \hspace{1cm} (2.1)

where \( t_{VRC} \) is the voltage-ramp corrected migration time, \( t_r \) is the migration time of the solute, and \( t_{ramp} \) is the time of the linear voltage-ramp (10.2 s).

The maximum of the first positive baseline disturbance caused by methanol at 214 nm was used as the \( t_0 \) marker. This value was used in the following equation to calculate electroosmotic mobility (EOF):

\[ \mu_{eo} = \frac{L_s L_t}{t_0 V} \]  \hspace{1cm} (2.2)
where \( L_d \) is the length of the capillary to the detector, \( L_t \) is the total length of the capillary, \( t_0 \) is the time of an neutral marker, and \( V \) is the applied voltage.

The retention factors of the solutes were calculated using the following equation:

\[
k = \frac{t_r (1 + \mu_r) - t_0}{t_0 - \left( \frac{\mu_{eo} + \mu_{ep,ves}}{\mu_{eo}} \right) t_r}
\] (2.3)

where \( \mu_{ep,ves} \), the electrophoretic mobility of the vesicle pseudostationary phase, is found by subtracting the electroosmotic mobility \( (\mu_{eo}) \) from the net mobility of the vesicle \( (\mu_{ves}) \), i.e., \( \mu_{ves} - \mu_{eo} \). The net (observed) vesicle mobility, \( \mu_{ves} \), is calculated via Eq. (2.2), with the migration time of the vesicle \( (t_{ves}) \) substituted for \( t_0 \). Finally, the relative electrophoretic mobility \( (\mu_r) \) is the ratio of \( \mu_{ep,analyte}/\mu_{eo} \).

A homologous series of alkylphenones (acetophenone through heptanophenone) were analyzed and their retention factors calculated in order to determine \( t_{ves} \), using the method of Bushey and Jorgenson [42] in which the logarithm of the retention factor vs. carbon number of a homologous series is assumed to yield a linear relationship. The retention time of heptanophenone is used as a starting value for \( t_{ves} \). Then multiple iterations are performed to maximize \( r^2 \) to determine \( t_{ves} \) [36].

Polar group selectivity is defined here as the quotient of the retention factor of a substituted benzene \( (k_s) \) divided by the retention factor of benzene \( (k_b) \):

\[
\alpha_{PG} = \frac{k_s}{k_b}
\] (2.4)

As \( \alpha_{PG} \) approaches unity, the resolution of benzene and the substituted benzene in question approaches zero. Similarly, as the \( \alpha_{PG} \)'s for two benzene derivatives approach the same value, the resolution for those compounds also approaches zero.
Efficiency was calculated using the Foley-Dorsey equation [43]:

\[ N = \frac{41.7 \left( \frac{t_r}{W_{0.1}} \right)^2}{\left( \frac{b_{0.1}}{a_{0.1}} \right) + 1.25} \]  

(2.5)

where \( t_r \) is the migration time, \( W_{0.1} \) is the peak width at 10% peak height, and \( a_{0.1} \) and \( b_{0.1} \) are the respective widths of the first and second halves of the peak at 10% peak height.

The change in retention factor with respect to the change in ACN content was calculated using the equation below:

\[ \% \text{Change} = \frac{k_{\text{mod}} - k_{\text{unmod}}}{k_{\text{unmod}}} \times 100\% \]  

(2.6)

where \( k_{\text{mod}} \) is the retention factor of the solute using the 10% ACN modified vesicles and \( k_{\text{unmod}} \) is the retention factor of the solute using the unmodified vesicles.

The LFER coefficients were normalized by dividing each coefficient by the following normalization parameter [44]:

\[ \sigma = \sqrt{e^2 + s^2 + a^2 + b^2 + v^2} \]  

(2.7)

2.3 Results and Discussion

2.3.1 Vesicle Size Comparison

Prior to size measurement, the vesicles were filtered using 0.45 µm nylon filters. The mean diameter of each vesicle system was then measured three times and the averages are presented in Table 2.1. Unmodified CTAB/SOS had a smaller mean diameter than
Table 2.1. Effect of counterion and organic modifier on mean diameter and electrophoretic parameters.

<table>
<thead>
<tr>
<th>Vesicle System a)</th>
<th>Mean Diameter (nm)</th>
<th>EOF b) $\mu_{eo}$ (x 10^{-4} cm^2/Vs)</th>
<th>Electrophoretic mobility b) $\mu_{ep}$ (x 10^{-4} cm^2/Vs)</th>
<th>Elution Range ($t_{ves}/t_0$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified CTAB/SOS</td>
<td>85 ± 6</td>
<td>4.17</td>
<td>-3.72</td>
<td>9.32 ± 0.19</td>
</tr>
<tr>
<td>0.5 % v/v 2-amino-1-butanol modified CTAB/SOS</td>
<td>105 ± 10</td>
<td>4.02</td>
<td>-3.52</td>
<td>8.06 ± 0.12</td>
</tr>
<tr>
<td>10% v/v Acetonitrile modified CTAB/SOS</td>
<td>111 ± 3</td>
<td>3.27</td>
<td>-2.80</td>
<td>7.04 ± 0.10</td>
</tr>
<tr>
<td>Unmodified CTAC/SOS</td>
<td>96 ± 3</td>
<td>4.28</td>
<td>-3.79</td>
<td>8.22 ± 0.06</td>
</tr>
<tr>
<td>0.5 % v/v 2-amino-1-butanol modified CTAC/SOS</td>
<td>74 ± 4</td>
<td>3.97</td>
<td>-3.44</td>
<td>7.38 ± 0.12</td>
</tr>
<tr>
<td>10% v/v Acetonitrile modified CTAC/SOS</td>
<td>83 ± 3</td>
<td>3.35</td>
<td>-2.80</td>
<td>6.05 ± 0.12</td>
</tr>
</tbody>
</table>

a) All vesicle systems are in a 1:3.66 mole ratio CTAB or CTAC to SOS in a 10 mM HEPES buffer at pH 7.2.
b) The uncertainties in the EOF and electrophoretic mobility were all less than 7 x 10^{-6} cm^2/Vs.
unmodified CTAC/SOS vesicles. However, the opposite was observed for the corresponding modified vesicles. For both the AB modified and 10% ACN modified vesicles, the CTAB/SOS vesicles were larger than the CTAC/SOS vesicles. For the AB modified vesicles, the mean diameter of CTAB/SOS vesicles increased by 24% whereas the mean diameter of the CTAC/SOS vesicles decreased by 23%. For the 10% ACN case, the mean diameter of the CTAB/SOS vesicles increased by approximately 30% whereas the mean diameter of the CTAC/SOS vesicles decreased by approximately 14%. The presence of organic modifier had an overall larger effect on the CTAB/SOS vesicles. These size results are somewhat different from the results of Pascoe and Foley [36]. In particular, no difference in mean diameter of CTAB/SOS vesicles was reported for the addition of AB, nor 10% ACN. The disagreement among the results can be attributed to a difference in the way the vesicles were prepared, specifically the time when the organic modifier was added during the vesicle preparation. In this work, the organic modifier was added after vesicle formation occurred, contrary to that of the previous work. This may also account for other differences between the research results, including LFER coefficients.

2.3.2 Electrophoretic Parameters

The electrophoretic parameters were calculated based upon the equations presented in Section 2.2.5. Shown in Table 2.1 are the electrophoretic parameters for the six vesicle systems that were investigated.
2.3.2.1 Electroosmotic Flow

Both unmodified vesicle systems showed similar values for EOF ($\mu_{eo}$), with the CTAB/SOS vesicle system exhibiting a 3% lower EOF. For the AB modified vesicles, the EOFs were also very similar. The extent to which EOF decreased in the presence of AB was 4% for the CTAB/SOS vesicles and 8% for the CTAC/SOS vesicles. EOF was affected the most by the presence of acetonitrile. There was a 22% decrease in EOF for both the CTAB/SOS vesicles and the CTAC/SOS vesicles in the presence of 10% ACN. The ACN-mediated decrease in EOF is due to an increase in the viscosity of the bulk aqueous phase and a decrease in the zeta potential of the capillary [45], the potential at the shear plane within the electrical double layer.

The trend in the EOF values that was observed going from unmodified vesicles to modified vesicles regardless of counterion was that the presence of organic modifier decreased EOF. Pascoe et al. [36] reported similar results, although a somewhat smaller reduction in EOF was observed in the presence of 10% ACN.

2.3.2.2 Electrophoretic Mobility of the Vesicles ($\mu_{ep,ves}$)

Table 2.1 reports the electrophoretic mobility of the vesicles. The electrophoretic mobilities of the unmodified vesicles are statistically the same, which was not expected due to the differences in the mean diameters of the vesicles. However, the mobilities may be similar due to similar average charge densities around the net negative vesicles. The vesicles have net negative charges due to the excess SOS that is present in their compositions. Overall, the charge to frictional drag ratio of the vesicles is similar despite the identity of the counterion.
With the addition of organic modifier, the electrophoretic mobilities of both kinds of vesicles decreased, but the magnitude depended on the identity and concentration of the modifier. With 0.5 % AB, the electrophoretic mobilities of CTAB/SOS and CTAC/SOS vesicles decreased by 5% and 8%, respectively. With 10% ACN, the respective electrophoretic mobilities decreased by 22% and 26%. From these results, it seems that both organic modifiers have a slightly smaller effect on the electrophoretic mobility of CTAB/SOS vesicles than they do on CTAC/SOS vesicles.

### 2.3.2.3 Elution Range

As shown in Table 2.1, the largest elution range of all six vesicle systems studied was found for the unmodified CTAB/SOS vesicles. The elution ranges of the unmodified vesicles were consistently higher than the organic modified vesicles. This indicates that EOF is not decreasing as rapidly as $\mu_{ep,ves}$ for the organic modified vesicle systems. AB, which is positively charged at pH 7.2, could interact with the negatively charged vesicles. Based on the molar ratios of AB to vesicles, there could be $1.12 \times 10^5$ AB molecules associated with a single vesicle at a given time. This would lower the net charge of the vesicle (and possibly increase its frictional drag), thereby decreasing its electrophoretic mobility. ACN, which interacts more with the bulk phase than the vesicle aggregates, has been shown to slightly reduce EOF [46] compared to small chain alcohol organic modifiers. Since the viscosity of a water-10 % acetonitrile mixture is slightly higher than the viscosity of water alone [47], the electrophoretic mobility of the vesicles decreased. In these results, the effect of increased viscosity on the electrophoretic mobility is greater than the effect on EOF. The local viscosity of the electrical double layer can increase due
to adsorption of solvent molecules at the interface of the capillary wall and the electrolyte solution [48]. The adsorption, described by Schwer and Kenndler [45], would lead to a reduction in EOF caused by the displacement of hydroxide ions by the solvent molecules. Since electrophoretic mobility is decreasing faster than EOF, it may be that the viscosity of the bulk solution is slightly higher than that of the electrical double layer, depending upon the number of solvent molecules adsorbed to the surface. Since acetonitrile interacts through dipole forces, which are weaker than hydrogen-bonding forces, it could be likely that not many acetonitrile molecules are present near the capillary wall, thus unable to contribute to a higher local viscosity in the electrical double layer. The most significant change in elution range was exhibited by the 10% ACN modified vesicles compared to the unmodified vesicles.

2.3.3 Polar Group Selectivity

Table 2.2 presents the polar group selectivities of eight substituted benzenes for the vesicle systems investigated. For the unmodified CTAB/SOS vesicles, the polar group selectivity of phenol was close to unity, which means it would not be resolved from benzene. Similarly, the selectivity of phenol was also close to unity for the unmodified CTAC/SOS vesicles and would probably not be well resolved from benzene. For the AB modified CTAC/SOS vesicles, nitrobenzene rather than phenol (with unmodified CTAB/SOS vesicles) had a polar group selectivity close to unity. Further comparisons within a given vesicle system showed that (i) aniline and benzaldehyde had nearly identical polar group selectivities in unmodified CTAB/SOS vesicle
Table 2.2. Comparison of the polar group selectivity of the vesicle systems investigated.

<table>
<thead>
<tr>
<th>Substituted Benzenes</th>
<th>Unmodified CTAB/SOS</th>
<th>0.5 % v/v 2-amino-1-butanol modified CTAB/SOS</th>
<th>10% v/v Acetonitrile modified CTAB/SOS</th>
<th>Unmodified CTAC/SOS</th>
<th>0.5 % v/v 2-amino-1-butanol modified CTAC/SOS</th>
<th>10% v/v Acetonitrile modified CTAC/SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl alcohol</td>
<td>0.18 ± 0.001</td>
<td>0.20 ± 0.005</td>
<td>0.12 ± 0.001</td>
<td>0.19 ± 0.001</td>
<td>0.21 ± 0.002</td>
<td>0.18 ± 0.001</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.30 ± 0.001</td>
<td>0.30 ± 0.001</td>
<td>0.17 ± 0.003</td>
<td>0.28 ± 0.004</td>
<td>0.31 ± 0.003</td>
<td>0.25 ± 0.002</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>0.32 ± 0.003</td>
<td>0.35 ± 0.002</td>
<td>0.35 ± 0.002</td>
<td>0.34 ± 0.001</td>
<td>0.38 ± 0.001</td>
<td>0.30 ± 0.001</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.31 ± 0.002</td>
<td>0.57 ± 0.003</td>
<td>0.27 ± 0.02</td>
<td>0.31 ± 0.003</td>
<td>0.29± 0.002</td>
<td>0.32 ± 0.002</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>0.90 ± 0.003</td>
<td>0.90 ± 0.002</td>
<td>0.47 ± 0.007</td>
<td>0.85 ± 0.01</td>
<td>1.01 ± 0.04</td>
<td>0.75 ± 0.005</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.98 ± 0.006</td>
<td>1.34 ± 0.01</td>
<td>0.62 ± 0.003</td>
<td>0.94 ± 0.01</td>
<td>1.65 ± 0.0  4</td>
<td>0.76 ± 0.02</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>5.43 ± 0.02</td>
<td>4.82 ± 0.01</td>
<td>3.94 ± 0.03</td>
<td>4.73 ± 0.2</td>
<td>6.76 ± 0.08</td>
<td>4.28 ± 0.08</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>8.17 ± 0.2</td>
<td>6.29 ± 0.09</td>
<td>6.58 ± 0.06</td>
<td>7.49 ± 0.2</td>
<td>10.20 ± 0.2</td>
<td>8.08 ± 0.2</td>
</tr>
</tbody>
</table>

a) Conditions as in Table 2.1.
solutions, while (ii) acetophenone and benzaldehyde and (iii) nitrobenzene and phenol had nearly identical or identical values in 10% ACN modified CTAC/SOS, meaning that none of these pairs of compounds would be resolved from each other; the results suggest that the presence of a non-hydrogen bonding molecular dipole like ACN may result in reduced differences in other types of intermolecular interactions, a minor disadvantage that is compensated by other beneficial effects discussed in later sections.

The fact that the polar group selectivity several of the compounds is significantly different among the six vesicle systems with different counterions or Class I or II modifiers means that both counterions and modifiers have a rather profound effect on selectivity in vesicle EKC.

2.3.4 Hydrophobic (Methylene) Selectivity

Hydrophobic selectivity is a measure of the ability of the pseudostationary phase to resolve compounds that differ by one or more methylene groups. This is measured by the antilogarithm of the slope of a graph of log k vs. carbon number for a homologous series of compounds. In this study, alkylphenones from acetophenone to heptanophenone were used. Presented in Table 2.3 are the hydrophobic selectivities that were observed. No significant differences were observed between the unmodified or ACN-modified pairs of vesicles. The addition of AB decreased the methylene selectivity for the CTAB/SOS vesicles, but had no observable effect on the CTAC/SOS vesicles. The decrease in methylene selectivity for the CTAB/SOS vesicles can be attributed to the insertion of the 2-amino-1-butanol into the vesicles, which creates a more hydrophilic environment [36]. It is unclear why the 2-amino-1-butanol had no effect on the methylene selectivity of the CTAC/SOS vesicles.
Table 2.3. Comparison of methylene selectivity and efficiency.

<table>
<thead>
<tr>
<th>Vesicle Systema)</th>
<th>Methylene Selectivity $\alpha_{CH2}$</th>
<th>Efficiencya) N/m</th>
<th>Efficiencyb) N/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified CTAB/SOS</td>
<td>3.06 ± 0.003</td>
<td>82,000</td>
<td>14,000</td>
</tr>
<tr>
<td>0.5 % v/v 2-amino-1-butanol modified CTAB/SOS</td>
<td>2.93 ± 0.04</td>
<td>95,000</td>
<td>16,000</td>
</tr>
<tr>
<td>10% v/v Acetonitrile modified CTAB/SOS</td>
<td>2.75 ± 0.05</td>
<td>107,000</td>
<td>140,000</td>
</tr>
<tr>
<td>Unmodified CTAC/SOS</td>
<td>3.05 ± 0.02</td>
<td>63,000</td>
<td>11,000</td>
</tr>
<tr>
<td>0.5 % v/v 2-amino-1-butanol modified CTAC/SOS</td>
<td>3.06 ± 0.02</td>
<td>31,000</td>
<td>19,000</td>
</tr>
<tr>
<td>10% v/v Acetonitrile modified CTAC/SOS</td>
<td>2.76 ± 0.02</td>
<td>101,000</td>
<td>140,000</td>
</tr>
</tbody>
</table>

a) Values represent the average of a minimum of 4 results for propiophenone ($0.66 < k < 1.08$ with CTAB/SOS vesicles and $0.66 < k < 1.05$ with CTAC/SOS vesicles, where $k$ is the retention factor). The % RSDs of the efficiency range from 11 to 33 % for propiophenone.

b) Values represent the average of a minimum of 4 results for heptanophenone ($36.5 < k < 91.5$ with CTAB/SOS vesicles and $37.7 < k < 88.2$ with CTAC/SOS vesicles, where $k$ is the retention factor). The % RSDs of the efficiency range from 17 to 24% for heptanophenone.
The lowest methylene selectivity was observed for the 10% ACN-modified vesicles, and is due to the decrease in the polarity of the bulk aqueous phase resulting from the presence of ACN. Therefore, the difference in polarity between the (polar) bulk aqueous phase and the (nonpolar) vesicle aggregates phase is less, which leads to the lower methylene selectivities observed.

2.3.5 Efficiency Comparison

Efficiency (plate count) data for representative hydrophilic and hydrophobic compounds, propiophenone and heptanophenone, are also provided in Table 2.3. Efficiencies for heptanophenone were very similar for the corresponding unmodified and modified CTAB/SOS and CTAC/SOS vesicles, while moderate differences in N were observed for propiophenone, giving CTAB/SOS vesicles a slight edge in overall separation efficiency. The most striking feature of this table, however, is the fact that the efficiencies for heptanophenone using the 10% ACN modified vesicles increased by an average of 91%. Figure 2.1 shows the electropherogram of unmodified CTAC/SOS vesicles compared to the electropherogram of 10% ACN modified CTAC/SOS vesicles. This finding is in contrast to the effect of ACN on the efficiency of strongly retained analytes using SDS micelles investigated by Seals and Davis [39]. Their work provided evidence that decreased efficiency in SDS BGEs with low concentrations of ACN was caused by longitudinal diffusion. In this research, perhaps improved mass transfer in conjunction with reduced retention factor leads to the observed increased efficiency. Heptanophenone’s retention factor (k) was reduced from 91.5 to 36.5 when 10% ACN was added to the aqueous CTAB/SOS vesicle solution. Similarly, it decreased from 88.0 to 37.7 when 10% ACN was added to the aqueous CTAC/SOS solution. The reduction in k upon the addition of 10%
Figure 2.1. Effect of 10% acetonitrile modified CTAC/SOS vesicles on the efficiencies of later migrating alkylphenones (B) compared to unmodified CTAC/SOS vesicles (A). A 10 mM HEPES buffer at pH 7.2 was used for both types of vesicles. Analytes are a homologous series of alkylphenones from acetophenone to heptanophenone. Separation conditions: A new fused silica capillary of 31.2 cm total length, 20.0 cm length to the detector, 50 µm ID, and 363 µm OD at 25°C was used for each vesicle system. An applied voltage of 14 kV was used and injection was done hydrodynamically for 2.0 s at 13.8 mbar (0.2 psi). Detection was done using PDA at 246 nm.
ACN was not as dramatic for propiophenone, for which \( k \) decreased from 1.08 to 0.66 and 1.06 to 0.66 in the CTAB/SOS and CTAC/SOS vesicle solutions, respectively. Irrespective of the reason(s), the increased efficiency in the presence of ACN represents a significant advantage of vesicle EKC over micellar EKC using SDS micelles for strongly retained analytes. With its much higher \( N \) and larger elution range, VEKC can provide superior resolution for hydrophobic compounds.

### 2.3.6 Change in Retention Factor (\( k \)) with Respect to ACN Content

Changes in solute retention factors upon the incorporation of 10% ACN into the vesicle solutions are presented in Table 2.4 along with the differences between the values calculated for CTAB/SOS vesicles and CTAC/SOS vesicles. Changes in \( k \), regardless of counterion, were negative for all compounds except for acetophenone using the CTAB/SOS vesicles; the change in \( k \) for acetophenone was not statistically significant, however. The results of a \( t \) test yielded a value of 2.22, while the \( t_{table} \) value at 95 \% was 2.447 for 6 degrees of freedom. The changes were expected to be negative since the addition of ACN affects the bulk aqueous phase, making it less polar. This decreased polarity of the bulk aqueous phase caused the compounds to spend less time partitioning into the vesicles, which lead to shorter migration times and therefore smaller retention factors. The small % change of 4.2 for acetophenone using CTAB/SOS vesicles indicated that using ACN-modified vesicles yielded no significant impact for this solute. The difference between the % changes was negative for all but ten solutes, which meant CTAB/SOS had larger % changes in retention factor when ACN was added than CTAC/SOS. This may indicate that ACN had more influence on the environment of the CTAB/SOS vesicles than on the environment of the CTAC/SOS vesicles.
Table 2.4. Percentage change in solute retention factor upon the incorporation of 10% ACN into the specified vesicle solutions.\textsuperscript{a)}

<table>
<thead>
<tr>
<th>Solutes</th>
<th>CTAB/SOS vesicles</th>
<th>CTAC/SOS vesicles</th>
<th>Difference $\Delta$CTAB/SOS-$\Delta$CTAC/SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>-43.0</td>
<td>-28.4</td>
<td>-14.6</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>-34.4</td>
<td>-22.7</td>
<td>-11.6</td>
</tr>
<tr>
<td>m-Nitroaniline</td>
<td>-65.7</td>
<td>-47.8</td>
<td>-18.0</td>
</tr>
<tr>
<td>Phenol</td>
<td>-39.1</td>
<td>-34.2</td>
<td>-4.9</td>
</tr>
<tr>
<td>1-Nitrobutane</td>
<td>-16.1</td>
<td>-5.8</td>
<td>-10.3</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>-16.1</td>
<td>-16.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Benzonitrile</td>
<td>-3.8</td>
<td>-21.2</td>
<td>17.4</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>4.2</td>
<td>-29.3</td>
<td>33.5</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>-49.0</td>
<td>-28.2</td>
<td>-20.8</td>
</tr>
<tr>
<td>$p$-Cresol</td>
<td>-21.8</td>
<td>-33.9</td>
<td>12.1</td>
</tr>
<tr>
<td>$m$-Cresol</td>
<td>-37.7</td>
<td>-34.6</td>
<td>-3.0</td>
</tr>
<tr>
<td>1-Nitropentane</td>
<td>-0.8</td>
<td>-2.9</td>
<td>2.2</td>
</tr>
<tr>
<td>Anisole</td>
<td>-48.8</td>
<td>-34.8</td>
<td>-14.0</td>
</tr>
<tr>
<td>Methyl benzoate</td>
<td>-9.9</td>
<td>-27.7</td>
<td>17.8</td>
</tr>
<tr>
<td>Benzene</td>
<td>-2.8</td>
<td>-18.3</td>
<td>15.6</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>-4.2</td>
<td>-39.1</td>
<td>34.9</td>
</tr>
<tr>
<td>4-Nitrotoluene</td>
<td>-40.5</td>
<td>-25.4</td>
<td>-15.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>-46.4</td>
<td>-45.9</td>
<td>-0.4</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>-4.8</td>
<td>-44.4</td>
<td>39.6</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>-25.5</td>
<td>-26.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>-21.7</td>
<td>-11.9</td>
<td>-9.8</td>
</tr>
<tr>
<td>$p$-Xylene</td>
<td>-39.0</td>
<td>-31.1</td>
<td>-7.8</td>
</tr>
<tr>
<td>Benzo phenone</td>
<td>-56.2</td>
<td>-28.5</td>
<td>-27.7</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>-85.1</td>
<td>-1.8</td>
<td>-83.3</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>-74.0</td>
<td>-48.3</td>
<td>-25.7</td>
</tr>
<tr>
<td>Butylbenzene</td>
<td>-79.6</td>
<td>-60.7</td>
<td>-18.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Solutes are listed in order of increasing octanol-water partition coefficient ($\log P_{\text{ow}}$ [49, 50]).
Even though both ACN modified vesicle systems had the same electrophoretic mobility, the CTAB/SOS vesicles were larger than the CTAC/SOS vesicles.

### 2.3.7 LFER Analysis

LFER has been in existence since 1921 [51], but it did not receive much attention until the mid to late 1930’s [52, 53]. There are several LFER models, but the one that our research group employs was developed by Abraham [54] who based his model on modification of the solvatochromic method [55]. Abraham’s solvation model is based on the cavity model of solution [56] which was described in Chap. 1. The following equation is used to describe all of the contributing factors to solute-solvent interactions:

\[
\log k = c + vV + eE + sS + aA + bB
\]  

(2.7)

where \( k \) is the retention factor, \( V, E, S, A, \) and \( B \) are the solute descriptors, \( v, e, s, a, \) and \( b \) are the system constants, and \( c \) is the y-intercept. Among the solute descriptors, \( V \) is the McGowan’s characteristic volume and can be calculated [57]. \( E \) represents excess molar refraction. It can be calculated using the refractive index of the solute at 20°C for the sodium D-line [47]. \( S \) represents dipolarity/polarizability, and \( A \) and \( B \) represent hydrogen bond acidity and hydrogen bond basicity, respectively. Shown in Table 2.5 are the solute descriptors used for the present study. After residual analysis was performed, three outliers were identified and removed from the solute set. The three outliers were aniline, m-nitroaniline, and butylbenzene. The cross-correlation table for the remaining 23 solutes is shown in Table 2.6.

The system constant \( v \) is the difference in the ease of cavity formation for the solute between the buffer and the aggregate phase (vesicles), \( e \) is the difference in capacity
Table 2.5. LFER solutes \(^a\) and descriptors [56, 58].

<table>
<thead>
<tr>
<th>Solute</th>
<th>V</th>
<th>E</th>
<th>S</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>0.8162</td>
<td>0.955</td>
<td>0.96</td>
<td>0.26</td>
<td>0.50</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>0.9230</td>
<td>0.832</td>
<td>0.95</td>
<td>0.37</td>
<td>0.56</td>
</tr>
<tr>
<td>(m)-Nitroaniline</td>
<td>0.9904</td>
<td>1.200</td>
<td>1.71</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.7751</td>
<td>0.805</td>
<td>0.89</td>
<td>0.60</td>
<td>0.30</td>
</tr>
<tr>
<td>1-Nitrobutane</td>
<td>0.8464</td>
<td>0.227</td>
<td>0.95</td>
<td>0</td>
<td>0.29</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.8750</td>
<td>0.820</td>
<td>1.00</td>
<td>0</td>
<td>0.39</td>
</tr>
<tr>
<td>Benzonitrile</td>
<td>0.8711</td>
<td>0.742</td>
<td>1.11</td>
<td>0</td>
<td>0.33</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>1.0139</td>
<td>0.818</td>
<td>1.01</td>
<td>0</td>
<td>0.48</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>0.8909</td>
<td>0.871</td>
<td>1.11</td>
<td>0</td>
<td>0.28</td>
</tr>
<tr>
<td>(p)-Cresol</td>
<td>0.9160</td>
<td>0.820</td>
<td>0.87</td>
<td>0.57</td>
<td>0.31</td>
</tr>
<tr>
<td>(m)-Cresol</td>
<td>0.9160</td>
<td>0.822</td>
<td>0.88</td>
<td>0.57</td>
<td>0.34</td>
</tr>
<tr>
<td>1-Nitropentane</td>
<td>0.9880</td>
<td>0.212</td>
<td>0.95</td>
<td>0</td>
<td>0.29</td>
</tr>
<tr>
<td>Anisole</td>
<td>0.9160</td>
<td>0.708</td>
<td>0.75</td>
<td>0</td>
<td>0.29</td>
</tr>
<tr>
<td>Methyl benzoate</td>
<td>1.0726</td>
<td>0.733</td>
<td>0.85</td>
<td>0</td>
<td>0.46</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.7164</td>
<td>0.610</td>
<td>0.52</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>1.1548</td>
<td>0.804</td>
<td>0.95</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>4-Nitrotoluene</td>
<td>1.0320</td>
<td>0.870</td>
<td>1.11</td>
<td>0</td>
<td>0.28</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.8573</td>
<td>0.601</td>
<td>0.52</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>Butyrophenone</td>
<td>1.2957</td>
<td>0.797</td>
<td>0.95</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>0.8388</td>
<td>0.718</td>
<td>0.65</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>0.8914</td>
<td>0.882</td>
<td>0.73</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>(p)-Xylene</td>
<td>0.9982</td>
<td>0.613</td>
<td>0.52</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>Benzophenone</td>
<td>1.4808</td>
<td>1.447</td>
<td>1.50</td>
<td>0</td>
<td>0.50</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1.0854</td>
<td>1.340</td>
<td>0.92</td>
<td>0</td>
<td>0.20</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>1.3242</td>
<td>1.360</td>
<td>0.99</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>Butylbenzene</td>
<td>1.2800</td>
<td>0.600</td>
<td>0.51</td>
<td>0</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^a\) Solutes are listed in order of increasing octanol-water partition coefficient (log \(P_{ow}\) [49, 50]).
Table 2.6. Cross correlation table of solutes used for LFER Analysis of the CTAB/SOS and CTAC/SOS vesicles.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>E</th>
<th>S</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.374</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.326</td>
<td>0.214</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.075</td>
<td>0.001</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.215</td>
<td>0.021</td>
<td>0.382</td>
<td>0.025</td>
<td>1.000</td>
</tr>
</tbody>
</table>
of the buffer and the aggregate phase to interact with the solutes’ $n$- or $\pi$- electrons, $s$ is the difference in dipolarity/polarizability between the aggregate phase and the bulk aqueous phase, $a$ is the difference in hydrogen bond basicity, and $b$ is the difference in hydrogen bond acidity. The system constants are calculated using multiple linear regression.

2.3.7.1 LFER characterization of the vesicle systems

Table 2.7 lists the results of the LFER analysis of the unmodified and organic modified CTAB/SOS vesicles and CTAC/SOS vesicles. The normalized LFER coefficients are shown in Table 2.8. Closer inspection of the normalized LFER coefficients leads to the conclusion that the two vesicle systems are not interacting identically in the presence of the organic modifiers. The two system constants that are contributing the most to retention are $v$ and $b$. First, consider the system constant $v$, the difference in the ease of cavity formation. In all cases, the value of normalized $v$ is positive, which means it is easier to form a cavity for the solutes in the aggregate phase than in the bulk aqueous phase. For the unmodified vesicles, CTAB/SOS vesicles have a slightly lower value of normalized $v$ than CTAC/SOS vesicles, indicating that the CTAC/SOS vesicles are very similar in polarity compared to the CTAB/SOS vesicles. This is consistent with the methylene selectivity data in which the values were almost identical. When AB is present, again the values for normalized $v$ for both types of vesicles are similar, seeming to indicate that AB has a similar effect on the two types of vesicles. When ACN is present, CTAB/SOS has a larger $v$ than CTAC/SOS. This data seems to suggest that the counterion present does not have a large role in determining the extent to which the organic modifier affects the vesicles.
Table 2.7. LFER coefficients for CTAB/SOS vesicles and CTAC/SOS vesicles with organic modifiers.\(^a\)

<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Modifier</th>
<th>(v)</th>
<th>(e)</th>
<th>(s)</th>
<th>(A)</th>
<th>(b)</th>
<th>(c)</th>
<th>n</th>
<th>(r^2)</th>
<th>SE</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/SOS</td>
<td>No modifier</td>
<td>3.32 (0.07)</td>
<td>0.59 (0.04)</td>
<td>-0.71 (0.05)</td>
<td>0.84 (0.05)</td>
<td>-3.78 (0.08)</td>
<td>-1.72 (0.05)</td>
<td>23</td>
<td>0.987</td>
<td>0.07</td>
<td>1343</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>2-Amino-1-butanol</td>
<td>2.89 (0.09)</td>
<td>0.43 (0.05)</td>
<td>-0.62 (0.06)</td>
<td>0.75 (0.06)</td>
<td>-3.17 (0.10)</td>
<td>-1.50 (0.06)</td>
<td>23</td>
<td>0.973</td>
<td>0.09</td>
<td>630</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>10% Acetonitrile</td>
<td>3.14 (0.13)</td>
<td>0.16 (0.07)</td>
<td>-0.74 (0.09)</td>
<td>0.75 (0.08)</td>
<td>-3.11 (0.15)</td>
<td>-1.55 (0.11)</td>
<td>23</td>
<td>0.942</td>
<td>0.13</td>
<td>277</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>No modifier</td>
<td>3.25 (0.07)</td>
<td>0.42 (0.04)</td>
<td>-0.78 (0.05)</td>
<td>0.83 (0.04)</td>
<td>-3.47 (0.08)</td>
<td>-1.59 (0.05)</td>
<td>23</td>
<td>0.987</td>
<td>0.07</td>
<td>1308</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>2-Amino-1-butanol</td>
<td>3.18 (0.11)</td>
<td>0.43 (0.06)</td>
<td>-0.80 (0.08)</td>
<td>0.88 (0.07)</td>
<td>-3.59 (0.12)</td>
<td>-1.45 (0.08)</td>
<td>23</td>
<td>0.968</td>
<td>0.11</td>
<td>524</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>10% Acetonitrile</td>
<td>3.00 (0.07)</td>
<td>0.38 (0.04)</td>
<td>-0.57 (0.05)</td>
<td>0.72 (0.05)</td>
<td>-3.58 (0.08)</td>
<td>-1.61 (0.05)</td>
<td>23</td>
<td>0.984</td>
<td>0.07</td>
<td>1043</td>
</tr>
</tbody>
</table>

\(^a\) Values in parentheses represent standard deviations.
Table 2.8. Normalized LFER coefficients for CTAB/SOS vesicles and CTAC/SOS vesicles with organic modifiers.\(^{a)}\)

<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Modifier</th>
<th>(v_u)</th>
<th>(e_u)</th>
<th>(s_u)</th>
<th>(a_u)</th>
<th>(b_u)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/SOS</td>
<td>No modifier</td>
<td>0.64 (0.02)</td>
<td>0.11 (0.01)</td>
<td>-0.14 (0.01)</td>
<td>0.16 (0.01)</td>
<td>-0.73 (0.02)</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>0.5 % 2-Amino-1-butanol</td>
<td>0.65 (0.02)</td>
<td>0.10 (0.01)</td>
<td>-0.14 (0.01)</td>
<td>0.17 (0.02)</td>
<td>-0.72 (0.03)</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>10% Acetonitrile</td>
<td>0.69 (0.03)</td>
<td>0.03 (0.02)</td>
<td>-0.16 (0.02)</td>
<td>0.17 (0.02)</td>
<td>-0.69 (0.04)</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>No modifier</td>
<td>0.66 (0.02)</td>
<td>0.09 (0.01)</td>
<td>-0.16 (0.01)</td>
<td>0.17 (0.01)</td>
<td>-0.71 (0.02)</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>0.5 % 2-Amino-1-butanol</td>
<td>0.64 (0.03)</td>
<td>0.09 (0.01)</td>
<td>-0.16 (0.02)</td>
<td>0.18 (0.01)</td>
<td>-0.72 (0.03)</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>10% Acetonitrile</td>
<td>0.63 (0.02)</td>
<td>0.08 (0.01)</td>
<td>-0.12 (0.01)</td>
<td>0.15 (0.01)</td>
<td>-0.75 (0.02)</td>
</tr>
</tbody>
</table>

\(^{a)}\) Values in parentheses represent standard deviations.
Next consider normalized $b$, the difference in hydrogen bond acidity between the vesicles and the bulk aqueous phase, for both vesicle systems. Given the composition of the vesicles, the negative values of normalized $b$, indicating that the bulk aqueous phase is a better acid than the vesicles, was expected. For the CTAB/SOS vesicles, the value of $b$ is similar between the unmodified vesicles and both types of organic modified vesicles. The same is true between the unmodified CTAC/SOS vesicles and the organic modified CTAC/SOS vesicles. It is unexpected that the presence of AB does not affect $b$ more than the presence of 10% ACN for both vesicle systems. ACN modifies the bulk aqueous phase, whereas AB modifies the vesicles by insertion into the bilayer.

Shifting to normalized $a$, the difference in hydrogen bond basicity between the vesicles and the bulk aqueous phase, one notices positive values, which mean that the vesicles are able to participate as hydrogen bond acceptors more readily than the bulk aqueous phase. Organic modified CTAB/SOS vesicles leads to the same $a$ value for the AB modified CTAB/SOS vesicles and for the ACN modified CTAB/SOS vesicles. Organic modified CTAC/SOS vesicles leads to a similar $a$ value for AB modified CTAC/SOS vesicles and a lower $a$ value for ACN modified CTAC/SOS vesicles.

An analysis of $e$, the difference in capacity of the buffer and the aggregate phase to interact with the solutes’ $n$- or $\pi$- electrons, reveals a dramatic decrease in normalized $e$ for the CTAB/SOS vesicles in the presence of 10% ACN. Since the $e$ values are positive, they signify that the aggregate interacts more than the bulk phase with the solutes’ $n$- or $\pi$- electrons. In the presence of AB, there were similar trends between
the CTAB/SOS vesicles and the CTAC/SOS vesicles. The value of $e$ was identical or almost the same for the AB modified CTAC/SOS vesicles and the AB modified CTAB/SOS vesicles, respectively.

Finally, an examination of normalized $s$, the difference in dipolarity/polarizability between the aggregate phase and the bulk aqueous phase, shows negative values, meaning that the dipolarity/polarizability is greater for the bulk aqueous phase than it is for the vesicles. The CTAC/SOS vesicles have larger $s$ coefficients compared to the CTAB/SOS vesicles in all instances except in the presence of 10% ACN.

In summary, the LFER results show distinct differences in the magnitudes of the chemical interactions that contribute to retention for the different vesicle systems. The most influential coefficients are $v$ and $b$, which show opposite trends for CTAB/SOS vs. CTAC/SOS.

Another way to compare the LFER coefficients is to calculate the $d$ parameter, which was introduced in Chap. 1. If the value of $d$ is greater than 0.25, then the two systems can be considered chemically different [59]. The calculated $d$ values are shown in Table 2.9. None of the calculated $d$ values are greater than 0.25, indicating that none of the vesicle systems are chemically different from one another.

2.4 Concluding Remarks

The nature of the counterion for the vesicles studied does not appear to dramatically affect the separations of the compounds investigated. However, even though the chromatography is very similar between the unmodified and organic modified
Table 2.9. Calculated $d$ values between vesicle systems.

<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Modifier</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/SOS</td>
<td>No modifier</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>0.5 % 2-Amino-1-butanol</td>
<td>2</td>
<td>0.03</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>10% Acetonitrile</td>
<td>3</td>
<td>0.11</td>
<td>0.08</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>No modifier</td>
<td>4</td>
<td>0.05</td>
<td>0.03</td>
<td>0.06</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>0.5 % 2-Amino-1-butanol</td>
<td>5</td>
<td>0.04</td>
<td>0.03</td>
<td>0.08</td>
<td>0.03</td>
<td>0</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>10% Acetonitrile</td>
<td>6</td>
<td>0.05</td>
<td>0.05</td>
<td>0.11</td>
<td>0.07</td>
<td>0.06</td>
</tr>
</tbody>
</table>
CTAB/SOS vesicles and the unmodified and organic modified CTAC/SOS vesicles, the mechanism by which these vesicle systems interact with the solutes used for LFER analysis is quite different. In addition, the CTAB/SOS vesicles, whether unmodified or modified, had larger elution ranges compared to the CTAC/SOS vesicles.

The presence of 10% acetonitrile for both CTAB/SOS vesicles and CTAC/SOS vesicles improves the efficiency of heptanophenone, a representative hydrophobic compound, by an average of 91%. The effect of acetonitrile on retention is larger using CTAB/SOS vesicles than CTAC/SOS vesicles.
References


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Chapter 3: Fluorinated Vesicles as a Novel Pseudostationary Phase in Electrokinetic Chromatography

3.1 Introduction

Improved sensitivity has been a long-time goal of capillary electrophoresis. A review by Simonet et al. has classified the approaches to meeting this goal into three different areas: 1) better detectors for CE, 2) online capillary pre-concentration methods, and 3) online coupled flow-injection pre-concentration systems [1]. Some of the detectors that have been used for CE in order to improve the sensitivity are mass spectrometry (MS) detectors, laser-induced fluorescence (LIF) detectors, and electrochemical detectors such as conductivity and amperometric detectors [1]. Each of these has its own drawbacks, such as the complications of the CE to MS interface, the need for a fluorescently active compound or tag [2], or the difficulty of operation and lack of robustness [1].

Some of the online capillary pre-concentration methods that have been described include field-amplified sample stacking (FASS), sweeping, isotachophoresis (ITP), and solid-phase extraction (SPE) [1]. Table 3.1 lists some of the online capillary pre-concentration methods and their corresponding enhancement in sensitivity. FASS involves using a less concentrated buffer for the sample compared to the BGE buffer concentration. The electric field varies according to the local conductivity (concentration for a given buffer), and the analytes are focused once they cross the boundary between the higher and lower electric field. FASS is a simple technique and can also be used with zones of different pH or viscosity. One of the only disadvantages is the technique’s potential for complicated sample quantitation [1] due...
Table 3.1. Online capillary pre-concentration methods and their corresponding enhancement in sensitivity [3].

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>FASS</td>
<td>10-20</td>
</tr>
<tr>
<td>Sweeping</td>
<td>100-5,000</td>
</tr>
<tr>
<td>tITP</td>
<td>100-500,000</td>
</tr>
<tr>
<td>SPE</td>
<td>100-10,000</td>
</tr>
</tbody>
</table>
to the possibility of broadened peaks or sample loss from the capillary [4]. Another disadvantage is that the use of a BGE with limited conductivity.

Sweeping involves the use of a pseudostationary phase (PSP) in the BGE and the absence of that PSP in the sample. The sample becomes concentrated through the extent of its interactions with the PSP, which have been identified as partitioning and complexation [5]. With this pre-concentration technique, there are no serious difficulties so it may be widely applied, provided the analytes interact to a sufficient degree with the PSP that is chosen.

ITP involves the use of a leading electrolyte and a terminating electrolyte, with the sample sandwiched in between the two. The leading electrolyte has a higher mobility, and the terminating electrolyte has a lower mobility compared to the sample [6]. In CE, transient (t) ITP is typically employed. When voltage is applied, the components of the sample quickly align between the two electrolytes via electrophoretic migration; upon alignment all components move toward the detector at the same speed. One aspect that must be considered is the variation of the migration time due to differences in sample concentration and composition; an internal standard is therefore recommended [6]. Additionally, detection of the analytes has the potential to be complicated.

SPE as an online capillary pre-concentration method involves the insertion of a microcartridge in the inlet of the capillary [1]. Usually the packing of the microcartridge is reversed-phase material, such as C$_{18}$ coated particles [1]. There are two disadvantages of using the SPE technique: 1) the preparation of the
microcartridge (difficult); and 2) the short capillary lifetimes when exposed to complex sample matrices [1].

The online coupled flow-injection pre-concentration systems make use of an additional instrument coupled to the CE. These can be interfaced either by a split-flow interface or a programmable arm. This approach is useful, but more complex due to the additional instrumentation required. The difference between this technique and the one previously described is that SPE is completed separately before the sample is injected into the capillary.

We chose an approach to identify and apply smaller vesicles to the EKC methods with which we were familiar. Here we investigated the potential of fluorinated vesicles as a PSP in EKC. Previously, small unilamellar vesicles (SUVs) comprised of nonstoichiometric mixtures of oppositely-charged, hydrocarbon surfactants were used as PSPs for EKC analysis [7-10]. However, the mean diameters of these vesicles ranged from 76 to 110 nm. Sizes on this order contribute to light scattering, which leads to decreased sensitivity with optical detection. The fluorinated vesicles used in these experiments were developed by Iampietro and Kaler at the University of Delaware [11]. No other group has reported using vesicles composed of the cationic surfactant cetyltrimethylammonium bromide (CTAB) and the anionic surfactant sodium perfluorooctanoate (FC7) for use in EKC. These vesicles were chosen based upon their reported mean diameter of 46 nm [12]. This represents a reduction in size by almost 50% compared to the CTAB/SOS and CTAC/SOS vesicles previously investigated [13].
Fluorinated vesicles have been investigated for use as drug carriers [14, 15] and for the production of polymerized microcapsules [16]. Vesicles composed of a mixture of fluorinated and hydrogenated surfactants display more stability compared to vesicles composed of purely hydrogenated surfactants [17]. In addition, fluorosurfactants contribute improved resistance to corrosive environments and bio fluids [18].

In addition to the fluorinated vesicles’ smaller size, the interactions between the vesicles and the analytes in terms of selectivity was also of interest. Vesicles with a fluorinated component have stiffer bilayers than vesicles with purely hydrocarbon components [12]. The stiffness of the fluorinated vesicles’ bilayers could potentially contribute to selectivity differences. Consequently, linear free energy relationship (LFER) analysis was utilized to quantitate the interactions responsible for retention.

3.2 Materials and Methods

3.2.1 Chemicals and Reagents

CTAB, HEPES, and the solutes used in LFER analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). FC7 was purchased from Alfa Aesar (Ward Hill, MA, USA). HPLC grade methanol was purchased from Fisher (Pittsburgh, PA, USA). To prepare the buffer, water was obtained from a Barnstead E-pure Water system (Dubuque, IA, USA).

3.2.2 Instrumentation

The experiments were performed on a Beckman Coulter P/ACE™ MDQ Series Capillary Electrophoresis System (Fullerton, CA, USA) equipped with a diode array
Detector. Data at wavelengths of 214 and 246 nm were collected. The temperature was maintained at 25°C for the capillary cartridge as well as the compartment that holds both the sample and buffer solutions. An applied voltage of 16 kV was used for all separations to maintain a Joule heating level of 1.5 W/m or less. The samples were injected for 2 seconds at 13.8 mbar (0.2 psi), corresponding to an injection volume of 1.5 nL, assuming the viscosity of the buffer is approximately the same as that of water at the same temperature. All data were collected and processed using 32 Karat™ Software Version 5.0 by Beckman Coulter.

A Horiba LB 500 Dynamic Light Scattering Particle Size Analyzer (Irvine, CA, USA) was used to measure the size of the vesicles. The temperature was set at 25°C for all measurements taken. Before each sample was analyzed, it was filtered using a 0.45 µm pore-size nylon membrane filter of 13 mm diameter from Whatman (Clifton, NJ, USA). The average of a minimum of three measurements/sample was reported for each batch of vesicles prepared.

A HP 8453 UV-Visible Spectrometer, model number G1103A was used to obtain the UV-Visible spectra of the fluorinated vesicles.

A new 31.2 cm x 50 µm ID fused silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used for each preparation of the fluorinated vesicle systems. Prior to first use, each capillary was conditioned for 5 min with HPLC-grade water, 10 min with 1.0 M sodium hydroxide, 5 min with 0.1 M sodium hydroxide, 3 min with HPLC-grade water, and 15 min with background electrolyte (BGE) all at an applied pressure of 2070 mbar (30 psi). Between each injection the capillary was
rinseed with 0.1 M NaOH for 1 min, HPLC-grade water for 2 min, and BGE for 1.5 min all at 2070 mbar. Then 15 kV was applied between the inlet and outlet buffer vials for 1.5 min.

3.2.3 Vesicle Preparation

The fluorinated vesicle system was prepared in a 1:3.3 mole ratio of CTAB:FC\textsubscript{7} for a total surfactant concentration of 48 mM. Initially, CTAB was added to a small amount of water and an appropriate amount of stock HEPES buffer adjusted to pH 7.2 by 1.0 M LiOH. The CTAB solution was stirred and heated on a low setting until all of the solid dissolved. Next the heat was turned off and the FC\textsubscript{7} was stirred into the still warm CTAB solution. More water was added and the solution was stirred until all of the FC\textsubscript{7} dissolved. The resulting solution looked cloudy and was vortex mixed for approximately 2 minutes. The vesicles were allowed to equilibrate for a minimum of 12 hours before use. Prior to use, the vesicles were filtered using a 0.45 μm pore-size nylon membrane filter of 13 mm diameter from Whatman (Clifton, NJ, USA).

3.2.4 Sample Preparation

Solutions ranging from 2-8 mg/mL were prepared for all solutes, except biphenyl, butyl benzene, and naphthalene, by dissolution of the appropriate amount in a solution comprised of 4 mL of aqueous HEPES buffer (10 mM) and 6 mL of HPLC grade methanol. The solvent system for the remaining three solutes consisted 3 mL of aqueous HEPES buffer (10 mM) and 7 mL of HPLC grade methanol.
3.2.5 Calculations

The calculations for voltage-ramp corrected migration time, electroosmotic mobility (EOF), retention factor, polar group selectivity, and efficiency can be found in Chapter 2.

A homologous series of alkylphenones (acetophenone through hexanophenone) were analyzed and their retention factors calculated in order to determine $t_{ves}$, using the method of Bushey and Jorgenson [19] in which the logarithm of the retention factor vs. carbon number of a homologous series is assumed to yield a linear relationship. The retention time of hexanophenone is used as a starting value for $t_{ves}$. Then multiple iterations are performed to maximize $r^2$ to determine $t_{ves}$ [20].

3.3 Results and Discussion

3.3.1 Vesicle Size

The size of the CTAB/FC$_7$ vesicles compared to the CTAB/SOS vesicles can be found in Table 3.2. The CTAB/FC$_7$ vesicles had a mean diameter of 48 nm, which is very close to the mean diameter of 46 nm reported in the literature [12]. It is also about half of the size of the CTAB/SOS vesicles. Thus, one of the goals of the investigation (to create smaller vesicles that scatter less light) was met. Further evidence of the smaller size of the fluorinated vesicles was obtained through the acquisition of the UV-visible absorption spectra of each type of vesicle. The buffer is the same concentration for both vesicle systems, but the concentration of surfactant for the two vesicle systems is different since the vesicles form at different concentrations of surfactant. Figure 3.1 shows the UV-visible absorption spectra of CTAB/SOS vesicles compared to CTAB/FC$_7$ vesicles. The fluorinated vesicles may
Table 3.2. Mean diameter and electrophoretic parameters for CTAB/FC\textsubscript{7} and CTAB/SOS vesicles.

<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Mean Diameter (nm)</th>
<th>EOF $\mu_{eo}$ ($\times 10^{-4}$ cm$^2$/Vs)</th>
<th>Electrophoretic mobility $\mu_{ep}$ ($\times 10^{-4}$ cm$^2$/Vs)</th>
<th>Elution Range ($t_{ve}/t_o$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/FC\textsubscript{7}</td>
<td>48 ± 4</td>
<td>6.46</td>
<td>-5.22</td>
<td>5.99 ± 0.97</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>85 ± 6</td>
<td>3.58</td>
<td>-3.22</td>
<td>10.00 ± 0.22</td>
</tr>
</tbody>
</table>
Figure 3.1. Comparison of UV-visible spectra for A) CTAB/SOS vesicles and B) CTAB/FC vesicles. The background for both spectra was 10 mM HEPES buffer pH 7.2.
have been smaller than the CTAB/SOS vesicles because the two types of vesicles are stabilized though different processes depending on the value of the bending constant $K$ [12]. CTAB/FC$_7$ vesicles are stabilized through spontaneous curvature, which means that only one radius is favored energetically [12]. However, CTAB/SOS vesicles are stabilized through Helfrich-undulation repulsions, indicating that the interbilayer potential is repulsive [12].

3.3.2 Electroosmotic Flow
For comparison purposes, the corresponding data for CTAB/SOS vesicles as well as for CTAB/FC$_7$ vesicles has also been included in Table 3.2. The EOF using CTAB/FC$_7$ vesicles was faster than that observed for the CTAB/SOS vesicles. Part of the reason for this difference is due to the higher voltage at which the CTAB/FC$_7$ vesicles were analyzed (16 kV vs. 14 kV). Since the fluorinated vesicles generated less current, a higher voltage could be applied while still maintaining a manageable level of Joule heating. Another possible cause for the faster EOF using fluorinated vesicles may be that there is less unbound CTAB available to coat the capillary surface. It is well-known that CTAB binds to surface silanols and can reduce, or even reverse EOF at sufficiently high concentrations of unbound CTAB [21]. There is a higher concentration of CTAB (14.8 mM) in the CTAB/SOS vesicles compared to the concentration of CTAB (11.2 mM) in the CTAB/FC$_7$ vesicles so it could be possible that unbound CTAB binds to the capillary.

3.3.3 Electrophoretic Mobility of the Vesicles ($\mu_{ep,ves}$)
The electrophoretic mobility of the CTAB/FC$_7$ vesicles was higher than that of the CTAB/SOS vesicles. The cause may be attributed to the smaller size of the
3.3.4 Elution Range

Although the electrophoretic mobility of the fluorinated vesicles is about 60% higher than the CTAB/SOS vesicles, the EOF in the presence of CTAB/FC\textsubscript{7} vesicles was 80% higher, meaning that the fluorinated vesicles had a faster net mobility, which is inversely proportional to the elution range (migration window). Consequently, the CTAB/FC\textsubscript{7} vesicles had a lower elution range compared to the CTAB/SOS vesicles.

3.3.5 Polar Group Selectivity

The polar group selectivity of the CTAB/FC\textsubscript{7} vesicles and the CTAB/SOS vesicles is shown in Table 3.3. The general trend of the data was that the retention of both polar and nonpolar substituted benzenes was reduced relative to benzene using the fluorinated vesicles compared to the CTAB/SOS vesicles for all compounds except benzaldehyde (statistically equivalent) and acetophenone. There are some significant selectivity differences between the two pseudostationary phases. First, benzyl alcohol and aniline could not be separated by the CTAB/FC\textsubscript{7} vesicles, whereas they could be separated by the CTAB/SOS vesicles. Conversely, acetophenone and benzaldehyde could be separated by the CTAB/FC\textsubscript{7} vesicles, but could not be separated by the CTAB/SOS vesicles.

3.3.6 Hydrophobic (Methylene) Selectivity

Hydrophobic selectivity is a measure of the difference in the hydrophobicity of the pseudostationary phase and the bulk electrolyte. Other chromatographic figures of...
Table 3.3. Polar Group Selectivity Using CTAB/FC₇ Vesicles Compared to CTAB/SOS Vesicles

<table>
<thead>
<tr>
<th>Substituted Benzenes</th>
<th>CTAB/FC₇</th>
<th>CTAB/SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl alcohol</td>
<td>0.13 ± 0.04</td>
<td>0.18 ± 0.001</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.13 ± 0.03</td>
<td>0.30 ± 0.001</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>0.42 ± 0.06</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.34 ± 0.06</td>
<td>0.32 ± 0.001</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>0.63 ± 0.07</td>
<td>0.90 ± 0.003</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.24 ± 0.07</td>
<td>0.98 ± 0.006</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>3.20 ± 0.5</td>
<td>5.43 ± 0.02</td>
</tr>
<tr>
<td>Bromobenzene</td>
<td>4.22 ± 0.3</td>
<td>8.17 ± 0.16</td>
</tr>
</tbody>
</table>
merit such as efficiency, retention, and elution range being the same, the greater the hydrophobic selectivity, the greater the resolution between compounds that differ primarily in hydrophobicity (e.g., homologs). If the bulk electrolyte is the same (e.g., aqueous buffer of specified composition) among the PSPs of interest, then the methylene selectivity can be taken as a direct measure of the hydrophobicity of the PSP. The higher the value of the hydrophobic selectivity, the higher the hydrophobicity of the pseudostationary phase or the more nonpolar the pseudostationary phase is. The hydrophobic selectivity of the CTAB/FC\(_7\) and the CTAB/SOS vesicles is given in Table 3.4. The fluorinated vesicles had a hydrophobic selectivity of 2.33 compared to 3.06 for the CTAB/SOS vesicles. The lower value signifies that the CTAB/FC\(_7\) vesicles are more polar than the CTAB/SOS vesicles. Also, the fluorinated vesicles cannot separate a homologous series of compounds as well as the nonfluorinated vesicles can. Figure 3.2 shows the electropherograms of a homologous series of alkylphenones using CTAB/FC\(_7\) and CTAB/SOS vesicles. The resolution of the alkylphenones is much greater using the CTAB/SOS vesicles compared to the CTAB/FC\(_7\) vesicles.

### 3.3.7 Efficiency Comparison

The efficiency of CTAB/FC\(_7\) and CTAB/SOS vesicles using propiophenone and heptanophenone as test compounds is presented in Table 3.4. The efficiency using CTAB/SOS vesicles was almost two times and five times higher than the efficiency using CTAB/FC\(_7\) vesicles for propiophenone and heptanophenone, respectively. This significant difference in efficiency values could be caused by the mixed structures of the CTAB/FC\(_7\) aggregates. The fluorinated aggregates are not only in
Figure 3.2. Comparison of a homologous series of alkylphenones from acetophenone to heptanophenone using (A) CTAB/FC\textsubscript{7} vesicles and (B) CTAB/SOS vesicles. The buffer for both types of vesicles was 10 mM HEPES at pH 7.2. Separation conditions: A new fused silica capillary of 31.2 cm total length, 20.0 cm length to the detector, 50 \(\mu\)m ID, and 363 \(\mu\)m OD at 25\(^\circ\)C was used for each vesicle system. An applied voltage of 16 kV was used for the CTAB/FC\textsubscript{7} vesicles while 14 kV was used for the CTAB/SOS vesicles. Injection was done hydrodynamically for 2.0 s at 13.8 mbar (0.2 psi). Detection was done using PDA at 246 nm.
Table 3.4. Comparison of Methylene Selectivity and Efficiency Using CTAB/FC\textsubscript{7} and CTAB/SOS vesicles.

<table>
<thead>
<tr>
<th>Vesicle System\textsuperscript{a)}</th>
<th>Methylene Selectivity $\alpha_{\text{CH}_2}$</th>
<th>Efficiency\textsuperscript{a)} N/m</th>
<th>Efficiency\textsuperscript{b)} N/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/FC\textsubscript{7}</td>
<td>2.33 ± 0.14</td>
<td>44,000</td>
<td>3,000</td>
</tr>
<tr>
<td>CTAB/SOS</td>
<td>3.06 ± 0.003</td>
<td>82,000</td>
<td>14,000</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Values represent the average of a minimum of 4 results for propiophenone.

\textsuperscript{b)} Values represent the average of a minimum of 4 results for heptanophenone.
the shape of spheres, but also the shapes of cylinders and flat disks [22]. These other shapes may contribute to slower mass transfer, which caused the peaks to be broader.

### 3.3.8 LFER Analysis

The fluorinated vesicles were also investigated through LFER analysis. The results of LFER analysis for 3 batches of CTAB/FC\textsubscript{7} vesicles are presented in Table 3.5 along with the LFER coefficients for CTAB/SOS vesicles, CTAB micelles, and lithium perfluorooctanesulfonate (LPFOS) micelles for comparison purposes. LFER analysis using Abraham’s model of solvation [23] was employed for these calculations. The following equation was used:

\[
\log k = c + vV + eE + sS + aA + bB
\]

where \( k \) is the retention factor, \( V, E, S, A, \) and \( B \) are the solute descriptors, \( c \) is the \( y \)-intercept, and \( v, e, s, a, \) and \( b \) are the system coefficients. The solute descriptors for the 26 compounds that were analyzed were presented in Table 2.5. After a combination of residual analysis and root mean square error of cross-validation (RMSECV) analysis, it was determined that \( m \)-nitroaniline and butylbenzene were outliers. These two solutes were removed from the set used to determine the LFER coefficients. The cross-correlation table of the remaining 24 compounds is given in Table 3.6.

The largest two system coefficients for the fluorinated vesicles were \( v \) and \( b \), which were also the largest for the unfluorinated CTAB/SOS vesicles. The values for \( v \) were all positive, indicating that it was easier to form a cavity for the solutes in the pseudostationary phase than in the BGE. The range of \( v \) values for the CTAB/FC\textsubscript{7} vesicles was from 2.58 to 2.88. This range of values was smaller than the \( v \)
Table 3.5. LFER coefficients for CTAB/FC$_7$ vesicles, CTAB/SOS vesicles, CTAB micelles, and LPFOS micelles.\textsuperscript{a)}

<table>
<thead>
<tr>
<th>Surfactant System</th>
<th>$v$</th>
<th>$e$</th>
<th>$s$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>n</th>
<th>$r^2$</th>
<th>SE</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 1)</td>
<td>2.58 (0.06)</td>
<td>0.32 (0.04)</td>
<td>-0.73 (0.05)</td>
<td>-0.15 (0.04)</td>
<td>-2.61 (0.07)</td>
<td>-1.62 (0.05)</td>
<td>24</td>
<td>0.982</td>
<td>0.07</td>
<td>989</td>
</tr>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 2)</td>
<td>2.88 (0.07)</td>
<td>0.33 (0.04)</td>
<td>-0.86 (0.06)</td>
<td>-0.15 (0.05)</td>
<td>-2.88 (0.08)</td>
<td>-1.74 (0.05)</td>
<td>24</td>
<td>0.981</td>
<td>0.08</td>
<td>950</td>
</tr>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 3)</td>
<td>2.86 (0.08)</td>
<td>0.26 (0.05)</td>
<td>-0.84 (0.07)</td>
<td>-0.20 (0.06)</td>
<td>-2.82 (0.10)</td>
<td>-1.75 (0.06)</td>
<td>24</td>
<td>0.974</td>
<td>0.10</td>
<td>681</td>
</tr>
<tr>
<td>CTAB/SOS Vesicles</td>
<td>3.32 (0.07)</td>
<td>0.59 (0.04)</td>
<td>-0.71 (0.05)</td>
<td>0.84 (0.05)</td>
<td>-3.78 (0.08)</td>
<td>-1.72 (0.05)</td>
<td>23</td>
<td>0.987</td>
<td>0.07</td>
<td>1343</td>
</tr>
<tr>
<td>CTAB micelles\textsuperscript{b)}</td>
<td>2.71 (0.05)</td>
<td>1.11 (0.09)</td>
<td>-0.76 (0.05)</td>
<td>0.82 (0.04)</td>
<td>-2.44 (0.06)</td>
<td>-1.83 (0.06)</td>
<td>49</td>
<td>0.988</td>
<td>0.08</td>
<td>690</td>
</tr>
<tr>
<td>LPFOS micelles\textsuperscript{b)}</td>
<td>1.97 (0.10)</td>
<td>-0.11 (0.13)</td>
<td>-0.24 (0.10)</td>
<td>-0.88 (0.08)</td>
<td>-0.46 (0.11)</td>
<td>-1.41 (0.09)</td>
<td>62</td>
<td>0.941</td>
<td>0.19</td>
<td>180</td>
</tr>
</tbody>
</table>

\textsuperscript{a)} Values in parentheses represent standard deviations.
\textsuperscript{b)} Values obtained from Reference [24].
Table 3.6. Cross correlation table of solutes used for LFER Analysis of the CTAB/FC7 vesicles.

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>E</th>
<th>S</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.332</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.301</td>
<td>0.217</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.088</td>
<td>0.002</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.150</td>
<td>0.028</td>
<td>0.372</td>
<td>0.038</td>
<td>1.000</td>
</tr>
</tbody>
</table>
coefficient for the CTAB/SOS vesicles, which was 3.32. The larger \( v \) coefficient of the CTAB/SOS vesicles appears to be linked to the higher hydrophobic selectivity that was observed for these vesicles compared to the CTAB/FC\(_7\) vesicles.

The range of values for the \( b \) coefficient using the CTAB/FC\(_7\) vesicles was from -2.88 to -2.61. The negative sign signifies that the PSP (vesicles) participates less in hydrogen bond acidity interactions than the BGE. Since the BGE is mainly water, this value is appropriate for the systems investigated.

For the \( e \) coefficient, the range of values using the CTAB/FC\(_7\) vesicles was from 0.26 to 0.33. The positive values indicate that capacity of the aggregate phase to interact with the solutes’ \( n \)- or \( \pi \)-electrons is higher than that of the BGE. These values were slightly higher than half the value of \( e \) obtained for the CTAB/SOS vesicles, which was 0.59. The reason for the difference is most likely caused by the presence of the fluorinated surfactant in the CTAB/FC\(_7\) vesicles. It has been reported that the \( e \) coefficient for systems with fluorocarbons is usually negative [25]. This is supported by the data of the LPFOS micelles. While this is not the case here, the values of \( e \) are drastically smaller than those of the nonfluorinated vesicles.

The values for the \( s \) coefficient were all negative which means the dipolarity/polarizability of the BGE is larger than that of the vesicle phase. Again, this is reasonable since water comprises most of the BGE and water is a polar substance. The vesicles are composed of surfactant molecules with both polar and nonpolar domains, which is why they have less ability to participate in dipolarity/polarizability interactions. The range of \( s \) values for the CTAB/FC\(_7\)
vesicles was from -0.86 to -0.73 while the s value for the CTAB/SOS vesicles was -0.71. The magnitude of s is greater for the CTAB/FC\textsubscript{7} vesicles because its fluorinated surfactant is more hydrophobic than the hydrogenated surfactants of the CTAB/SOS vesicles [18].

Finally, the range of values for the a coefficient using CTAB/FC\textsubscript{7} vesicles was from -0.20 to -0.15 while the value for the a coefficient using CTAB/SOS was 0.84. Here the two different vesicle systems have the opposite sign. This means that the CTAB/FC\textsubscript{7} vesicles participate less in hydrogen bond basicity interactions, but the CTAB/SOS vesicles participate more in hydrogen bond basicity interactions. The positive coefficient of the vesicles containing trimethylalkylammonium systems has been previously observed by Poole et al. [26].

Another technique for interpreting the LFER coefficients is to normalize the values and then calculate the d value between the different combinations of vesicle systems. Table 3.7 shows the normalized values of the LFER coefficients using CTAB/FC\textsubscript{7} and CTAB/SOS vesicles, as well as CTAB micelles and LPFOS micelles. A survey of the normalized coefficients shows that generally, the values for the CTAB/FC\textsubscript{7} are clustered together. The true significance of the normalized LFER coefficients is the ability to use them to calculate values for the d parameter.

The values for d using the normalized LFER coefficients for both CTAB/FC\textsubscript{7} and CTAB/SOS are presented in Table 3.8. First, the d values calculated between the three batches of CTAB/FC\textsubscript{7} reveal values of 0.02, 0.03, and 0.02. All of these values are less than 0.25, indicating that all 3 batches are chemically similar to each other as
Table 3.7. Normalized LFER coefficients for CTAB/FC\textsubscript{7} vesicles, CTAB/SOS vesicles, CTAB micelles, and LPFOS micelles.

<table>
<thead>
<tr>
<th>Surfactant System</th>
<th>$v_u$</th>
<th>$e_u$</th>
<th>$s_u$</th>
<th>$a_u$</th>
<th>$b_u$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/FC\textsubscript{7} vesicles (batch 1)</td>
<td>0.69 (0.02)</td>
<td>0.08 (0.01)</td>
<td>-0.19 (0.01)</td>
<td>-0.04 (0.01)</td>
<td>-0.70 (0.02)</td>
</tr>
<tr>
<td>CTAB/FC\textsubscript{7} vesicles (batch 2)</td>
<td>0.69 (0.02)</td>
<td>0.08 (0.01)</td>
<td>-0.21 (0.01)</td>
<td>-0.04 (0.01)</td>
<td>-0.69 (0.02)</td>
</tr>
<tr>
<td>CTAB/FC\textsubscript{7} vesicles (batch 3)</td>
<td>0.69 (0.03)</td>
<td>0.06 (0.01)</td>
<td>-0.20 (0.02)</td>
<td>-0.05 (0.01)</td>
<td>-0.69 (0.03)</td>
</tr>
<tr>
<td>CTAB/SOS Vesicles</td>
<td>0.64 (0.02)</td>
<td>0.11 (0.01)</td>
<td>-0.14 (0.01)</td>
<td>0.16 (0.01)</td>
<td>-0.73 (0.02)</td>
</tr>
<tr>
<td>CTAB Micelles</td>
<td>0.68 (0.02)</td>
<td>0.28 (0.02)</td>
<td>-0.19 (0.01)</td>
<td>0.21 (0.01)</td>
<td>-0.61 (0.02)</td>
</tr>
<tr>
<td>LPFOS Micelles</td>
<td>0.89 (0.06)</td>
<td>-0.05 (0.06)</td>
<td>-0.11 (0.04)</td>
<td>-0.40 (0.04)</td>
<td>-0.21 (0.05)</td>
</tr>
</tbody>
</table>

\(^a\) Values in parentheses represent standard deviations.
Table 3.8. Calculated $d$ values between surfactant systems.

<table>
<thead>
<tr>
<th>Surfactant System</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 1)</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 2)</td>
<td>2</td>
<td>0.02</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB/FC$_7$ vesicles (batch 3)</td>
<td>3</td>
<td>0.03</td>
<td>0.02</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAB/SOS vesicles</td>
<td>4</td>
<td>0.22</td>
<td>0.22</td>
<td>0.24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CTAB micelles</td>
<td>5</td>
<td>0.33</td>
<td>0.33</td>
<td>0.34</td>
<td>0.22</td>
<td>0</td>
</tr>
<tr>
<td>LPFOS micelles</td>
<td>6</td>
<td>0.66</td>
<td>0.65</td>
<td>0.64</td>
<td>0.82</td>
<td>0.83</td>
</tr>
</tbody>
</table>
was expected. Next, the $d$ values calculated between the CTAB/FC$_7$ vesicles and the CTAB/SOS vesicles were 0.22, 0.22, and 0.24, with an average of 0.23. Since the mean value of $d$ is lower than 0.25, the fluorinated vesicles and the unfluorinated vesicles can be considered chemically similar to each other. A comparison of the CTAB/FC$_7$ vesicles to the CTAB micelles gave $d$ values of 0.33, 0.33, and 0.34. All of the values are greater than 0.25, so all of the CTAB/FC$_7$ vesicle batches can be considered chemically different than the CTAB micelles. The CTAB/FC7 vesicles can also be considered chemically different from the LPFOS micelles since the $d$ values were 0.66, 0.65, and 0.64. The CTAB/SOS vesicles were not chemically different from the CTAB micelles according to the $d$ value of 0.22. In contrast, the CTAB/SOS vesicles and the CTAB micelles were chemically different from the LPFOS micelles according to the respective $d$ values of 0.82 and 0.83.

### 3.4 Concluding Remarks

Overall, the CTAB/FC$_7$ vesicles were smaller and scattered less light than the CTAB/SOS vesicles. However, the CTAB/FC$_7$ vesicles proved to be more difficult to work with compared to the CTAB/SOS vesicles. Despite their smaller size, the data collected at short wavelengths such as 214 nm contained more variation in the baseline. The baseline disturbances made it difficult to identify the peaks. In addition, smaller numbers of compounds could be present in the same sample when using the CTAB/FC$_7$ vesicles due to the smaller elution range and therefore decreased resolution.
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Chapter 4: Electrokinetic Chromatography Using Surfactant Vesicles as a Predictor for Intestinal Permeability

4.1 Introduction

Drug discovery is a time and money-consuming endeavor. In 2004 the cost to bring a new drug to market was reported as high as $0.8 to 1.7 billion [1]. Not only must the potential candidates be nontoxic and effective, but they must also be able to permeate the intestine in the case of an orally administered drug. The ability to reach the desired target is a key aspect of the success of a new drug. Therefore, the ability to rapidly screen the potential drug candidates for intestinal permeability is vital to determine whether they should be pursued or dismissed. With this in mind, the pharmaceutical industry is always looking for fast, easy, accurate techniques for screening compounds.

4.1.1 Background of Intestinal Permeability

Intestinal permeability is an extremely complex process. Figure 4.1 shows 5 different pathways for intestinal absorption. For the drug to enter through passive absorption, it must go through the cell membrane of the enterocytes, (cells lining the intestine) or between two enterocytes [2]. Active transport and facilitated diffusion are two more ways for the drug to enter through the intestinal membrane. Another path is when the drug is carried through the cell membrane by a transporter. The drug could be metabolized and then both the drug and its metabolites would be absorbed. Yet another method is via transport by a vesicle. Since there are such a large number of possible pathways to transverse the intestinal lining, it is not surprising that a single accurate model has not emerged [2].
Figure 4.1. Different pathways for intestinal absorption. 1) passive transcellular path; 2) passive paracellular path; 3) carrier assisted path 4) carrier-mediated flux path; and 5) vesicle transport path [3].
4.1.1.1 Models to Predict Cell Membrane Permeability

There are several different models that have been used to predict cell membrane permeability, including parallel artificial membrane permeability assay (PAMPA), cell-based systems, for example Caco-2 and Mardin-Darby canine kidney (MDCK) cells, liquid chromatographic methods using immobilized artificial membrane (IAM) columns, in silico methods, and vesicle and liposome electrokinetic chromatography. The two most widely used methods in the pharmaceutical industry are PAMPA and Caco-2 cells [2]. The aforementioned methods complement each other. Typically, a high throughput method will not be as accurate as a more predictive method. This is the reason multiple methods are used simultaneously.

PAMPA, which was developed by Kansy et al. in 1998, is a high throughput intestinal permeability predictor method [4]. Thousands of compounds can be screened per week using this low-cost, easy and rapid assay [2]. Figure 4.2 shows the steps of the method. Each well of a 96-well microtiter plate is filled with buffer. Then the microtiter plate is covered with a microtiter filter plate, which has a hydrophobic filter material as a support. Half of the wells of the microtiter filter plate are impregnated with lecithin in an organic solvent, such as dodecane. The other wells are used as a reference and contain methanol-buffer solution. UV measurements are taken to determine the concentration of the drug in each well [4]. A limitation of PAMPA is that it only models passive transport. Consequently, it underestimates the absorption of actively transported drugs [2].

Caco-2 cells are from human colon adenocarcinoma and they are the most popular cell line for predicting intestinal permeability. These cells are grown in monolayers
Figure 4.2. PAMP A schematic showing the steps of the method. The microtiter plate is placed on the buffer-filled 96-well plate. Then half of the wells are impregnated with lecithin phospholipids. Finally, the sample and reference wells are measured by UV analysis [4].
on a porous membrane support and are very similar to the human intestinal epithelium. Labor and cost of the method are two of its drawbacks. The cost of analysis of one sample by Caco-2 is approximately $20.00 [5]. In addition, like PAMPA, it is a passive model for intestinal absorption and can give negative results for compounds that are easily transferred across the intestinal membrane [6].

There have also been chromatographic-based permeability predictor models. Detroyer, et al. described a fast monolithic micellar liquid chromatography system to determine drug permeability [7]. They concluded that their predictions were best for compounds that were highly absorbed, meaning it was not a universally applicable technique. Pascoe et al. reported using vesicle electrokinetic chromatography (VEKC) as an intestinal permeability model [8]. Their results showed good correlations between the logarithm of the retention factor values and the logarithm of Caco-2 intestinal permeability values. Another example of EKC analysis, but with liposomes, instead of vesicles, was provided by Wang et al. [9]. In this study, the researchers correlated the logarithm of the retention factors measured for the pharmaceutical compounds to the drug oral fraction absorbed ($F_a$). They observed a sigmoidal relationship between the two factors and suggested that their method needed to be improved by increasing the number of drugs studied and having equal numbers of low, medium, and high percent absorption values [9].

Given the promising results from other researchers, this work further pursues the potential of using unilamellar surfactant vesicles as a predictor for intestinal permeability. The unilamellar surfactant vesicles are similar to the brush border membrane vesicles of the small intestine. The intestinal membrane is highly
negatively charged, with one report estimating 1000-2000 negative charges/vesicle in the intestinal membrane [10]. The approach is to measure the retention factors of the pharmaceutical compounds using a vesicle PSP, and then to correlate the retention data to both Caco-2 permeability and intestinal permeability, using a logarithmic scale to space the data more evenly for purposes of both regression and visual presentation. Two types of vesicles were used in this work: cetyltrimethylammonium bromide (CTAB)/sodium n-octyl sulfate (SOS) vesicles and cetyltrimethylammonium chloride (CTAC)/SOS vesicles. The retention factors obtained were also correlated with octanol/water partition coefficients to compare the vesicles to others that have been previously reported.

4.2 Materials and Methods

4.2.1 Chemicals and Reagents

CTAB, CTAC, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES), acebutolol hydrochloride, alprenolol, atenolol, caffeine, chloramphenicol, hydrocortisone, indomethacin, metoprolol tartrate, nadolol, oxprenolol hydrochloride, pindolol, progesterone, promethazine, propranolol hydrochloride, terbutaline hemisulfate, verapamil hydrochloride, and warfarin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ibuprofen was obtained from Acros (Morris Plains, New Jersey, USA). SOS was purchased from Alfa Aesar (Ward Hill, MA, USA). HPLC grade methanol was purchased from Fisher (Pittsburgh, PA, USA). Water from a Barnstead Nanopure Water system (Dubuque, IA, USA) was used to prepare the buffer.
4.2.2 Instrumentation

A Beckman Coulter P/ACE™ MDQ Series Capillary Electrophoresis System (Fullerton, CA, USA) was used to perform the experiments. A diode array detector was used to collect wavelengths of 200, 236, and 246 nm. The thermostatted capillary cartridge and sample and buffer compartments were set at 25°C. Joule heating was kept to 1.5 W/m or less by using an applied voltage of 14 kV for all separations. The samples were injected for 4 seconds at 13.8 mbar (0.2 psi) corresponding to a volume of 3 nL. The data was collected and processed using 32 Karat™ Software Version 5.0.

A new 31.2 cm x 50 µm ID fused silica capillary from Polymicro Technologies (Phoenix, AZ, USA) was used for each different preparation of vesicles. Before every first use, each capillary was conditioned with 5 min of HPLC-grade water, 10 min of 1.0 M sodium hydroxide, 5 min of 0.1 M sodium hydroxide, 3 min of HPLC-grade water, and 15 min of background electrolyte (BGE) all at an applied pressure of 2070 mbar (30 psi). Between each injection the capillary was rinsed with 0.1 M NaOH for 1 min, HPLC-grade water for 2 min, and BGE for 1.5 min all at 2070 mbar. Then 15 kV was applied between the inlet and outlet buffer vials for 1.5 min.

4.2.3 Vesicle Preparation

The vesicles were prepared in a 1:3.66 mole ratio of CTAB:SOS or CTAC:SOS, giving a final total surfactant concentration of 69 mM. The solid CTAB was weighed and then transferred to a 100 mL beaker using a small amount of water and stock HEPES buffer to make the final concentration of buffer 10 mM. The HEPES buffer was adjusted to pH 7.2 by addition of 1.0 M LiOH. At first the solution was cloudy
white, but after stirring and heating on a setting of 2, it became clear and colorless. As soon as all of the CTAB dissolved, the heat was turned off. While the solution was still warm, solid white SOS was added to the beaker along with additional water. The solution had a bluish tinge to it after all of the solid SOS dissolved. The solution was transferred to an appropriately sized volumetric flask and diluted to the mark with water. Then the solution was vortex mixed for approximately 2 min. The vesicles were not used until at least 12 hours had passed to allow the vesicles to equilibrate. The equilibration time was previously determined [11]. This same procedure was used to prepare the CTAC/SOS vesicles. Prior to use, the vesicles were filtered using 13 mm GD/X disposable nylon membrane filters with 0.45 µm pore size from Whatman (Sanford, ME, USA). In addition, the vesicles were sonicated for 15 minutes in a Branson Model 1510 Ultrasonic Cleaner purchased from Branson Ultrasonic Corporation (Danbury, CT, USA).

4.2.4 Sample Preparation

The pharmaceutical compounds were prepared at concentrations of 0.3-0.8 mg/mL. First, the appropriate amount of solid was weighed. Then methanol was added to each sample followed by 10 mM HEPES so that the final ratio of methanol to HEPES was 6:4.

4.2.5 Calculations

The calculations for voltage-ramp corrected migration time, electroosmotic mobility (EOF), and retention factor can be found in Chapter 2. The electrophoretic mobility of the analyte is calculated by the following equation:
\[ \mu_{ep,analyte} \cong \mu_{ep,CZE} \left( \frac{t_{p,CZE}}{t_{p,VEKC}} \right) \]  

(4.1)

where \( \mu_{ep,CZE} \) is the electrophoretic mobility of the analyte measured under CZE conditions and is equal to \( \mu_{net} - \mu_{eo} \). \( \mu_{net} \) is calculated based upon Eq. (2.2) with the migration time of the analyte \( (t_R) \) substituted for \( t_0 \). \( t_{p,CZE} \) is the time it takes for a plug of acetone to reach the detector via pressure driven flow under CZE conditions. \( t_{p,VEKC} \) is the time it takes for a plug of acetone to reach the detector via pressure driven flow under VEKC conditions. In these experiments, the applied pressure was 0.7 psi. The electrophoretic mobility must be estimated because the migration time of the analyte is influenced by both chromatographic partitioning with the vesicles and its own electrophoretic mobility for charged analytes [12].

A homologous series of alkylphenones (acetophenone through valerophenone) were analyzed and their retention factors calculated in order to determine \( t_{ves} \), using the method of Bushey and Jorgenson [13] in which the logarithm of the retention factor vs. carbon number of a homologous series is assumed to yield a linear relationship. The retention time of valerophenone is used as a starting value for \( t_{ves} \). Then multiple iterations are performed to maximize \( r^2 \) to determine \( t_{ves} \) [11].

4.3 Results and Discussion

4.3.1 Correlation of VEKC to Caco-2 Permeability Using CTAB/SOS and CTAC/SOS Vesicles

Table 4.1 shows the 22 pharmaceutical compounds that were investigated along with their \( pK_a \) value, their average charge at pH 7.2, corresponding Caco-2 and intestinal permeability value, and logarithm P value. The chemical structures of the pharmaceutical compounds are provided in Figure 4.3.
Table 4.1  Pharmaceutical compounds with pKa, average charge at pH 7.2, Caco-2 permeability, intestinal permeability, and octanol-water partition coefficient.

<table>
<thead>
<tr>
<th>ID #</th>
<th>Compound</th>
<th>pKa&lt;sup&gt;a)&lt;/sup&gt;</th>
<th>Average Charge at pH 7.2</th>
<th>Caco-2&lt;sup&gt;b)&lt;/sup&gt; (10&lt;sup&gt;-6&lt;/sup&gt; cm/s)</th>
<th>P&lt;sub&gt;int&lt;/sub&gt;&lt;sup&gt;c)&lt;/sup&gt; (10&lt;sup&gt;-6&lt;/sup&gt; cm/s)</th>
<th>log P&lt;sub&gt;ow&lt;/sub&gt;&lt;sup&gt;d)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ketoprofen</td>
<td>4.23</td>
<td>-1</td>
<td>23.15</td>
<td>5.19</td>
<td>-0.25</td>
</tr>
<tr>
<td>2</td>
<td>Naproxen</td>
<td>4.84</td>
<td>-1</td>
<td>39.50</td>
<td>9.40</td>
<td>0.28</td>
</tr>
<tr>
<td>3</td>
<td>Salicylic acid</td>
<td>3.01</td>
<td>-1</td>
<td>22.00</td>
<td>9.24</td>
<td>2.26</td>
</tr>
<tr>
<td>4</td>
<td>Acetyl salicylic acid</td>
<td>3.48</td>
<td>-1</td>
<td>9.09</td>
<td>1.94</td>
<td>1.19</td>
</tr>
<tr>
<td>5</td>
<td>Ibuprofen</td>
<td>4.41</td>
<td>-1</td>
<td>52.50</td>
<td>18.10</td>
<td>3.50</td>
</tr>
<tr>
<td>6</td>
<td>Indomethacin</td>
<td>3.96</td>
<td>-1</td>
<td>20.40</td>
<td>6.94</td>
<td>4.27</td>
</tr>
<tr>
<td>7</td>
<td>Warfarin</td>
<td>4.50</td>
<td>-1</td>
<td>21.10</td>
<td>2.47</td>
<td>2.70</td>
</tr>
<tr>
<td>8</td>
<td>Caffeine</td>
<td>0.73</td>
<td>0</td>
<td>30.80</td>
<td>2.58</td>
<td>-0.07</td>
</tr>
<tr>
<td>9</td>
<td>Hydrocortisone</td>
<td>12.47</td>
<td>0</td>
<td>14.00</td>
<td>6.61</td>
<td>1.61</td>
</tr>
<tr>
<td>10</td>
<td>Chloramphenicol</td>
<td>11.03</td>
<td>0</td>
<td>20.60</td>
<td>12.09</td>
<td>1.14</td>
</tr>
<tr>
<td>11</td>
<td>Progesterone</td>
<td>9.33</td>
<td>0</td>
<td>23.70</td>
<td>22.65</td>
<td>3.87</td>
</tr>
<tr>
<td>12</td>
<td>Terbutaline</td>
<td>9.10</td>
<td>+1</td>
<td>0.47</td>
<td>2.95</td>
<td>0.48</td>
</tr>
<tr>
<td>13</td>
<td>Acebutolol</td>
<td>9.16</td>
<td>+1</td>
<td>4.46</td>
<td>3.13</td>
<td>1.71</td>
</tr>
<tr>
<td>14</td>
<td>Atenolol</td>
<td>9.17</td>
<td>+1</td>
<td>0.53</td>
<td>0.64</td>
<td>0.16</td>
</tr>
<tr>
<td>15</td>
<td>Oxprenolol</td>
<td>9.17</td>
<td>+1</td>
<td>65.50</td>
<td>14.65</td>
<td>2.10</td>
</tr>
<tr>
<td>16</td>
<td>Nadolol</td>
<td>9.14</td>
<td>+1</td>
<td>3.88</td>
<td>0.33</td>
<td>0.71</td>
</tr>
<tr>
<td>17</td>
<td>Propranolol</td>
<td>9.20</td>
<td>+1</td>
<td>21.80</td>
<td>24.05</td>
<td>2.98</td>
</tr>
<tr>
<td>18</td>
<td>Pindolol</td>
<td>9.17</td>
<td>+1</td>
<td>16.70</td>
<td>18.29</td>
<td>1.75</td>
</tr>
<tr>
<td>19</td>
<td>Metoprolol</td>
<td>9.16</td>
<td>+1</td>
<td>23.70</td>
<td>5.06</td>
<td>1.88</td>
</tr>
<tr>
<td>20</td>
<td>Alprenolol</td>
<td>8.98</td>
<td>+1</td>
<td>25.30</td>
<td>21.77</td>
<td>3.10</td>
</tr>
<tr>
<td>21</td>
<td>Promethazine</td>
<td>8.97</td>
<td>+1</td>
<td>not available</td>
<td>24.58</td>
<td>4.81</td>
</tr>
<tr>
<td>22</td>
<td>Verapamil</td>
<td>9.33</td>
<td>+1</td>
<td>26.30</td>
<td>12.96</td>
<td>3.79</td>
</tr>
</tbody>
</table>

<sup>a)</sup> pKa data from [14].
<sup>b)</sup> Caco-2 data from [15-20]
<sup>c)</sup> P<sub>int</sub> data from [21].
<sup>d)</sup> log P<sub>ow</sub> from [22, 23].
Figure 4.3. Chemical structures of the pharmaceutical compounds investigated.
Verapamil

Chloramphenicol

Hydrocortisone

Indomethacin

Progesterone

Warfarin

Figure 4.3. (continued). Chemical structures of the pharmaceutical compounds investigated.
Representative electropherograms are presented in Figure 4.4 using CTAB/SOS vesicles and Figure 4.5 using CTAC/SOS vesicles. Also, the Caco-2 permeability was unavailable for promethazine so that compound was not included in the Caco-2 correlations.

### 4.3.1.1 Correlations Using Cationic Compounds

The pharmaceutical compounds were separated by charge class (cations, neutrals, anions) before their retention factors were correlated to the logarithm of the Caco-2 permeabilities (log $P_{\text{Caco-2}}$). Figure 4.6 shows the correlation of log $P_{\text{Caco-2}}$ with the logarithm of the retention factors of the cationic pharmaceutical compounds using CTAB/SOS vesicles (log $k_{\text{CTAB/SOS,cations}}$). Nonlinear regression analysis using a four-parameter dose-response curve was performed using GraphPad Prism version 5.01 for Windows, (GraphPad Software, San Diego, California, USA). The general equation of the line used to fit the data is as follows:

$$Y = Bottom + \frac{Top - Bottom}{1 + 10^{(\log EC50 - X \cdot HillSlope)}}$$

where Bottom and Top are the lower and upper plateaus of the sigmoidal curve, respectively, EC50 is the $X$ value that gives a response halfway between the Top and the Bottom or the inflection point, and HillSlope is the steepness of the curve [24]. The parameters of the best fit line are shown in Table 4.2. Figure 4.7 shows how similar the results are using CTAC/SOS vesicles to correlate log $P_{\text{Caco-2}}$ with the logarithm of the retention factors of the cationic pharmaceutical compounds (log $k_{\text{CTAC/SOS,cations}}$). The parameters of the best fit line are shown in Table 4.2. Sigmoidal relationships have previously been observed by Wang et al. [9] who correlated the logarithm of the retention factor using liposomes to the logarithm of the human fraction absorbed ($Fa$).
Figure 4.4. Electropherograms showing separation of pharmaceutical compounds using CTAB/SOS vesicles with 10 mM HEPES buffer pH 7.2. Compound identification according to Table 4.1. Separation conditions: A new fused silica capillary of 31.2 cm total length, 20.0 cm length to the detector, 50 µm ID, and 363 µm OD at 25°C was used. An applied voltage of 14 kV was used and injection was done hydrodynamically for 4.0 s at 13.8 mbar (0.2 psi). Detection was done using PDA at 236 nm.
Figure 4.5. Electropherograms showing separation of pharmaceutical compounds using CTAC/SOS vesicles with 10 mM HEPES buffer pH 7.2. Compound identification according to Table 4.1. Separation conditions as in Figure 4.4.
Figure 4.6. Correlation of the Caco-2 permeabilities of the cationic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. The average relative standard deviation (RSD) in log k was 7.9 %, with a range of 1.6 % to 19.4 %. Composition of vesicle solution: 1:3.66 mole ratio of CTAB:SOS, 69 mM total concentration, in 10 mM HEPES buffer (pH 7.2).
Figure 4.7. Correlation of the Caco-2 permeabilities of the cationic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. The average relative standard deviation (RSD) in log k was 5.9 %, with a range of 1.7 % to 20.3 %. Composition of vesicle solution: 1:3.66 mole ratio of CTAC:SOS, 69 mM total concentration, in 10 mM HEPES buffer (pH 7.2).
Table 4.2. Parameters for the Nonlinear Regression Analysis of the Retention Factors Cationic Compounds Correlated with Caco-2 Permeabilities

<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Top</th>
<th>Bottom</th>
<th>log(EC50)</th>
<th>HillSlope</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/SOS</td>
<td>1.5 ± 0.1</td>
<td>-0.3 ± 0.3</td>
<td>-0.5 ± 0.1</td>
<td>2.7 ± 2.0</td>
<td>0.878</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>1.5 ± 0.2</td>
<td>-0.3 ± 0.4</td>
<td>-0.5 ± 0.2</td>
<td>2.2 ± 1.0</td>
<td>0.870</td>
</tr>
</tbody>
</table>
4.3.1.2 Correlations Using Neutral Compounds
The graph of the correlation between the logarithm of the retention factors of the neutral compounds with the logarithm of the Caco-2 permeabilities using CTAB/SOS vesicles and CTAC/SOS vesicles are shown in Figures 4.8 and 4.9, respectively. In both examples, the relationship is nonlinear with the four neutral compounds that were employed. The data were fit with a quadratic, but this has not physical meaning. The $R^2$ values of the fits were 0.731 and 0.733 using the CTAB/SOS vesicles and the CTAC/SOS vesicles, respectively. It could be that the literature value of Caco-2 permeability for caffeine was incorrect. Other values were used, but none of them were able to improve the relationship.

4.3.1.3 Correlations Using Anionic Compounds
The graphs of the correlations using anionic compounds for retention factors with logarithm of Caco-2 permeabilities are shown in Figures 4.10 and 4.11. It can be observed that no apparent linear or sigmoidal trend was present. This could be due to the fact that the retention factors of the anionic compounds were difficult to calculate. One of the striking features of the graph is that the anionic compounds were clustered around the same location, whereas both the neutral and cationic compounds were more dispersed. There were difficulties in measuring the retention factors of the anionic compounds. Normally, the viscosity correction to determine the electrophoretic mobility of the analyte would be sufficient, but in these experiments, the retention factors of the anionic compounds were either very close to zero or negative, which does not make chemical sense. Garcia et al. [14] indicated the increased amount of error associated with measuring retention factor for neutral compounds for which the migration time was similar to either $t_0$ or $t_{psp}$. An electrophoretic drag correction was considered for the vesicles, but this is also difficult to determine. One possible reason for the difficulty in measuring the
Figure 4.8. Correlation of the Caco-2 permeabilities of the neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. The average relative standard deviation (RSD) in log k was 8.0 %, with a range of 5.2 % to 12.1 %. Conditions as in Figure 4.6.
Figure 4.9. Correlation of the Caco-2 permeabilities of the neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. The average relative standard deviation (RSD) in log k was 4.4 %, with a range of 0.92 % to 10.5 %. Conditions as in Figure 4.7.
Figure 4.10. Correlation of the Caco-2 permeabilities of the anionic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. The average relative standard deviation (RSD) in log k was 16.1%, with a range of 0.65% to 40.4%. Conditions as in Figure 4.6.
Figure 4.11. Correlation of the Caco-2 permeabilities of the anionic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. The average relative standard deviation (RSD) in log k was 12.4 %, with a range of 2.6 % to 26.0 %. Conditions as in Figure 4.7.
retention factors of the anionic analytes was the electrostatic repulsions between the negatively charged analytes and the negatively charged vesicles.

**4.3.2 Correlation of VEKC to Intestinal Permeability Using CTAB/SOS and CTAC/SOS Vesicles**

**4.3.2.1 Correlations Using Cationic Compounds**

The correlation of the logarithm of the intestinal permeabilities with the logarithm of the retention factor using CTAB/SOS vesicles is shown in Figure 4.12. The data are extremely similar in profile to the correlations with Caco-2 permeability. They are both well-fit by a sigmoidal function. One major difference are the positions of nadolol and terbutaline. These two compounds switch position going from correlations with Caco-2 to intestinal permeability. Also, the data for the CTAC/SOS vesicles are similar to that of the CTAB/SOS vesicles. The graph for the correlation of the cationic compounds using CTAC/SOS vesicles is given in Figure 4.13. The parameters for the sigmoidal lines are given in Table 4.3.

**4.3.2.2 Correlations Using Neutral Compounds**

For the neutral compounds, a linear relationship was observed for the correlations with the logarithm of retention factor with the logarithm of the intestinal permeabilities using both the CTAB/SOS vesicles and the CTAC/SOS vesicles. Figures 4.14 and 4.15 show the graphs using CTAB/SOS and CTAC/SOS, respectively. The $R^2$ value that was obtained was 0.924 for both types of vesicles and the slopes were statistically different from zero. The $R^2$ value reported here was higher than the $R^2$ value of 0.727 obtained by Pascoe et al. [8] using CTAB/SOS vesicles for a set of 36 proprietary compounds. This difference could be due to the fact that 36 compounds were included in the study reported by Pascoe et al. compared to the 4 neutral compounds reported here.
Figure 4.12. Correlation of the intestinal permeabilities of the cationic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.13. Correlation of the intestinal permeabilities of the cationic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
<table>
<thead>
<tr>
<th>Vesicle System</th>
<th>Top</th>
<th>Bottom</th>
<th>log(EC50)</th>
<th>HillSlope</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB/SOS</td>
<td>1.3 ± 0.1</td>
<td>-0.17 ± 0.3</td>
<td>-0.33 ± 0.1</td>
<td>2.4 ± 1.6</td>
<td>0.865</td>
</tr>
<tr>
<td>CTAC/SOS</td>
<td>1.3 ± 0.1</td>
<td>-0.08 ± 0.2</td>
<td>-0.24 ± 0.1</td>
<td>3.9 ± 3.7</td>
<td>0.868</td>
</tr>
</tbody>
</table>
Figure 4.14. Correlation of the intestinal permeabilities of the neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.15. Correlation of the intestinal permeabilities of the neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
4.3.2.3 Correlations Using Anionic Compounds

No clear correlations of retention factor to intestinal permeabilities for the anionic compounds could be established using either the CTAB/SOS vesicles nor the CTAC/SOS vesicles. Figure 4.16 shows the graph of log $P_{int}$ vs. log $k$ using CTAB/SOS vesicles. The reason for the lack of correlation is also the same as those previously given in Section 4.3.1.3. Figure 4.17 contains the graph of intestinal permeability vs. retention factor using CTAC/SOS vesicles.

4.3.3 Correlation of VEKC Retention Factors with Octanol-Water Partition Coefficients using CTAB/SOS and CTAC/SOS Vesicles

VEKC has been previously used to predict logarithm $P_{ow}$ for both pesticides[25] and pharmaceutical compounds [8, 26]. To show that the vesicles presented here were similar to those reported in the past, the retention factors obtained using CTAB/SOS vesicles were correlated to logarithm $P_{ow}$ values.

4.3.3.1 Correlations Using Cationic Compounds

The graphs of the correlations of logarithm $P_{ow}$ for the cationic compounds with the logarithm of the retention factor obtained using CTAB/SOS and CTAC/SOS vesicles can be found in Figures 4.18 and 4.19, respectively. There is a linear relationship observed in both of these plots. The $R^2$ value using CTAB/SOS vesicles is 0.863 compared to an $R^2$ of 0.889 using the CTAC/SOS vesicles. These values are very similar to the correlation coefficient of 0.879 reported by Razak et al. who also used CTAB/SOS vesicles [26], as well as the correlation coefficient of 0.850 reported by Pascoe et al. for CTAB/SOS vesicles [8].
Figure 4.16. Correlation of the intestinal permeabilities of the anionic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.17. Correlation of the intestinal permeabilities of the anionic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
Figure 4.18. Correlation of the octanol-water partition coefficients of the cationic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.19. Correlation of the octanol-water partition coefficients of the cationic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
4.3.3.2 Correlations Using Neutral Compounds

Similar to the results correlating the logarithm of \( P_{ow} \) for the cationic compounds, there is also a linear relationship using the neutral compounds. Figure 4.20 shows the correlation of logarithm of \( P_{ow} \) vs. the retention factors of the neutral compounds using CTAB/SOS vesicles. Figure 4.21 has the equivalent graph using CTAC/SOS vesicles. The \( R^2 \) for both of the regression lines is 0.959, which is better than the \( R^2 \) values found for the cationic compounds.

4.3.3.3 Correlations Using Anionic Compounds

Finally, the correlation for the anionic compounds’ retention factors and the logarithm of \( P_{ow} \) using CTAB/SOS vesicles is given in Figure 4.22. The corresponding graph using CTAC/SOS vesicles is given in Figure 2.23. Following the same trend as was observed for the correlations with Caco-2 and intestinal permeabilities, there was no clear relationship between \( \log P_{ow} \) and \( \log k \) for the anionic compounds. It is apparent that the charge of the compound plays a major role in the strength of the correlations from this data.

4.4 Concluding Remarks

The correlations with retention factor were better for intestinal permeabilities compared to correlations with Caco-2 permeabilities. The results of all the correlations with the logarithm of the retention factor indicate that the correlations are better for neutral and positively charged analytes than for negatively charged ones. For this approach to succeed, the compounds should be run at a pH where they are neutral. It would be ideal to have a method where all of the compounds could be run at the same pH to facilitate rapid analysis. This does not
Figure 4.20. Correlation of the octanol-water partition coefficients of the neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.21. Correlation of the octanol-water partition coefficients of the neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
Figure 4.22. Correlation of the octanol-water partition coefficients of the anionic compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure 4.6.
Figure 4.23. Correlation of the octanol-water partition coefficients of the anionic compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure 4.7.
seem possible under the conditions described in this report. Another alternative would be to use reversed mode CE with a positively charged vesicle and short end injection for analysis of the anionic compounds. As for the correlations of logarithm $k$ with logarithm $P_{ow}$, a similar trend was observed regarding the anionic compounds. These compounds are related to logarithm $P_{ow}$ in a nonlinear fashion. The correlation would most likely be linear if the compounds were analyzed under conditions where they would be neutral as demonstrated by Klotz et al. [25] for the correlation of organic acids to $P_{ow}$ at pH 2 using CTAB/SOS vesicles.

One other interesting aspect of this investigation is that both CTAB/SOS vesicles and CTAC/SOS vesicles have provided similar results in the correlations. This proves that not only CTAB/SOS vesicles are useful passive models of cell membranes, but CTAC/SOS vesicles are as well. In order to improve the model, a larger number of compounds should be investigated at a pH in which they are neutral. In addition, cholesterol could be added to these vesicles to possibly improve the correlations, although previous additions were by no means universally successful and increased the complexity of the PSP [8].
References

1 Food and Drug Administration, Challenges and Opportunity on the Critical Path to New Medical Products. FDA Report., Rockville, MD 2004.


Chapter 5: Conclusions and Recommendations for Future VEKC Experiments

VEKC using synthetic vesicles formed from cationic and anionic surfactants has been investigated for the purpose of i) identifying the effect of cationic surfactant counterion; ii) improving the efficiency of highly hydrophobic compounds in order to facilitate the measurement of their retention factors; iii) developing smaller vesicles in order to reduce light scattering in the low UV in order to improve sensitivity; and iv) developing a high throughput method for estimating intestinal permeability. The cationic surfactant counterion did not dramatically affect the chromatographic results, but it did provide interesting effects in terms of selectivity. Next, the smaller vesicles that were developed (CTAB/FC7) were not as effective at improving sensitivity as originally hoped. The results using the fluorinated vesicles contained noisy baselines and the sensitivities were worse compared to the CTAB/SOS vesicles. In contrast, there were some promising results with respect to the correlations of retention factor with intestinal permeability for cationic and neutral analytes. The method needs improvement however if it is to be employed by the pharmaceutical industry.

One thing that could be done to improve the correlations of retention factor with intestinal permeability would be to use a pH where the compounds are neutral. The effect of pH has been examined by Razak et al. in terms of correlations of retention factor with log $P_{ow}$ [1]. Another suggestion would be to add cholesterol to the vesicles to make them more similar to the epithelial cells of the intestine.
To further the development of VEKC, it would be useful to identify a reliable marker for the vesicles that could be added to each sample. This *in-situ* monitoring of the time of the vesicle would be beneficial because any changes would be identified as they occur. The approach used for the experiments described here took an average $\mu_{ep,ves}$ from 8 or less injections. While $\mu_{ep,ves}$ is not expected to change drastically, there are some slight deviations from one injection to the next. Additionally, if there were a large change in $\mu_{ep,ves}$, then that would be an indication of a larger problem. Some suggestions for a marker for the vesicles would be dodecanophenone or octylbenzene [2]. Of course, adding a marker to every sample would increase the run times, which may be a disadvantage which would have to be weighed against the possible improvement caused by the accuracy of the marker.

It would be interesting to see the effects of using other vesicles in EKC analyses. Some suggestions for other vesicles to use would be those that are composed of double-tailed surfactants, such as didodecyldimethyl ammonium bromide (DDAB)/bis(2-ethylhexyl) sodium sulfosuccinate (AOT) vesicles [3]. A completely fluorinated vesicle may also be worthwhile to explore for different selectivity and improved efficiency. Extreme care would need to be taken with the handling and disposal of any perfluorinated components, though. A good source of other potential vesicles is the review by Segota and Tezak [4]. Many different surfactant vesicles are available, as well as phospholipid vesicles.

The area of chiral analysis using VEKC has been steadily growing over recent years [5, 6]. Consequently, chiral vesicles would be another interesting research subject. Only
one group has reported the successful creation and implementation of chiral vesicles in CE. There is a great need for rapid chiral analysis. Previously vesicles composed of CTAB and dodecoxycarbonylvaline (DDCV) with HEPES buffer showed no enantioselectivity [7]. Perhaps if the buffer were changed to sodium phosphate or another buffer that was not zwitterionic and/or bulky, then the vesicles would exhibit enantioselectivity. If the lack of stereoselectivity is due to strong interactions of the oppositely-charged head groups, however, switching to a different type of buffer may not lead to an improvement.

The variable of temperature has not been investigated with respect to VEKC analysis. It would be interesting to see if temperature has any effect on the correlations with intestinal permeability. Since internal body temperature is higher than room temperature (at which all of the VEKC experiments have been performed), the results may change for the better.

Still another interesting experiment would be to use the vesicles in EKC using a laser induced fluorescence (LIF) detector. These experiments could demonstrate the carrier properties of the vesicles if a fluorescently active compound were encapsulated within it. One possible drawback would be that the counterion of the vesicles could act as a fluorescence quencher, though. Also, it might be interesting to compare the intensity of the fluorescence signal in the presence and absence of vesicles or use whole column detection with a charge coupled device (CCD) camera and a µTotal Analysis System (TAS).
One type of characterization that should be conducted on the vesicles is cryogenic transmission electron microscopy (cryo-TEM). This would provide a more accurate image of the vesicles and would give a better idea of the true shape of the vesicles. For the most part, the vesicles are probably spherical, but there could be other shapes present, which may influence the separations in VEKC analysis.

A more comprehensive study should be conducted to resolve whether the results in VEKC are better when the samples are prepared in a mixture of buffer and organic solvent versus the vesicle solution. In microemulsion electrokinetic chromatography (MEEKC), the samples are prepared in the microemulsion solution to avoid disrupting the microemulsion. It could be that more reproducible results would be obtained by simply changing the diluent of the analytes to the vesicle solution.

Overall, VEKC is a valuable mode of CE analysis with increased elution range and different selectivity compared to micellar electrokinetic chromatography (MEKC) and MEEKC. VEKC has potential as a high throughput estimator of intestinal permeability and log P_{ow}. There are still several areas of VEKC that can be developed including a study to find a suitable marker to estimate the time of the vesicles, chiral analysis, and temperature analysis. The future of VEKC is unlimited with respect to all of the vesicles that have not been tried, as well. Since it is such a fast, inexpensive analysis technique, the breadth and number of VEKC applications should continue to increase.
References


Appendix A: Correlations of Caco-2 and Intestinal Permeability with Retention Factors of Cationic and Neutral Compounds Combined

A.1 Correlation of VEKC to Caco-2 Permeability Using CTAB/SOS Vesicles

Since there was difficulty in measuring the retention factors of the anionic compounds, they were omitted from the correlation. After residual analysis was completed for the remaining data, it was determined that atenolol, caffeine, oxprenolol, and terbutaline were outliers. All of these compounds had residuals > 0.5. The correlation of the logarithm of the Caco-2 permeabilities to the retention factors of the remaining neutral and cationic compounds is shown in Figure A.1. The $R^2$ value was 0.465 with a 95% confidence interval of 0.184 to 0.324, which does not include 0, indicating that the slope is statistically significant.

A.2 Correlation of VEKC to Caco-2 Permeability Using CTAC/SOS Vesicles

The correlation coefficient with the anionic compounds removed for the correlation of Caco-2 permeabilities to retention factors using CTAC/SOS vesicles was 0.456, which was slightly lower than the correlation coefficient obtained using the retention factors obtained with the CTAB/SOS vesicles. See Figure A.2. The 95% confidence interval of the slope was 0.384 to 0.523, which indicated that the value was statistically significant and not 0.

A.3 Correlation of VEKC to Intestinal Permeability Using CTAB/SOS Vesicles

For the following results, the data for nadolol was not used since its residual was > 0.5. In addition, there were difficulties determining the retention factors for ketoprofen and
Figure A.1. Correlation of the Caco-2 permeabilities of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Compounds as in Table 4.1. Composition of vesicle solution: 1:3.66 mole ratio of CTAB:SOS, 69 mM total concentration, in 10 mM HEPES buffer (pH 7.2).
Figure A.2. Correlation of the Caco-2 permeabilities of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Compounds as in Table 4.1. Composition of vesicle solution: 1:3.66 mole ratio of CTAC:SOS, 69 mM total concentration, in 10 mM HEPES buffer (pH 7.2).
salicylic acid so these compounds were also omitted. The graph of the logarithm of intestinal permeability ($P_{\text{int}}$) versus the logarithm of the retention factor ($k$) using CTAB/SOS vesicles using all cationic, neutral, and anionic compounds is shown in Figure A.3. The $R^2$ value was 0.707, indicating a slight correlation. Razak et al. had expressed concern about correlating the retention factors of anionic compounds to logarithm $P_{\text{ow}}$ using anionic vesicles [1]. Figure A.4 shows the correlation of logarithm $P_{\text{int}}$ versus logarithm $k$ using CTAB/SOS vesicles excluding anionic compounds. When the anionic compounds were excluded, the $R^2$ value increased to 0.849. This demonstrated that there were electrostatic interactions taking place between the anionic pharmaceutical compounds and the anionic vesicles that reduced the correlation between $P_{\text{int}}$ and $k$ for these compounds. The $R^2$ results of all of the replicates using CTAB/SOS vesicles for correlation of logarithm $P_{\text{int}}$ to logarithm $k$ are shown in Table A.1. The correlations became slightly lower with time as shown by the progression from replicate 1 to replicate 3. When a fresh preparation of vesicles was used (replicate 4), the correlation returned to its previous level. This could be caused by changes in vesicle morphology, such as aggregation of smaller vesicles to form larger ones.

A.4 Correlation of VEKC to Intestinal Permeability Using CTAC/SOS Vesicles

In order to examine any possible effects caused by the counterion of the cationic surfactant in the vesicles, the retention factors of the pharmaceutical compounds were also measured using CTAC/SOS vesicles. For these results, the data for nadolol was omitted since its residual was $> 0.5$. Similar to the CTAB/SOS vesicle results, there were difficulties determining the retention factors for ketoprofen, salicylic acid, and
Figure A.3. Correlation of the intestinal permeabilities of a diverse set of cationic, neutral, and anionic pharmaceutical compounds with their corresponding retention factors obtained using electrokinetic chromatography (EKC) with CTAB/SOS vesicles as the pseudostationary phase (PSP). Compounds as in Table 4.1. Composition of vesicle solution: 1:3.66 mole ratio of CTAB:SOS, 69 mM total concentration, in 10 mM HEPES buffer (pH 7.2).
Figure A.4. Correlation of the intestinal permeability of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure A.1.
Table A.1. $R^2$ values for replicate CTAB/SOS vesicle correlation of log $P_{int}$ to log $k$.

<table>
<thead>
<tr>
<th>Replicate #</th>
<th>$R^2$ using all compounds</th>
<th>$R^2$ excluding anionic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$0.707^{a)}$</td>
<td>0.849</td>
</tr>
<tr>
<td>2</td>
<td>$0.640^{a)}$</td>
<td>0.781</td>
</tr>
<tr>
<td>3</td>
<td>$0.607^{a)}$</td>
<td>0.776</td>
</tr>
<tr>
<td>4</td>
<td>$0.676^{a)}$</td>
<td>0.796</td>
</tr>
</tbody>
</table>

*a) Excluding ketoprofen and salicylic acid since the measured retention factors were negative and excluding nadolol since it was an outlier.
acetylsalicylic acid so these compounds were also omitted. The graph of the best
correlation using all of the compounds in shown in Figure A.5. The $R^2$ value for this
correlation was 0.625, which was slightly lower than the best correlation using the
CTAB/SOS vesicles. The graph with the best correlation excluding the anionic
compounds for CTAC/SOS vesicles is shown in Figure A.6. The $R^2$ value for this
correlation was 0.791, which falls in the middle of the range of the $R^2$ values for the
CTAB/SOS vesicles. Table A.2 contains the $R^2$ values for additional correlations using
CTAC/SOS vesicles. The correlations were lower than expected based upon the results
reported by Pascoe et al. [2]. Using CTAB/SOS vesicles, they reported an $R^2$ value of
0.727. However, one major difference between these results and the previous results is
that the majority of compounds used by Pascoe et al. were neutral whereas 4 out of 21
compounds were neutral in the present study. The correlations seem to be heavily
dependent upon the charge of the analytes under investigation.

A.5 Correlation of VEKC to logarithm $P_{ow}$ using CTAB/SOS and CTAC/SOS Vesicles

In order to determine the best fit of the data, the anionic compounds were omitted as
outliers. Figure A.7 shows the correlation of logarithm $P_{ow}$ vs. logarithm $k$ for all of the
neutral and cationic pharmaceutical compounds using CTAB/SOS vesicles. The $R^2$ value
was 0.911. The increase in correlation coefficient is probably due to the set of test
compounds that was investigated and the removal of the anionic compounds. The
retention factors obtained using CTAC/SOS vesicles were also correlated to logarithm
$P_{ow}$ data. Again the anionic compounds were removed so a linear regression line could
be calculated. Figure A.8 shows the results of the graph of logarithm $P_{ow}$ vs. logarithm $k$
Figure A.5. Correlation of the intestinal permeabilities of a diverse set of cationic, neutral, and anionic pharmaceutical compounds with their corresponding retention factors obtained using electrokinetic chromatography (EKC) with CTAC/SOS vesicles as the pseudostationary phase (PSP). Compounds as in Table 4.1. Conditions as in Figure A.2.

\[ y = 0.370x + 0.891 \]

\[ R^2 = 0.625 \]
Figure A.6. Correlation of the intestinal permeability of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure A.2.
Table A.2. $R^2$ values for replicate CTAC/SOS vesicle correlation of log $P_{im}$ to log $k$.

<table>
<thead>
<tr>
<th>Replicate #</th>
<th>$R^2$ using all compounds</th>
<th>$R^2$ excluding anionic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.625 $^a$</td>
<td>0.791</td>
</tr>
<tr>
<td>2</td>
<td>0.570 $^a$</td>
<td>0.773</td>
</tr>
<tr>
<td>3</td>
<td>0.599 $^a$</td>
<td>0.778</td>
</tr>
</tbody>
</table>

a) Excluding ketoprofen, salicylic acid, and acetylsalicylic acid since the measured retention factors were negative and nadolol since it was an outlier.
Figure A.7. Correlation of the octanol water partition coefficients of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAB/SOS vesicles. Conditions as in Figure A.1.
Figure A.8. Correlation of the octanol water partition coefficients of the cationic and neutral compounds of Table 4.1 with their EKC retention factors using CTAC/SOS vesicles. Conditions as in Figure A.2.
using CTAC/SOS vesicles for only the neutral and cationic compounds. The correlation coefficient was 0.907, which is very similar to the result using CTAB/SOS vesicles.
References


Appendix B: Attempts at Creating New Vesicles

Several attempts were made at creating new vesicles from cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS). However, all of these attempts resulted in the creation of micelles or unstable saturated solutions instead. Table B.1 lists the components and concentrations of the vesicle attempts. The motivation for creation of the vesicles was to create a system with common chemicals that most laboratories would have readily available.

The appropriate amount of surfactant was mixed together in 10 mM phosphate buffer (pH 7.2) and then the resulting solution was analyzed using a Horiba LB 500 Dynamic Light Scattering Particle Size Analyzer (Irvine, CA, USA). If the solution was very cloudy, if precipitate was present, or if the solution contained multiple phases, then the size of the particles in solution was not measured.

A few papers have described the combined use of CTAB and SDS as pseudostationary phases in electrokinetic chromatography [1, 2] and as capillary coatings [3], but the results described in the papers regarding CTAB/SDS vesicles [4-6] could not be replicated. For example, the 5 mM CTAB and 20 mM SDS combination was chosen based upon the results reported by Ying et al. [6], while the 15 mM CTAB and 25 mM SDS combination was chosen based upon the results of Tomasic et al. [5]. The difficulty duplicating the other groups’ results could be related to the fact that unlike the literature sources, the solutions were prepared in 10 mM Phosphate buffer. Another difference is
Table B.1. Compositions of CTAB/SDS vesicle attempts.

<table>
<thead>
<tr>
<th>[CTAB] mM</th>
<th>[SDS] mM</th>
<th>Buffer</th>
<th>Appearance</th>
<th>Mean Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>10</td>
<td>10 mM Phosphate</td>
<td>Clear and colorless</td>
<td>0.004-0.005 µm</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>10 mM Phosphate</td>
<td>Milky white</td>
<td>2.5-3 µm</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>10 mM Phosphate</td>
<td>Milky white</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>10 mM Phosphate</td>
<td>Milky white</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>0.6</td>
<td>10 mM Phosphate</td>
<td>Slightly cloudy</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>1.5</td>
<td>10 mM Phosphate</td>
<td>Slightly cloudy</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>0.3</td>
<td>10 mM Phosphate</td>
<td>Slightly cloudy</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>No buffer</td>
<td>Clear and colorless</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>1.5</td>
<td>No buffer</td>
<td>Clear and colorless</td>
<td>0.004-0.006 µm</td>
</tr>
</tbody>
</table>
that the surfactants were used as received whereas some of the references stated that the surfactants were recrystallized before use [5]. No buffer was used for two of the vesicle attempts, but the resulting particle size of the components in solution was not indicative of the presence of vesicles. If the experiments were to be repeated, then the surfactants should be purified to test whether that was the source of the discrepancy.
References


### Appendix C: List of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{0.1}$</td>
<td>width of the first half of the peak at 10% peak height</td>
</tr>
<tr>
<td>$b_{0.1}$</td>
<td>width of the second half of the peak at 10% peak height</td>
</tr>
<tr>
<td>$\alpha_{PG}$</td>
<td>polar group selectivity</td>
</tr>
<tr>
<td>$\alpha_{CH2}$</td>
<td>methylene selectivity</td>
</tr>
<tr>
<td>CH$_2$</td>
<td>methylene group</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>permittivity of a vacuum</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>relative permittivity of the buffer</td>
</tr>
<tr>
<td>$\eta$</td>
<td>viscosity</td>
</tr>
<tr>
<td>$n$</td>
<td>carbon number</td>
</tr>
<tr>
<td>$\sigma^2$</td>
<td>variance or standard deviation squared</td>
</tr>
<tr>
<td>$\mu_{eo}$</td>
<td>electroosmotic mobility</td>
</tr>
<tr>
<td>$\mu_{ep}$</td>
<td>electrophoretic mobility</td>
</tr>
<tr>
<td>$\mu_{ep,analyte}$</td>
<td>electrophoretic mobility of the analyte</td>
</tr>
<tr>
<td>$\mu_{ep,CZE}$</td>
<td>electrophoretic mobility under capillary zone electrophoresis conditions</td>
</tr>
<tr>
<td>$\mu_{ep,ves}$</td>
<td>electrophoretic mobility of the vesicle</td>
</tr>
<tr>
<td>$\mu_{ep,PSP}$</td>
<td>electrophoretic mobility of the pseudostationary phase</td>
</tr>
<tr>
<td>$\mu_{net}$</td>
<td>net mobility</td>
</tr>
<tr>
<td>$\mu_{ves}$</td>
<td>net mobility of the vesicle</td>
</tr>
<tr>
<td>$\mu_r$</td>
<td>relative electrophoretic mobility</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>zeta potential</td>
</tr>
<tr>
<td>$\omega$</td>
<td>normalization parameter</td>
</tr>
<tr>
<td>$d$</td>
<td>distance parameter</td>
</tr>
<tr>
<td>E</td>
<td>electric field</td>
</tr>
<tr>
<td>H</td>
<td>plate height</td>
</tr>
<tr>
<td>k</td>
<td>retention factor</td>
</tr>
<tr>
<td>$k_1$</td>
<td>retention factor, peak #1</td>
</tr>
<tr>
<td>$k_2$</td>
<td>retention factor, peak #2</td>
</tr>
<tr>
<td>$k_{avg}$</td>
<td>average retention factor</td>
</tr>
<tr>
<td>$k_b$</td>
<td>retention factor of benzene</td>
</tr>
<tr>
<td>$k_s$</td>
<td>retention factor of a substituted benzene</td>
</tr>
<tr>
<td>$k_{mod}$</td>
<td>retention factor of the solute using 10% ACN modified vesicles</td>
</tr>
<tr>
<td>$k_{unmod}$</td>
<td>retention factor of the solute using unmodified vesicles</td>
</tr>
<tr>
<td>L</td>
<td>length of the capillary</td>
</tr>
<tr>
<td>$L_d$</td>
<td>length to the detector</td>
</tr>
<tr>
<td>$L_{tot}$</td>
<td>total capillary length</td>
</tr>
<tr>
<td>N</td>
<td>efficiency or number of theoretical plates</td>
</tr>
<tr>
<td>$P_{int}$</td>
<td>intestinal permeability</td>
</tr>
<tr>
<td>$P_{ow}$</td>
<td>octanol water partition coefficient</td>
</tr>
<tr>
<td>$r^2$</td>
<td>the square of the correlation coefficient</td>
</tr>
<tr>
<td>R</td>
<td>resolution</td>
</tr>
<tr>
<td>$t_0$</td>
<td>migration time of a neutral solute</td>
</tr>
<tr>
<td>$t_{mc}$</td>
<td>migration time of the micelle</td>
</tr>
<tr>
<td>$t_{ves}$</td>
<td>migration time of the vesicle</td>
</tr>
</tbody>
</table>
List of LFER Analysis Symbols

\[ V \] McGowan’s characteristic volume
\[ E \] excess molar refraction
\[ S \] dipolarity/polarizability
\[ A \] hydrogen bond acidity
\[ B \] hydrogen bond basicity
\[ c \] y-intercept
\[ v \] difference in the ease of cavity formation for the solute between the buffer and the aggregate phase (vesicles)
\[ e \] difference in capacity of the buffer and the aggregate phase to interact with the solutes’ n- or \( \pi \)-electrons
\[ s \] difference in dipolarity/polarizability between the aggregate phase and the bulk aqueous phase
\[ a \] difference in hydrogen bond basicity
\[ b \] difference in hydrogen bond acidity
\[ v_u \] normalized \( v \) coefficient
\[ e_u \] normalized \( e \) coefficient
\[ s_u \] normalized \( s \) coefficient
\[ a_u \] normalized \( a \) coefficient
\[ b_u \] normalized \( b \) coefficient
### Appendix D: List of Abbreviations

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>2-amino-1-butanol</td>
</tr>
<tr>
<td>ACN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>AOT</td>
<td>bis(2-ethylhexyl)sodium sulfosuccinate</td>
</tr>
<tr>
<td>BGE</td>
<td>background electrolyte</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>CEC</td>
<td>capillary electrochromatography</td>
</tr>
<tr>
<td>CGE</td>
<td>gel electrophoresis</td>
</tr>
<tr>
<td>CIEF</td>
<td>capillary isoelectric focusing</td>
</tr>
<tr>
<td>CITP</td>
<td>capillary isotachophoresis</td>
</tr>
<tr>
<td>CMC</td>
<td>critical micelle concentration</td>
</tr>
<tr>
<td>cryo-TEM</td>
<td>cryogenic transmission electron microscopy</td>
</tr>
<tr>
<td>CTAB</td>
<td>cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>CTAC</td>
<td>cetyltrimethylammonium chloride</td>
</tr>
<tr>
<td>CZE</td>
<td>capillary zone electrophoresis</td>
</tr>
<tr>
<td>DDAB</td>
<td>didodecyl(dimethyl ammonium bromide</td>
</tr>
<tr>
<td>DDCV</td>
<td>dodecoxy carbonylvaline</td>
</tr>
<tr>
<td>DMPC</td>
<td>dimyristoyl L-α-phosphatidylcholine</td>
</tr>
<tr>
<td>DMPG</td>
<td>dimyristoyl L-α-phosphatidyl-D,L-glycerol</td>
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<td>DPPC</td>
<td>1,2-dipalmitoyl-sn-glycero-3-phosphocholine</td>
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<tr>
<td>EKC</td>
<td>electrokinetic chromatography</td>
</tr>
<tr>
<td>EOF</td>
<td>electroosmotic flow</td>
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<tr>
<td>Eq.</td>
<td>equation</td>
</tr>
<tr>
<td>ER</td>
<td>elution range</td>
</tr>
<tr>
<td>Fa</td>
<td>fraction absorbed</td>
</tr>
<tr>
<td>FASS</td>
<td>field-amplified sample stacking</td>
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<tr>
<td>FC</td>
<td>sodium perfluoro octanoate</td>
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<td>FSCE</td>
<td>free solution CE</td>
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<tr>
<td>HEPES</td>
<td>N-(2-Hydroxyethyl)piperazine-N’(2-ethanesulfonic acid)</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td>IAM</td>
<td>immobilized artificial membrane</td>
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<tr>
<td>i.d.</td>
<td>inner diameter</td>
</tr>
<tr>
<td>IHP</td>
<td>inner Helmholtz plane</td>
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<tr>
<td>ITP</td>
<td>isotachophoresis</td>
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<tr>
<td>LFER</td>
<td>linear free energy relationship</td>
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<td>LIF</td>
<td>laser induced fluorescence</td>
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<td>MDCK</td>
<td>Mardin-Darby canine kidney</td>
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<tr>
<td>MEEKC</td>
<td>microemulsion electrokinetic chromatography</td>
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<tr>
<td>MEKC</td>
<td>micellar electrokinetic chromatography</td>
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<td>NaOH</td>
<td>sodium hydroxide</td>
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<td>MS</td>
<td>mass spectrometry</td>
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<td>outer Helmholtz plane</td>
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<td>OTAB</td>
<td>octyltrimethylammonium bromide</td>
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<tr>
<td>PA</td>
<td>phosphatidic acid</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PAMPA</td>
<td>parallel artificial membrane permeability assay</td>
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<td>PC</td>
<td>phosphatidylcholine</td>
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<td>PDA</td>
<td>photo diode array</td>
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<td>1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine</td>
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<td>PG</td>
<td>phosphatidyl glycerol</td>
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<tr>
<td>PI</td>
<td>phosphatidyl inositol</td>
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<td>POPC</td>
<td>1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine</td>
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<td>PS</td>
<td>phosphatidylserine</td>
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<td>PSP</td>
<td>pseudostationary phase</td>
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<tr>
<td>SDLIL</td>
<td>sodium N-[4-n-dodecyloxybenzoyl]-L-isoleucinate</td>
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<td>SDLL</td>
<td>sodium N-[4-n-dodecyloxybenzoyl]-L-leucinate</td>
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<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
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<tr>
<td>SOS</td>
<td>sodium n-octyl sulfate</td>
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<tr>
<td>SPE</td>
<td>solid-phase extraction</td>
</tr>
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<td>SPH</td>
<td>sphingomyelin</td>
</tr>
<tr>
<td>SUVs</td>
<td>small unilamellar vesicles</td>
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<tr>
<td>UV</td>
<td>ultraviolet</td>
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<tr>
<td>VEKC</td>
<td>vesicle electrokinetic chromatography</td>
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</table>
Vita

Stephanie Ann Schuster was born in Philadelphia, Pennsylvania on June 10, 1976. She attended La Salle University on full scholarship as a member of the Honors Program where she majored in Chemistry, Biochemistry and English. Stephanie graduated cum laude with a Bachelor of Arts degree in May of 1998. She was awarded the 1997 Undergraduate Award in Analytical Chemistry and the 1998 La Salle University Department of Chemistry and Biochemistry Outstanding Graduate Award. After graduation, Stephanie attended the Pennsylvania State University for one year of graduate work in chemistry. In 2001, Stephanie enrolled in the Ph. D. program at Drexel University after taking a two year hiatus to marry and start a family. Under the direction of Dr. Joe P. Foley, Stephanie has presented her research at several meetings including, the Frederick Conference on Capillary Electrophoresis, the Mid-Atlantic Regional Meeting of the ACS, the Eastern Analytical Symposium, and HPLC 2004 and 2006. She has also participated in the ACS Graduate Student Poster Session, Drexel’s College of Arts and Sciences Research Day and Drexel’s University-wide Research Day. Stephanie has published one manuscript:


Stephanie has also submitted another manuscript to *Electrophoresis* for publication.

While at Drexel, Stephanie also had the opportunity to participate in internships at the DuPont Stine-Haskell Research Center in Newark, DE and at Cephalon in West Chester, PA. She currently lives with her husband Tom and son Aidan in Philadelphia, PA.