6-2000

Forensic applications of capillary electrophoresis

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FORENSIC APPLICATIONS OF CAPILLARY ELECTROPHORESIS

By

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Submitted in partial fulfillment
of the requirements for
Honors in the Department of Chemistry

UNION COLLEGE
June, 2000

ABSTRACT

JAKUBOWSKI, JENNIFER Forensic Applications of Capillary Electrophoresis. Department of Chemistry, June 2000.

The separation by capillary zone electrophoresis (CZE) of the chiral narcotic propoxyphene is the goal of this work. A three-way collaboration between our research group, Professor Isiah Warner (LSU), and the New York State Forensic Investigation Center is underway, with the goal of separating controlled substances and developing techniques that the Forensic Center can apply in its investigations. Earlier studies by Warner's group have shown that using polymerized N-undecylenyl-L-amino acid and peptide derivatives as pseudo-stationary phases in CZE has resulted in the separation of chiral molecules. Our separations were attempted by combining the drug samples with polymerized N-undecylenyl-L-amino acid and peptide surfactants. Successful chiral separation of (+/-) 1,1' bi-2-naphthol was achieved using polymerized N-undecylenyl-L-valine. The bulk of the studies involved the attempted chiral separation of d/l-propoxyphene using this and other polymerized micelles. Preliminary studies using α- and β-cyclodextrins as chiral selectors show partial separation of the d/l-propoxyphene enantiomers.

ACKNOWLEDGEMENTS

I would like to acknowledge Professor T. C. Werner for his incredible guidance and support throughout my thesis work. Also, this thesis work would not have been possible without the excellent instruction provided by professors in the department of chemistry at Union College.

I would like to thank the members of Dr. Adrian's lab, especially Rich Fox and Julia Barkin, for their help with the surfactant syntheses.

I would like to acknowledge Michelle Nerozzi, my thesis partner, for a wonderful year. Her enthusiasm and support made this project a very enjoyable experience.

I would also like to thank Professor Isiah Warner's group, especially Fereshteh Haddadian, for their help with the polymer surfactants. We greatly appreciated Ms. Haddadian's assistance with the polymerization of the surfactants as well as the donation of several surfactant samples.

Lastly, I would like to acknowledge the New York State Forensic Investigation Center, under the direction of Keith Coonrod. Mr. Coonrod and his co-workers provided valuable information that made our collaboration possible.

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INTRODUCTION TO CAPILLARY ELECTROPHORESIS AND CHIRAL SEPARATIONS

Capillary Electrophoresis (CE) is a separation technique originally developed in the late 1960s that is gaining popularity. In general, CE involves a buffer-filled capillary where the sample is introduced at one end and a dc potential is applied to the electrode ends. In most applications of CE, the detector is located at the negative end of the capillary such that analytes move from the positive to negative capillary end.[1] Figure 1 shows the general schematic of a CE instrument.[2]

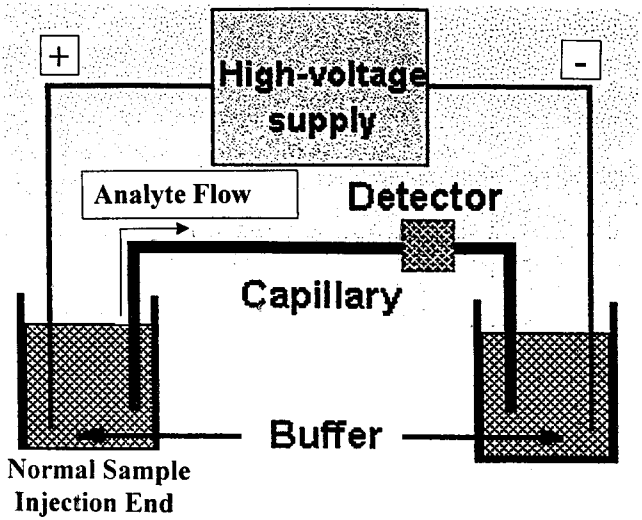


Figure 1: General Schematic of Capillary Electrophoresis Instrument[2]

The rate of migration of an analyte in a given direction depends on the charge to mass ratio, where the larger the charge to mass ratio, the faster the migration observed. This is the electrophoretic mobility (μ_e). Electrophoretic mobility and the bulk electroosmotic mobility (μ_{eof}) of the buffer solution determine the overall migration of an analyte.[1]

The electrophoretic mobility (μ_e) is represented by equation 1:

$$\mu_e = \frac{q}{6 \pi r \eta} \quad (1)$$

where q is the charge of the analyte, r is the analyte radius, and η is the viscosity of the buffer. The electrophoretic mobility is directly proportional to charge (q) and inversely proportional to analyte radius (r). Therefore, when comparing ions of the same size, those with the higher charge will migrate faster, while for ions of the same charge, the smaller ions migrate faster since less friction is experienced. Additionally, the electrophoretic mobility is zero for neutral species, positive for cationic analytes, and negative for anionic analytes. Thus, if the only component of analyte migration were the electrophoretic mobility, only positively charged species would migrate towards the detector (negative end of the capillary).[3]

The electroosmotic mobility (μ_{eof}) results from the general migration of buffer cations towards the negative cathode and allows all species (positive, negative, and neutral) to move toward the negative detector end. Equation 2 determines the electroosmotic mobility of analytes:

$$\mu_{eof} = \frac{\varepsilon_o \varepsilon \zeta}{4 \pi \eta} \quad (2)$$

where ε is the dielectric constant of the buffer, ε_o is the dielectric constant of a vacuum, ζ is the zeta potential, and again, η is the viscosity of the buffer. The zeta potential is inversely proportional to the buffer concentration, but directly proportional to the number of charges on the capillary wall.[3]

Except at low pH (< 4), the silica capillary surface achieves a negative charge due to ionization of silanol groups (Si-OH). Therefore, the buffer cations are attracted to this negatively charged capillary surface, and an electric double layer is formed at the silica/solution surface. The buffer cations remaining in solution move to the cathode, carrying the bulk solution along with them, resulting in a very flat flow profile.[1] Electroosmotic flow is represented in Figure 2:[2]

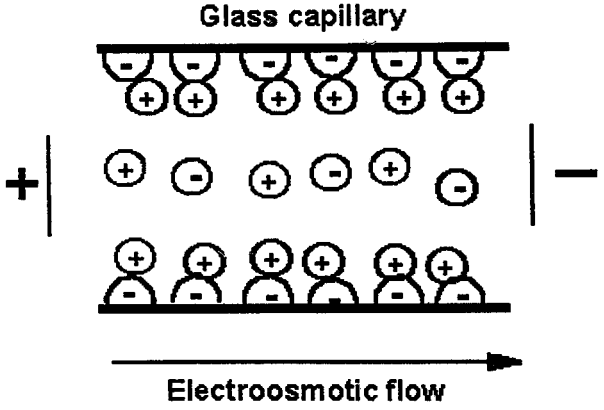


Figure 2: Electroosmotic Flow[2]

The two analyte migration components, electrophoretic and electroosmotic mobility, can be combined in order to represent the net mobility of a species (μ_{net}) as shown in the following relationships:[1]

For Cations: $\mu_{net+} = \mu_{eof} + \mu_e$ (3)

For Neutral Species: $\mu_{neto} = \mu_{eof}$ (4)

For Anions: $\mu_{net-} = \mu_{eof} - \mu_e$ (5)

Again, since the charge is equal to zero for neutral species, the μ_e is also zero and the net mobility is determined only by the μ_{eof}. For anionic species, the μ_e is subtracted from the μ_{eof}, whereas for cationic analytes the net mobility is simply the sum of the μ_{eof} and the μ_e.[1]

It is important to note that the analyte flow velocity (v_{net}) in cm/sec. is dependent on the electric field (E) as well as the net mobility (μ_{net}) according to equation 6:[1]

$$v_{net} = \mu_{net} E \quad (6)$$

The net mobility can then be determined experimentally using the following relationship:

$$\mu_{net} = v_{net} / E = (L_d / t_m) / (V / L_t) \quad (7)$$

where L_d is the length of the capillary to the detector, L_t is the total length of the capillary, V is the voltage, and t_m is the migration time.[4]

The general net mobility equation is the sum of the electrophoretic and electroosmotic mobilities, as shown below[1]

$$\mu_{net} = \mu_e + \mu_{eof} \quad (8)$$

Therefore, the net flow velocity can be expressed as in equation 9 by combining equations 6 and 8:[1]

$$v_{net} = v_e + v_{eof} = \mu_e E + \mu_{eof} E \quad (9)$$

Figure 3 represents the effects of migration on various charged and neutral species, emphasizing that positive species will migrate the fastest (since the electrophoretic and electroosmotic velocities are in the same direction). Neutral species then migrate next since the electroosmotic velocity solely determines their migration rate. Lastly, negatively charged species will have the longest migration time since the electrophoretic and electroosmotic velocities are in opposite directions.[1]

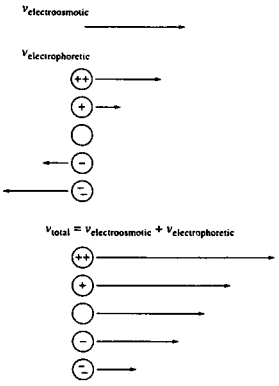


Figure 3: Total Analyte Velocities of Various Changed and Neutral Species[1]

The advantages of CE stem from the small capillary volume (on the order of microliters). As a result, there is very low solvent consumption, minimal sample losses, and a high number of theoretical plates. Also, mostly aqueous solvents are employed in CE. Very low sample volumes are needed (nanoliters); however, in order for there to be adequate detection by UV/Visible absorption the concentration must be high enough (at least about 10 ug/mL). Consequently, poor concentration limits of detection are often observed with UV detection. Techniques involving sample stacking, on-line isotachophoresis, as well as membrane preconcentration are currently being studied in order to improve the limit of detection. In our analysis, however, we are currently looking at species well above the limit of detection. We are focusing on achieving optimal resolution and separation efficiency.[1]

Capillary electrophoresis can be used to separate chiral pairs, or enantiomers, which are molecules having non-superimposable mirror images that have very similar properties. The separation of enantiomers is particularly important in the pharmaceutical industry since different enantiomers of drugs produce varying (and sometimes harmful) effects. For example, in the late 1950s a racemic mixture (containing both enantiomers) of thalidomide was taken during pregnancy. One thalidomide enantiomer was intended as a sedative, but the other enantiomer of thalidomide caused stunted limb development in utero. Thus, separation of enantiomers is vastly important in the drug industry.[5]

Chiral separations depend on enantioselective interactions with other species. Small differences in three-dimensional structure of enantiomers lead to significant differences in chemical interactions. Yet, since the chemical and physical properties of enantiomers are nearly identical in solution, the separation of enantiomers is very

challenging. β-Cyclodextrin, for example, has frequently been used to effect chiral separations since it can achieve selective complexation of enantiomers.[6] Cyclodextrins are shaped like a truncated cone with hydrophilic edges and a hydrophobic central cavity. Selective complexation of enantiomers can occur based on the size and shape of the cyclodextrin additive, resulting in chiral recognition.[4] Additionally, chiral surfactants have been used as mobile phase additives to separate species by micellar electrokinetic capillary chromatography (MECC). When chiral surfactants are added to the buffer phase, an optically active micelle is formed that will interact enantioselectively with added solutes.[7]

Two enantiomers will have the same electrophoretic mobility if they are in a nonchiral buffer. This is why it is necessary to use chiral selectors, in this case polymerized surfactants, to separate enantiomers. As shown in Figure 4, a complex is formed with the polymer and the individual enantiomers. If each enantiomer has a sufficiently different binding constant with the micelle, then chiral separation is possible since the free and complexed solutes will have different electrophoretic mobilities. Thus, optimizing the mobility difference of the enantiomers is important for the best chiral separation.[7]

Figure 4 summarizes the interaction between an analyte (S) and monomer and polymer surfactants. Figure 4a shows the monomer form of the surfactant, where a dynamic equilibrium exists between the monomer subunits and the micelle. The monomer subunits consist of a long hydrocarbon tail with a polar head group that is chiral and negatively charged. There will be a critical micelle concentration (CMC) for the monomer form below which the micelle will break apart. The chiral center on the

head group must interact with the analyte enantiomers differently in order to achieve a chiral separation. As Figure 4a shows, the solute enters quite far into the micelle cavity, making it difficult for effective interaction between the chiral solute and chiral head group. This will limit the ability to separate enantiomers as well as cause peak broadening due to slow mass transfer into and out of the micelle.[7]

Figure 4b shows the polymerized micelle where covalent bonds join monomer units together. There is an absence of the monomer subunits; therefore there is no dynamic equilibrium between monomer subunits and the micelle. Thus, the polymerized surfactant can be used at any concentration; there is no CMC. Also, the solute does not penetrate as deeply into the micelle, allowing better interaction between the chiral center of the analyte and the chiral head groups on the micelle. Since the micelle is highly negatively charged, the interaction with the solute will give it a highly negative charge. This causes the electrophoretic mobility to be negative and the net mobility to decrease. Thus, interaction of the analyte species with the micelle causes migration times to increase compared to when the analyte is alone.[7]

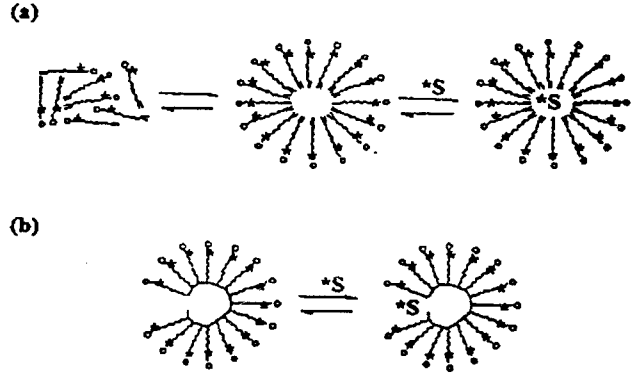


Figure 4: Diagram of Surfactant, Micelle, and Solute Interactions. (a) Normal (nonpolymerized) micelle. (b) Polymerized micelle. S, solute; asterisk denotes chiral center[7]

These chiral surfactant polymers are of interest to us because our contacts at the New York State Forensic Investigation Center are looking to develop a method to separate chiral drug species. Dr. Isiah Warner's group at Louisiana State University has extensively researched the use of polymerized micelles in capillary electrophoretic separations. Thus, a three-way collaboration was established between Dr. Warner's group at LSU, the New York State Forensic Investigation Center, and the Union College research group under the direction of Professor Werner. The main goal of this collaborated project was to develop a method for the chiral separation of various drugs using polymerized surfactants.

Dr. Warner's group notes that there are several advantages to using polymerized surfactants. First, the polymerized species are much more stable and rigid with a controllable size, such that the complexation between the solute and the micelle is enhanced. As Figure 4 illustrates, covalent bonds are formed between monomer units, eliminating the dynamic equilibrium that normally exists between the monomers and solute. As discussed above, another major advantage is that the critical micelle concentration (CMC) for the polymerized micelles is zero. For non-polymerized micelles, the concentration of the surfactant must be above the CMC to be effective; otherwise the micelle will be broken up. The micelle polymers, however, can be used at any concentration since the CMC is zero. Additionally, monomeric micelles often cause peak broadening in MECC due to a slow mass transfer rate of the solute between the micelle and solvent. The more compact structures of polymerized micelles do not allow the solute species to go very deeply into the micelle, thus creating an increased mass transfer rate.[7]

In order to understand the general procedure for using the CE system, we began with a procedure outlined in the *Journal of Chemical Education* for CE analysis of Diet Pepsi.[8] This preliminary study allowed us to explore several variables in CE. Specifically, we did an analysis of three components in Diet Pepsi: caffeine, aspartame, and benzoic acid. We sought to do a study of the effect of buffer concentration and capillary length on the appearance of peaks as well as the repeatability of migration times from injection to injection. We tried to establish the best parameters for optimization of separation in this Diet Pepsi analysis.

A CE workshop given by Susan DiPrima of Agilent Technologies on November 18, 1999 at the New York State Forensic Investigation Center provided us with additional information on how to set up our methods. Also, a trip to Louisiana State University in mid-December of 1999 allowed us to consult with Dr. Isiah Warner and Fereshteh Haddadian on our syntheses of polymerized N-Undecylenyl-L-amino acid and peptide derivatives. We ultimately sought to establish a method for separation of chiral narcotic species, using the surfactants developed by Warner's group, that the Forensic Center workers can apply to their analyses.

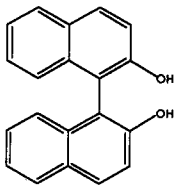
During fall term, we synthesized the monomer form of N-undecylenyl-L-valine based on a published synthesis procedure.[9] Ms. Haddadian assisted us with the polymerization of this species, using the Cobalt-60 source at LSU. Several articles published by Dr. Warner's group showed that polymerized surfactants can be used as a pseudo-stationary phase in CE analysis for the separation of chiral enantiomers.[4,7]

This report will present an explanation of the use of polymerized surfactants in chiral separations. It should be noted, however, that Michelle Nerozzi's thesis report also analyzes this aspect of our research.[10]

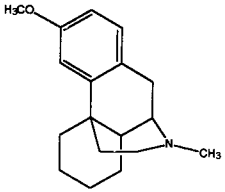
Warner's initial evaluation of the use of polymerized surfactants employed poly-(sodium N-undecylenyl-L-valinate) (poly-(LSUV)) for the chiral separation of (+/-)-1,1'-bi-2-naphthol (BOH). We used the parameters outlined in Warner's paper for our first analysis using this polymerized surfactant. We looked at the effects of changing surfactant concentration, temperature, and voltage on the separation efficiency and resolution of (+/-)-1,1'-bi-2-naphthol to establish the optimal conditions for separation of the two BOH enantiomers. The structure of BOH is shown in Figure 5.[7]

After this preliminary study using poly-(LSUV), we wanted to begin chiral analysis of narcotic species, including methorphan and propoxyphene. Dextromethorphan is the main active ingredient in many over-the-counter cough syrups. Since we were unable to obtain a sample of levomethorphan, a chiral separation study of methorphan could not be performed.

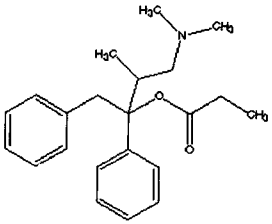
Both d- and l-propoxyphene are available commercially as the napsylate salts. The d-propoxyphene enantiomer is an illegal substance, while the l- form is available over-the-counter. This is one of the reasons the New York State Forensic Investigation Center wanted a method developed for the chiral separation of propoxyphene. In order for the caseworkers to determine if a substance was used illegally, they need to be able to separate the legal l-form from the illegal d-form. Propoxyphene napsylate (d-form) is an analgesic commercially known as Darvon for the relief of mild to moderate pain.[11] The structures of the chiral drugs are as shown in Figure 5.[12, 13]



Binaphthol (BOH)[7]



Methorphan[13]



Propoxyphene[12]

Figure 5: Figure of Analytes Used in Polymer Surfactant Analysis

For the analysis of the two chiral forms of propoxyphene, we tried several different conditions in order to achieve a chiral separation, including changing the buffer pH, using different buffers (borate buffer, Tris buffer, and phosphate buffer), changing poly-(LSUV) concentration, and adding CAPS, as recommended by many of Warner's articles. Since the propoxyphene is positively charged at most of the pHs we used, adsorption of these cations onto the negatively-charged capillary wall causes peak broadening. CAPS is added in order to minimize this adsorption and sharpen peaks.[14] Michelle Nerozzi's thesis looks at the effect of these parameter changes on the attempted chiral separation and peak shape of the propoxyphene enantiomers using poly-(LSUV).[10]

Based on Warner's articles, it seemed that the placement of a bulkier amino acid on the surfactant would yield better enantiomeric separation.[15] Therefore, we decided to synthesize poly-N-undecylenyl-L-*tert*-leucine (poly-(LSUTBL)), whose bulky amino acid group might be expected to achieve better enantiomeric resolution of the propoxyphene species. The synthesis was performed using the synthetic route described in the literature, as well as an addendum to the procedure developed by Fereshteh Haddadian at LSU.[9] Ms. Haddadian also polymerized our synthesized monomers using the Cobalt-60 gamma radiation source at LSU. Michelle Nerozzi's thesis analyses in more detail the synthesis of the poly-N-undecylenyl-L-*tert*-leucine species.[10]

We also decided to synthesize poly-N-undecylenyl-L-leucine-L-valine (poly-(L,L)-SULV)) since several articles published by Warner's group indicated the merit of this surfactant for chiral separations. For example, Warner showed that the poly-(LSULV) provided much better chiral resolution than poly-(LSUVL) for bi-2-naphthol enantiomers.[16] Furthermore, it should be noted that the poly-(LSULV) contains two

chiral centers. Michelle Nerozzi's thesis analyses in more detail the synthesis of the poly-(LSULV) surfactant.[10]

A paper written by Dr. Warner's group (not yet published) looked at the effect of number and placement of chiral centers, amino acid order, and steric effects. In this paper three cationic β-blockers (propranolol, alprenolol, and oxprenolol) are analyzed with nineteen various polymerized dipeptide species. It was found that an increase in the size of the R-group on the C-terminal amino acid (the outer amino acid) resulted in improved enantiomeric resolution of most of the β-blocker species. It is also concluded that the β-blockers bind preferentially to the C-terminal (outer) amino acid.[15] We therefore realized that while Warner showed an improved resolution with the poly-(LSULV) surfactant versus the poly-(LSUVL) surfactant for the bi-2-naphthol enantiomers, separation of our propoxyphene enantiomers would be more likely to occur with poly-(LSUVL). The poly-(LSUVL) has a larger C-terminal (outer) amino acid which is expected to produce better enantiomeric separation of d/l-propoxyphene since the propoxyphene has a similar structure to the β-blocker species.[15] Since the synthesis of poly-(LSULV) was already underway, we decided to attempt separation of our propoxyphene species with the poly-(LSULV) surfactant despite Warner's results.

Using the three synthesized polymers, we focused on achieving chiral separation of d- and l-propoxyphene. We also obtained three samples of surfactants, namely, poly-N-undecylenyl-L-glycine-alanine (poly-(LSUGA)), poly-N-undecylenyl-L-glycine-valine (poly-(LSUGV)), and poly-N-undecylenyl-L-valine-L-leucine (poly-(LSUVL)), which were recommended by Ms. Haddadian (LSU) for the chiral separation of the propoxyphene species. Ms. Haddadian noted that the difference in our propoxyphene

species and the β-blockers she studied was that propoxyphene has no possible hydrogen bond donor sites, whereas the β-blockers have an –OH group at the chiral center. She felt that since our analyte interacts with the micelle surface, surfactants with an achiral N-terminal amino acid (inner amino acid) and a chiral C-terminal amino acid (outer amino acid) could possibly separate the propoxyphene enantiomers. Thus, she recommended using poly-(LSUGA) and poly-(LSUGV).

This report contains the results of the analysis using poly-(LSULV) for the separation of bi-2-naphthol and d/l-propoxyphene as well as the results for using poly-(LSUTBL) for the attempted separation of the propoxyphene enantiomers. Michelle Nerozzi's thesis[10] reports the results of propoxyphene analysis using poly-(LSUV), poly-(LSUVL), poly-(LSUGA), and poly-(LSUGV).

In addition to the chiral analyses performed using polymerized chiral surfactants, preliminary studies were done using α-cyclodextrin, sulfated β-cyclodextrin, and methylated β-cyclodextrin as mobile phase additives. Further studies using cyclodextrins are currently underway.

In the future, the chiral separation of propoxyphene and other species will be analyzed. We are hopeful that eventually a chiral separation will be achieved using some of the surfactant species. It has also been proposed that the combination of surfactants and cyclodextrins in the buffer could separate the enantiomers of propoxyphene.

EXPERIMENTAL

Chemical and Reagent Sources:

Caffeine was obtained from Merck and Company. Aspartame (L-Aspartyl-L-phenylalanine methyl ester), benzoic acid, *tert*-leucine, boric acid, and N-hydroxysuccinimide were obtained from Aldrich Chemical Company. The L-valine used for our synthesis as well as Trizma base was obtained from Sigma Chemical Company. ICN Biomedicals Inc., supplied the L-leucyl-L-valine used in the poly-(LSULV) synthesis. CAPS (3-cyclohexylamino-1-propane sulfonic acid), a buffer additive, was purchased from FisherBiotech Company.

Poly-(LSUV), poly-(LSUTBL), and poly-(LSULV) were synthesized in our laboratory. Poly-(LSUVL), poly-(L-SUGA), and poly-(L-SUGV) were synthesized and provided by Dr. Warner's group at LSU. The 1,1'-bi-2-naphthol was obtained from Aldrich Chemical Company. The d- and l-propoxyphene napsylate were both obtained from USP chemicals. The α-cyclodextrin was obtained from Wacker. The sulfated β-cyclodextrin was obtained from Amaizo. Methylated β-cyclodextrin was obtained from Cerestar.

General Sample Preparation:

Since the bulk of the CE process is automated, the experimental procedure mainly involves sample preparation. CE requires that samples be very pure and filtered. Samples were prepared in high quality water obtained from a Millipore water system, then filtered through a 0.22 μm membrane. Thoroughly washed glassware rinsed with this water was used for samples. The solids were massed on an analytical balance and

volumetric flasks were used. The samples were then filtered again through a 0.2 μm syringe filter tip and put into clean, new plastic vials for every run.

Buffer Preparation and Handling:

Preparation of the buffers involved an equal amount of care, using the Millipore/filtered water and volumetric flasks. The acid form of the buffers was carefully weighed on a balance and then the buffer was diluted to about 80% of the volume with water. A calibrated pH meter was used to ensure that the pH of the buffer was accurate. The pH was adjusted with the addition of 1.0 M NaOH, the final dilution was made, and the pH re-tested. Since CE is so pH dependent, it is very important to make sure the pH of the buffer is consistent. Furthermore, to help keep the buffers fresh, they were stored in the cold room at 4 °C. Fresh buffer solutions were made up about every two weeks.

When ready for a run, the buffers used were warmed to room temperature, filtered again through the 0.2 um filter syringes into the vials, and placed in the CE tray. The buffer vials were replaced every four injections with fresh buffer. We considered using the buffer replenishment system so that we would not have to manually change the buffer vials every sequence. But the replenishment system required a large volume of buffer. Since we had a limited amount of our surfactants, we chose not to use this feature. Our first goal was to find the "ideal" buffer conditions to maximize separation and migration time repeatability.

General CE Instrument Maintenance:

A Hewlett-Packard HP-3D Capillary Electrophoresis System was employed throughout our thesis work. Maintenance of the CE instrument involves cleaning the electrodes and pre-punchers when necessary. At the CE workshop, it was recommended that the electrodes be cleaned when a yellow bar appears for the current. This indicates that there is a leakage current (the current is too high), most likely resulting from salt buildup on the electrodes.[3] Additionally, the lamp needs to be replaced after approximately 1000 hours of use.[17] At one point, we started having difficulty igniting the lamp; it would often take up to five attempts to successfully ignite the lamp. We discovered that the lamp had been used for over 900 hours and thus, we determined that we should replace the lamp.

Daily Instrument and Software Use:

To use the CE instrument, the power and the lamp are turned on (and allowed to warm up for about 30 minutes). The system is initialized and the method parameters are defined, depending on the analysis.

Properly saving the data in an organized fashion is vastly important. We found it very useful to put ample information in the "sample information" box since this is printed out on the electropherograms. We noted the concentrations of components, buffer strength, as well as any other parameters that we changed. Furthermore, it is important to change the file name under "sequence parameters" by creating a new subdirectory for each run (for example, ABC1, ABC2, etc.) in order to ensure that sample files were not

erased. Once the method is saved (with the change noted in the method name box that appears), the injection sequence is ready to run.

For preliminary analyses, we also determined how to turn off the integration feature. We were initially concerned with achieving separation, and not with specific migration times or peak areas. In order to conserve paper, we set the integration to the off position in the "Integration Events" table of the "Edit Integration Events" window. When we found the desired separation, the integration was turned back on. Additionally, we found it best to set the events table as "DAD default." We also learned how to turn off the tick marks, migration time, and baseline calculations in order to produce cleaner electropherograms by selecting the "Signal Options" feature on the "Specify Report" window of the method development.

Diet Pepsi Analysis:

Solutions of each of the single components were prepared and injections run in order to determine the elution order as well as migration times for the three species. As outlined in the *Journal of Chemical Education* article,[8] the following concentration solutions were made:

Component	Concentration, mg/l
Caffeine	101.8
Benzoic Acid	78.6
Aspartame	156.9

A borate buffer solution was made (25 mM concentration, pH 9.4). Calculations for the amount of boric acid, caffeine, aspartame, and benzoic acid used in the buffers and samples can be found in my lab notebook.

The method parameters defined were generally kept the same as in the Installation Qualification for the Diet Pepsi analysis. The only significant changes made were to set the wavelengths to correspond to the maximum absorbances of the three components and to change the voltage to 20 kV. The wavelengths used were 272 nm (caffeine), 229 nm (benzoic acid), and 210 nm (aspartame). After the vials were placed in the tray as shown in Table 1, injection sequences could begin.[17]

Table 1: Table of vials used

Vial #	Solution
1	1.0 N NaOH, wash
2	Water
3	---
4	Borate buffer
5	Borate buffer
6	Borate buffer
7	Sample
8	Waste, empty vial

Several runs were then completed with all three components, varying the buffer strength as well as the capillary length. The percent relative deviations of migration times from run to run were calculated in order to determine the best parameters for analysis.

Lastly, Diet Pepsi samples were studied using a calibration table constructed based on the single injections of caffeine, aspartame, and benzoic acid. An injection sequence was set up using the sequence mode of the software. A table was constructed where we put four injections per vial (#7). Then, the amounts of aspartame, benzoic acid, and caffeine could be calculated from the known amounts in the standards.

Binaphthol Analysis with poly-(LSUV):

At the CE workshop, we learned that the 1.0 N NaOH flush we were using as part of our preconditioning step was unnecessary, and only needed for conditioning a new column. We therefore changed the method to only have a four-minute buffer flush as the preconditioning step.

The experimental procedure for the chiral separation of (+/-)-1,1'-bi-2-naphthol (BOH) was performed as outlined in the *Advances in Chromatography*[4] as well as Warner and Wang's article.[7] A 25 mM borate buffer solution with pH 9.0 was prepared and used for all of the binaphthol analyses. Several solutions of varying concentrations (0.05%, 0.1%, 0.2%, and 0.5%) of poly-(LSUV) were prepared. (The synthesis procedure of this surfactant is explained in detail in Michelle Nerozzi's thesis.)[10] A 0.1 mg/ml solution of (+/-)-1,1'-bi-2-naphthol in 70% methanol / 30% water was prepared. All calculations for these solutions can be found in the CE notebook. The following table summarizes the parameters set up under the BI-NAPH.M method:

Table 2: Table of Parameters used in BOH Separation Study with poly-(LSUV)

Parameter	
Pressure injection	50 mbar for 4 seconds
Capillary Length and ID	64.5 cm long and 50 um ID
Capillary Temperature	20 °C or 25 °C, as noted
Detection Wavelength	290 and 210 nm
Voltage	12 kV or 20 kV, as noted

A study of the effects of changing the LSUV concentration on the separation of (+/-)-1,1'-bi-2-naphthol was performed. Later, a study of the effects of temperature and voltage changes was completed in order to find the optimal conditions for separation.

Additionally, the purpose of the study was to show that our synthesis of poly-(LSUV) was successful by achieving a chiral separation of the BOH enantiomers.

Tables of the percent relative standard deviation of the migration times from run to run as well as injection to injection were created. Resolution data were compiled and tables created showing these data. The resolution was calculated using the following formula, where t is the migration time and W is the width of the peak at the base:[17]

$$R = 2 * (t_2 - t_1) / (W_1 + W_2) \quad (10)$$

An R value of greater than or equal to 1.5 indicates baseline resolution. The %RSD data and resolution data collected were ultimately used to determine the optimal parameters for peak resolution and separation of (+/-)-1,1'-bi-2-naphthol.

Binaphthol Analysis with poly-(LSULV):

As explained in the introduction, the experimental procedure included the synthesis of poly-N-undecylenyl-L-leucine-valine (poly-(LSULV)), and poly-N-undecylenyl-L-*tert*-leucine as well. These synthesis procedures are explained in detail in Michelle Nerozzi's thesis.[10]

The next part of the experimental procedure involved the attempted chiral separation of (+/-)-1,1'-bi-2-naphthol with our synthesized poly-(LSULV). A 25 mM borate buffer solution with pH 9.0 was prepared and used for all of the binaphthol analyses. Several solutions of varying concentrations (0.005%, 0.01%, 0.025%, 0.05%, 0.1%, 0.3%, and 1.0%) of poly-(LSULV) were prepared. A 0.5 mg/ml solution of (+/-)-1,1'-bi-2-naphthol in 70% methanol / 30% water was prepared. All calculations for these

solutions can be found in the CE notebook. The following table summarizes the parameters set up under the BI-NAPH.M method:

Table 3: Table of Parameters used in BOH Separation Study with poly-(LSULV)

Parameter	
Pressure injection	50 mbar for 4 seconds
Capillary Length and ID	64.5 cm long and 50 um ID
Capillary Temperature	20 °C
Detection Wavelength	290 and 210 nm
Voltage	20 kV

Then a study of the effects of changing the LSULV concentration on the separation of (+/-)-1,1'-bi-2-naphthol was performed. Tables of the migration times, standard deviations, and percent relative standard deviations of the migration times from injection to injection were created. Additionally, the average peak widths were calculated in order to assess where the maximum interaction with the surfactant occurred.

D/L-Propoxyphene Analysis with poly-(LSULV):

Using our synthesized poly-(LSULV), we attempted chiral separation of d/l-propoxyphene. A 25 mM borate buffer solution (pH 8.5) containing 300 mM CAPS (to prevent adsorption of propoxyphene cations onto capillary wall) and varying concentrations (0.01% and 0.025%) of poly-(LSULV) were prepared. A 0.5 mg/ml solution of d/l-propoxyphene napsylate in 30% methanol / 70% water was prepared. All calculations for these solutions can be found in the CE notebook. The following table summarizes the parameters set up under the PROPOX.M method:

Table 4: Table of Parameters used in d/l-Propoxyphene Separation Study with poly-(LSULV)

Parameter	
Pressure injection	50 mbar for 4 seconds
Capillary Length and ID	64.5 cm long and 50 um ID
Capillary Temperature	25 °C
Detection Wavelength	220 and 260 nm
Voltage	20 kV

A limited study of the effects of changing the LSULV concentration on the separation of d/l-propoxyphene was performed. Tables of the migration times, standard deviations, and percent relative standard deviations of the migration times from injection to injection were created. Lastly, the average capacity factor, k', was calculated as follows:

$$k' = (t_m - t_o) / t_o \qquad (11)$$

where t_m is the migration time of the analyte species and t_o is the migration time of methanol, which is a neutral species carried through the capillary only by the electroosmotic mobility. The k' value was used as a measure of the interaction of propoxyphene with the surfactant, where larger k' values indicated more interaction.[1]

D/L-Propoxyphene Analysis with poly-(LSUTBL):

The chiral separation of d/l-propoxyphene was attempted using our synthesized poly-(LSUTBL). Several different 25 mM borate buffer solutions containing 300 mM CAPS were prepared at various pHs (pH 8.2 and pH 10.2) as well as with varying concentrations of poly-(LSUTBL) (0.00%, 0.01%, 0.05%, and 0.1%). A 0.5 mg/ml solution of d/l-propoxyphene in 30% methanol / 70% water was prepared. All

calculations for these solutions can be found in the CE notebook. The following table summarizes the parameters set up under the PROPOX.M method:

Table 5: Table of Parameters used in d/l-Propoxyphene Separation Study with poly-(LSUTBL)

Parameter	
Pressure injection	50 mbar for 4 seconds
Capillary Length and ID	64.5 cm long and 50 um ID
Capillary Temperature	25 °C
Detection Wavelength	220 and 260 nm
Voltage	20 kV

Several different studies were performed using the poly-(LSUTBL). First a study of the effect of changing the poly-(LSUTBL) concentration and pH on the separation of d/l-propoxyphene was performed. Tables of the migration times, standard deviations, and percent relative standard deviations of the migration times from injection to injection were created. Again, the average capacity factor, k', was included to compare the degree of propoxyphene/surfactant interaction.

Next, a 75 mM borate buffer solution was prepared (pH 10.2) containing 300 mM CAPS and various concentrations of poly-(LSUTBL) including 0.00%, 0.01%, and 0.05%. The average migration times, standard deviations, percent relative standard deviations, and capacity factors were tabulated. These data were compared to the same data taken with the 25 mM borate buffer in order to establish the effect of increasing buffer concentration on migration time data.

Lastly, various concentrations of d/l-propoxyphene napsylate (0.5 mg/ml, 0.125 mg/ml, and 0.063 mg/ml) were prepared. Migration time as well as peak width data were

collected and tabulated in order to complete a study of the effect of analyte concentration on peak width and peak shape.

Michelle Nerozzi's thesis[10] reports the results of propoxyphene analysis using poly-(LSUV), poly-(LSUVL), poly-(L-SUGA), and poly-(L-SUGV).

D/L-Propoxyphene Analysis using α- and β-Cyclodextrins:

In addition to the chiral analyses performed using polymerized chiral surfactants, preliminary studies were done using α-cyclodextrin, sulfated β-cyclodextrin as well as methylated β-cyclodextrin as mobile phase additives. This report contains tables of migration time data and resolution values for varying concentrations of methylated β-cyclodextrin (10, 20, and 30 mM). Michelle Nerozzi's thesis[10] reports the results of propoxyphene analysis using the α-cyclodextrin.

RESULTS

Diet Pepsi Analysis:

The following tables show the results of the CE analysis for our Diet Pepsi study. We prepared standards containing three components found in Diet Pepsi—caffeine (101.8 mg/l), aspartame (156.9 mg/l), and benzoic acid (78.6 mg/l). Since we were attempting to reproduce a study reported in the literature, we used the concentrations noted.[8] Originally, we were hoping to quantitate the amount of the three components in a Diet Pepsi sample by using a calibration table constructed from our standards. Since this was our first study using the new CE instrument, we focussed more on general trends that we observed than the quantitation of the Diet Pepsi.

We reported the migration times, standard deviations as well as the percent relative standard deviations of the migration times. Although a lot of data were collected, only a few tables will be presented with the major trends. We mainly studied how the migration times varied from injection to injection with different column length and buffer concentrations. We also optimized some aspects of our capillary preconditioning method.

Four injections per vial were taken and the results averaged. The migration time data of these averages are reported in the tables. Table 6 presents the effects on the migration times when the column length is changed.

Table 6: Effect of Capillary Length on the Migration Times (t_m) of Caffeine, Aspartame, and Benzoic Acid*

Capillary Length, cm	t_m (Caffeine), min. %RSD	t_m (Aspartame), min. %RSD	t_m (Benzoic Acid), min. %RSD
40.0	2.431 (+/-) 0.015 %RSD = 0.62 %	3.281 (+/-) 0.036 %RSD = 1.1 %	4.207 (+/-) 0.072 %RSD = 1.7 %
64.5	4.334 (+/-) 0.044 %RSD = 1.0 %	5.856 (+/-) 0.138 %RSD = 2.35 %	7.507 (+/-) 0.250 %RSD = 3.32 %
80.5	7.615 (+/-) 0.145 %RSD = 1.91 %	10.345 (+/-) 0.312 %RSD = 3.01 %	13.374 (+/-) 0.539 %RSD = 4.03 %

*The following conditions were employed in all runs in Table 6:

Voltage = 20 kV

Temperature = 20°C

Capillary Dimensions = L = 40.0 to 80.5 cm, as noted; ID= 50 μm

Buffer = 25 mM Borate Buffer, pH 9.4

Table 6 shows several trends that exist when the capillary length increases. The migration time of all components increase with a longer capillary, clearly since it will take species longer to migrate through a longer capillary. Also, there is a decrease in the reproducibility of migration times with the longer capillary and also with longer migration times. The recorded %RSD values are overall very high, since the normal %RSD values expected, according to Agilent Technologies literature, are between 0.1 and 0.3%.[3] We later learned at the CE workshop that this is likely because we were doing a 1.0 N NaOH wash between every injection. The NaOH wash is intended to condition a new column. As the studies done after the Diet Pepsi analysis will show, elimination of this NaOH wash decreased our %RSDs to within the acceptable range

Table 7 shows the effects of changing the buffer concentration on the migration times of the three Diet Pepsi components.

Table 7: Effect of Buffer Strength on the Migration Times (t_m) of Caffeine, Aspartame, and Benzoic Acid*

Buffer Strength, mM	t_m (Caffeine), min. %RSD	t_m (Aspartame), min. %RSD	t_m (Benzoic Acid), min. %RSD
20	6.881 (+/-) 0.109 %RSD = 1.59 %	9.277 (+/-) 0.377 %RSD = 4.06 %	11.835 (+/-) 0.692 %RSD = 5.85 %
25	7.615 (+/-) 0.145 %RSD = 1.91 %	10.345 (+/-) 0.312 %RSD = 3.01 %	13.374 (+/-) 0.539 %RSD = 4.03 %
50	8.733 (+/-) 0.090 %RSD = 1.0 %	12.216 (+/-) 0.214 %RSD = 1.75 %	16.665 (+/-) 0.390 %RSD = 2.34 %

*The following conditions were employed in all runs in Table 7:

Voltage = 20 kV

Temperature = 20°C

Capillary Dimensions = L = 80.5 cm; ID= 50 μm

Buffer = 20-50 mM Borate Buffer, as noted; pH 9.4

Table 7 again shows that the %RSDs are higher than expected which was caused by the NaOH wash between injections. The 50 mM borate buffer resulted in the most reproducible migration times, shown by the lower %RSD. Additionally, the overall run time increases with increasing buffer concentration since high buffer concentrations produce a lower electroosmotic flow, which will move the analyte components through the capillary more slowly, making the run time longer.

After the analysis of the aspartame, caffeine, and benzoic acid, a calibration table was constructed that could be used to quantitate the amount of these components in a

Diet Pepsi sample. Three different samples were run with four injections each. Table 8 shows the results of this study with the averages of the four injections presented.

Table 8: Table of expected Diet Pepsi Component Concentrations and Calculated Concentrations*

Sample #	Average Caffeine amt. Found (mg/L)	Average Aspartame amt. Found (mg/L)	Average Benzoic Acid found (mg/L)
1 (40 cm capillary)	87.97	456.7	157.6
2 (40 cm capillary)	75.38	335.5	123.2
3 (80.5 cm capillary)	90.09	252.6	330.9
Expected Concentrations[8]	89	162	475

*The following conditions were employed in all runs in Table 8:

Voltage = 20 kV

Temperature = 20°C

Capillary Dimensions = L = 40.0 cm or 80.5 cm, as noted; ID= 50 μm

Buffer = 25 mM Borate Buffer, as noted; pH 9.4

The calculated concentrations of the caffeine were the closest to the accepted value, while the calculated concentrations of aspartame and benzoic acid were not at all close to the accepted value. We did not analyze these data in great detail, however, since the main goal of this preliminary Diet Pepsi experiment was to learn basic CE principles.

Bi-2-Naphthol Separation Using Poly-(LSUV):

Wang and Warner reported the successful chiral separation of (+/-)-1,1'-bi-2-naphthol (BOH) using the polymerized surfactant, poly-N-undecylenyl-L-valine.[7] We sought to reproduce this study, with a few modifications, in order to evaluate the effectiveness of our polymerized LSUV in chiral separations. Our goal was to vary certain parameters, including poly-(LSUV) concentration, temperature, and voltage in order to optimize the conditions. The following tables represent the observed effects on migration time as well as on the enantiomeric resolution, as the various parameters were changed. Each run consists of a set of four injections from the same vial; separate runs indicate that different sample vials were used. The migration time data are given in the first group of tables for the different surfactant concentrations, voltages, and temperatures. Then, the second group of tables looks at the resolution data of all the set sequences.

Table 9 shows the effect of increasing poly-(LSUV) concentration on the migration times of the bi-2-naphthol enantiomers. Each sample is an identical solution of 0.5 mg/ml (+/-)-1,1'-bi-2-naphthol in 70% methanol / 30% water mixture. Four injections were performed using each sample and the results averaged. Migration times are recorded in minutes. The standard deviation and percent relative standard deviation (%RSD) of the migration times are also included in the tables.

Table 9: Effects of Increasing % poly-(LSUV) on Migration Times (t_m) of (+/-)1,1' Bi-2-Naphthol*

% poly-(LSUV)	t_m Values for:	Sample # 1	Sample # 2	Sample # 3	Sample # 4
0.05%	(-) BOH	10.962	10.989	10.984	11.027
	Std. Dev.	0.061	0.018	0.012	0.010
	%RSD	0.56	0.16	0.11	0.092
	(+) BOH	11.304	11.337	11.329	11.374
	Std. Dev.	0.067	0.020	0.015	0.012
	%RSD	0.59	0.18	0.13	0.11
0.1%	(-) BOH	12.540	12.622	12.676	12.726
	Std. Dev.	0.037	0.026	0.024	0.011
	%RSD	0.30	0.21	0.19	0.089
	(+) BOH	12.905	12.991	13.050	13.103
	Std. Dev.	0.038	0.029	0.026	0.012
	%RSD	0.30	0.22	0.20	0.093
0.2%	(-) BOH	14.627	14.685	14.685	14.774
	Std. Dev.	0.020	0.023	0.036	0.030
	%RSD	0.14	0.16	0.24	0.20
	(+) BOH	14.931	14.989	14.998	15.101
	Std. Dev.	0.021	0.028	0.042	0.033
	%RSD	0.14	0.18	0.28	0.22
0.5%	(-) BOH	18.558	18.674	18.818	18.963
	Std. Dev.	0.020	0.054	0.050	0.059
	%RSD	0.11	0.29	0.27	0.31
	(+) BOH	18.801	18.922	19.063	19.209
	Std. Dev.	0.019	0.048	0.053	0.057
	%RSD	0.10	0.25	0.28	0.30

*The conditions employed were as follows for the data in Table 9:

Voltage = 12 kV

Temperature = 20°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.0 containing 0.05 to 0.5 % poly-(LSUV)

The data in Table 9 present a trend that occurs when the concentration of the poly-(LSUV) is varied. There is a definite increase in the migration times of both enantiomers of BOH with an increase in surfactant concentration. This is expected since a greater micelle concentration will more effectively bind with the analyte, carrying the analyte more slowly through the capillary. Thus, it will take longer for analyte species to reach the detector and the migration time increases. Also, the fact that the chiral isomers of BOH were separated indicates that they bind differentially to the surfactant.

Table 10 shows the overall average data for the migration times of (+/-)1,1' bi-2-naphthol based on Table 9. The average migration time, standard deviation, and percent relative standard deviation of the four samples are presented for each poly-(LSUV) concentration.

Table 10: Summary of the Effect of poly-(LSUV) Concentration on 1,1'-Bi-2-Naphthol Migration Time

% poly-(LSUV)	t_m avg. (-) BOH (Std. Dev.), %RSD	t_m avg. (+) BOH (Std. Dev.), %RSD
0.05	10.990 (+/- 0.027) 0.25%	11.336 (+/- 0.029) 0.26%
0.1	12.641 (+/- 0.080) 0.63%	13.012 (+/- 0.085) 0.65%
0.2	14.693 (+/- 0.061) 0.41%	15.005 (+/- 0.071) 0.47%
0.5	18.753 (+/- 0.176) 0.94%	18.999 (+/- 0.176) 0.93%

It is important to note that Table 9 highlights the intrasample precision, whereas Table 10 shows the precision of intersample runs. According to literature from the Agilent Technologies CE workshop, the %RSDs are generally expected to be 0.1 to 0.3% within a given data set.[3] Table 9 indicates that within a given sample the %RSD is in this

expected range, while Table 10 shows that, between samples, the %RSDs are a bit higher than this expected range. This is not surprising since we have noted a general increase in migration times from injection to injection as well as sample to sample. This indicates an inherent problem in CE, whereby the initial equilibrium conditions are not fully re-established before the next sample is run. Perhaps increasing the time that the capillary is preconditioned with the buffer could alleviate this problem to an extent. However, we found that based on Warner's articles, a four-minute buffer preconditioning step was sufficient.[15] Since the focus of our project is on chiral separation, we did not attempt to optimize this parameter.

The next table shows the effects of increasing voltage to 20 kV and of increasing temperature from 20°C to 25°C on bi-2-naphthol migration times for buffers containing 0.05%, 0.1%, 0.2%, and 0.5% poly-(LSUV). The reported values in each case are overall averages of quadruplicate injections on two different sample vials.

Table 11: Effects Changing Temperature and Voltage on Migration Times of (+/-)1,1' bi-2-naphthol with Increasing Poly-(LSUV) Concentrations*

% poly-(LSUV)	Temperature, °C	t_m avg. [(-) BOH] (+/- Std. Dev.)	t_m avg. [(+) BOH] (+/- Std. Dev.)
0.05%	20	6.535 (+/- 0.001) %RSD = 0.01	6.742 (+/- 0.003) %RSD = 0.05
	25	5.791 (+/- 0.001) %RSD = 0.02	5.962 (+/- 0.001) %RSD = 0.01
0.1%	20	7.749 (+/- 0.030) %RSD = 0.38	7.981 (+/- 0.029) %RSD = 0.35
	25	6.738 (+/- 0.018) %RSD = 0.26	6.937 (+/- 0.016) %RSD = 0.23
0.2%	20	8.829 (+/- 0.028) %RSD = 0.32	9.023 (+/- 0.031) %RSD = 0.34
	25	7.728 (+/- 0.011) %RSD = 0.14	7.900 (+/- 0.010) %RSD = 0.12
0.5%	20	11.216 (+/- 0.073) %RSD = 0.65	11.367 (+/- 0.072) %RSD = 0.63
	25	10.312 (+/- 0.045) %RSD = 0.44	10.452 (+/- 0.044) %RSD = 0.42

*The following conditions were employed in all runs in Table 11:

Voltage = 20 kV

Temperature = 20°C or 25°C, as noted

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.0 containing 0.05 to 0.5 % poly-(LSUV)

Table 11 shows that increasing temperature had a positive effect on the precision of the migration times from sample to sample. The %RSDs were consistently lower at 25 °C than at 20 °C. Additionally, the migration times of both enantiomers of BOH decreased with an increase in temperature. This is expected since an increase in temperature generally causes the viscosity of the buffer to decrease, increasing the

electroosmotic mobility, and moving analyte species to the detector more quickly. Additionally, we predict that complexation of the BOH with the surfactant is strengthened at lower temperatures, which results in longer migration times at lower temperatures as observed. Also, the migration times increase with an increase in poly-(LSUV) concentration. Again, this is because a higher surfactant concentration will result in more interaction with the BOH enantiomers, causing an increase in migration time of both enantiomers. The BOH enantiomers were bound differentially to the surfactant, resulting in chiral separation.

Bi-2-Naphthol Separation Using Poly-(LSUV) Resolution Data:

The following tables show the resolution data calculated for the bi-2-naphthol sample runs. The resolution is calculated based on the width of the peak base (W) and the migration times (t_m) as shown in equation 10. A value for resolution greater than or equal to 1.5 indicates baseline resolution of the peaks, where higher R values indicate better peak separation.

Table 12 shows the effect of increasing poly-(LSUV) concentration on the resolution of the bi-2-naphthol enantiomers. Each sample is an identical solution of 0.5 mg/ml (+/-)-1,1'-bi-2-naphthol in 70% methanol / 30% water mixture. Four injections were performed using each sample and the results averaged. The table contains the calculated resolution values and standard deviations.

Table 12: Effects of Changing poly-(LSUV) Concentration on Resolution (R) of (+/-)1,1' Bi-2-Naphthol*

% poly-(LSUV)	Sample # 1 Resolution	Sample # 2 Resolution	Sample # 3 Resolution	Sample # 4 Resolution	Overall Avg. Resolution
0.05%	3.359 (+/- 0.115)**	3.269 (+/- 0.131)	3.243 (+/- 0.159)	3.108 (+/- 0.135)	3.245 (+/- 0.110)
0.1%	3.349 (+/- 0.159)	3.298 (+/- 0.041)	3.508 (+/- 0.228)	3.393 (+/- 0.225)	3.387 (+/- 0.158)
0.2%	2.583 (+/- 0.059)	2.502 (+/- 0.159)	2.511 (+/- 0.127)	2.665 (+/- 0.212)	2.565 (+/- 0.094)
0.5%	1.881 (+/- 0.039)	1.920 (+/- 0.066)	1.935 (+/- 0.060)	1.966 (+/- 0.068)	1.925 (+/- 0.048)

* The conditions employed were as follows:

Voltage = 12 kV

Temperature = 20°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.0 containing 0.05 to 0.5 % poly-(LSUV)

**** Sample Calculation for Resolution of Sample #1, injection #1 for poly-(LSUV) = 0.05%:**

$$R = 2 * (t_2 - t_1) / (W_1 + W_2)$$

$$R = 2 * (11.221 \text{ min.} - 10.890 \text{ min.}) / (0.0774 \text{ min.} + 0.1206 \text{ min.})$$

$$R = 3.343, \text{ injection 1}$$

$$R \text{ (sample \#1)} = \text{average of four injections} = (3.343 + 3.253 + 3.522 + 3.319) / 4 = 3.359$$

While the resolution values all indicate baseline resolution (R values greater than 1.5), the resolution is clearly better at lower poly-(LSUV) concentrations.

The next table shows the effects of increasing voltage to 20 kV and of increasing temperature from 20°C to 25°C on the resolution of bi-2-naphthol enantiomers for buffers containing 0.05%, 0.1%, 0.2%, and 0.5% poly-(LSUV).

The reported values in each case are averages of quadruplicate injections on two different sample vials as well as the overall averages of the resolution values and standard deviations calculated from the two sample vials.

Table 13: Effects Changing Temperature and Voltage on Resolution of (+/-)1,1' Bi-2-Naphthol*

% poly-(LSUV)	Temperature °C	Sample # 1 Resolution	Sample # 2 Resolution	Overall Resolution
0.05%	20	3.645 (+/- 0.171)	3.510 (+/- 0.216)	3.578 (+/- 0.095)
	25	3.270 (+/- 0.124)	3.004 (+/- 0.095)	3.137 (+/- 0.188)
0.1%	20	3.556 (+/- 0.234)	3.825 (+/- 0.130)	3.690 (+/- 0.190)
	25	2.957 (+/- 0.217)	3.034 (+/- 0.032)	2.995 (+/- 0.054)
0.2%	20	2.784 (+/- 0.074)	2.863 (+/- 0.044)	2.823 (+/- 0.056)
	25	2.491 (+/- 0.094)	2.583 (+/- 0.042)	2.537 (+/- 0.065)
0.5%	20	1.701 (+/- 0.206)	1.809 (+/- 0.087)	1.755 (+/- 0.076)
	25	1.733 (+/- 0.112)	1.616 (+/- 0.049)	1.675 (+/- 0.083)

*The following conditions were employed in all runs:

Voltage = 20 kV

Temperature = 20°C or 25°C, as noted

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.0 containing 0.05 to 0.5 % poly-(LSUV)

Table 13 shows once again that the resolution is better (higher calculated R values) at the lower poly-(LSUV) concentrations. Also, the resolution is improved

consistently with the lower temperature of 20°C. Our migration time precision data given in Tables 9-11 showed that the lowest %RSDs occurred at lower poly-(LSUV) concentrations and at 25 °C. This contradicts the resolution data since the resolution was the best at 20°C. But since the resolution values are all above 1.5, baseline resolution is achieved.

Thus, overall the samples injected at 25°C produced a better separation (achieved baseline resolution and had the most repeatable migration times). Also, the separation took less time, as shown by the lower migration times at 25°C. This is beneficial since faster separations are always desired for efficiency in chemical research.

Figure 6 shows a sample electropherogram illustrating the successful chiral separation of the BOH enantiomers. The resolution was calculated to be 3.486 for this sample injection, which is well above baseline resolution. The experimental conditions are noted under the figure.

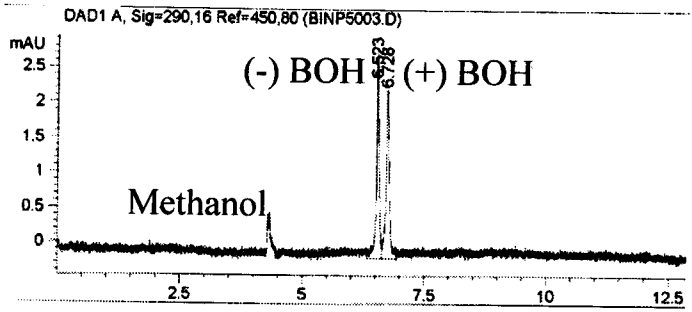


Figure 6: Sample BOH electropherogram with T = 20 °C, V = 20 kV, L = 64.5 cm, ID = 50 μm, Detection at 290 nm, and in a 25 mM borate buffer (pH 9.0) with 0.05% poly-(LSUV).

Bi-2-Naphthol Separation using Poly-(LSULV):

Warner reported the successful chiral separation of (+/-)-1,1'-bi-2-naphthol (BOH) using the polymerized surfactant, poly-N-undecylenyl-L-leucyl-valinate (poly-(LSULV)).[14] We sought to reproduce this study, with a few modifications, in order to evaluate the effectiveness of our polymerized LSULV in chiral separations. The following table represents the observed effects on migration time as well as peak width, as the surfactant concentration is varied.

Table 14 shows the effect of increasing poly-(LSULV) concentration on the migration times of bi-2-naphthol. Each run consists of a set of three injections from the same vial; separate runs indicate that different sample vials were used. Each sample is an identical solution of 0.5 mg/ml (+/-)-1,1'-bi-2-naphthol in 70% methanol / 30% water. Three injections were performed using each sample and the results averaged. The standard deviation and percent relative standard deviation (%RSD) are also included in the table.

Table 14: Effects of Increasing % poly-(LSULV) on Migration Times (t_m) of (+/-)1,1' Bi-2-Naphthol*

% poly-(LSULV)	t_m avg. [BOH], min. (+/- Std. Dev.)	Avg. Peak Width, min.
0.005%	5.403 (+/- 0.135) %RSD = 2.51	0.57
0.01%	5.549 (+/- 0.147) %RSD = 2.65	0.69
0.025%	6.924 (+/- 0.251) %RSD = 3.62	0.74
0.1%	7.455 (+/- 0.018) %RSD = 0.24	0.62
0.3%	9.737 (+/- 0.118) %RSD = 1.21	0.56
1.0%	12.915 (+/- 0.014) %RSD = 0.11	0.45

*The following conditions were employed in all runs in Table 14:

Voltage = 20 kV

Temperature = 20°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.0 containing 0.005 to 1.0 % poly-(LSULV)

Table 14 shows that no chiral separation of the BOH enantiomers was achieved since only one migration time is reported. An increase in migration time with increasing surfactant concentration is observed, indicating that there is increased interaction between the surfactant and the BOH species. This interaction, however, is not different for the two enantiomers and they are, therefore, not separated. The average peak width was used to establish the poly-(LSULV) concentration that most nearly separated the enantiomers

The widest peaks were seen at 0.025% poly-(LSULV). This is where the solute/surfactant interaction is the most differential for the two enantiomers.

Also, the standard deviation and %RSD data, especially at the lower poly-(LSULV) concentrations, show that the migration times were not very reproducible. The 1.0% poly-(LSULV) migration time data show the best reproducibility.

Since Warner's group reported chiral separation of the BOH enantiomers with this surfactant, we concluded that our synthesis was not successful as we were unable to achieve this separation.

D/L-Propoxyphene Separation Using Poly-(LSULV):

Since we were unable to achieve a chiral separation of (+/-)-1,1'-bi-2-naphthol (BOH) using the polymerized surfactant, poly-N-undecylenyl-L-leucyl-valinate (poly-(LSULV), we were fairly certain that the synthesis was unsuccessful. We still decided to attempt the separation of the d/l-propoxyphene even though we were doubtful a separation could be achieved using our poly-(LSULV).

Table 15 shows the effect of increasing poly-(LSULV) concentration on the migration times of d/l-propoxyphene. We chose to use 0.01% and 0.025% poly-(LSULV) since these concentrations produced the broadest peaks in the BOH study (Table 14). Each run consists of a set of three injections from the same vial; separate runs indicate that different sample vials were used. Each sample is an identical solution of 0.5 mg/ml d/l-propoxyphene in 30% methanol / 70% water mixture. Three injections were performed using each sample and the results averaged. The standard deviation and percent relative standard deviation (%RSD) of the migration times are included in the table. Also, the average capacity factor, k', is included in order to indicate the degree of interaction of the propoxyphene with the surfactant species. The average capacity factor, k', was calculated as shown in equation 11. Larger k' values indicate increased interaction.[1]

Table 15: Effects of Increasing % Poly-(LSULV) on Migration Times (t_m) of d/l-Propoxyphene*

% poly-(LSULV)	t_m avg. [d/l-Propoxyphene], min. (+/- Std. Dev.)	k'
0.01%	3.698 (+/- 0.012) %RSD = 0.31	-0.100**
0.025%	4.788 (+/- 0.002) %RSD = 0.04	-0.068

*The following conditions were employed in all runs in Table 15:

Voltage = 20 kV

Temperature = 25°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 8.5 containing 0.01 to 0.025 % poly-(LSULV), and 300 mM CAPS

****Sample Calculation of the Capacity Factor:**

$k' = (t_m - t_o) / t_o = (4.109 \text{ min.} - 3.698 \text{ min.}) / 4.109 \text{ min.} = -0.100$

Table 15 shows that, as expected, no chiral separation of the d/l-propoxyphene enantiomers was achieved since only one migration time is reported. Differential interaction of the two enantiomers with the surfactant is not observed, and they are, therefore, not separated,[7] although an increase in migration time with increasing surfactant concentration is observed. Additionally, the capacity factor is slightly higher (less negative) for the 0.025% poly-(LSULV), which is expected since we predict the higher concentration of surfactant to bind more.

The standard deviation and %RSD data shows that the migration times were quite reproducible, but this is not very important since a chiral separation was not achieved.

Thus, these data indicate that no chiral separation was obtained, which may be due to an unsuccessful synthesis.

D/L-Propoxyphene Separation Using Poly-(LSUTBL):

Poly-N-undecylenyl-L-*tert*-butyl-leucinate (poly-(LSUTBL)) was also synthesized in our lab. We attempted the separation of d/l-propoxyphene using the poly-(LSUTBL) surfactant using different surfactant concentrations, different pHs, as well as different buffer concentrations.

Table 16 shows the effect of changing poly-(LSUTBL) concentration, propoxyphene concentration, and pH on the migration times of d/l-propoxyphene. Each run consists of a set of three injections from the same vial; separate runs indicate that different sample vials were used. Each sample is a solution of 0.5 mg/ml d/l-propoxyphene in 30% methanol / 70% water mixture. Three injections were performed using each sample and the results averaged. The standard deviation and percent relative standard deviation (%RSD) are included in the table. Also, the average capacity factor, k' is included in order to indicate the degree of interaction of the propoxyphene with the surfactant species.

Table 16: Table of the Effects of Increasing Surfactant Concentration and pH on the Migration Times of d/l-Propoxyphene*

% Poly-(LSUTBL)	pH	Average t_m (Propox), min. %RSD	k'
0.00%	8.2	N/A	
	10.2	4.797 (+/-) 0.009 %RSD = 0.1%	0.054
0.01%	8.2	4.144 (+/-) 0.005 %RSD = 0.1%	-0.095
	10.2	5.559 (+/-) 0.079 %RSD = 1.42%	0.210
0.05%	8.2	5.091 (+/-) 0.020 %RSD = 0.40%	0.168
	10.2	6.066 (+/-) 0.038 %RSD = 0.63%	0.305
0.1%	8.2	6.111 (+/-) 0.040 %RSD = 0.66%	0.352
	10.2	N/A	

* The following conditions were employed in all runs in Table 16:

Voltage = 20 kV

Temperature = 25°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 8.2 or 10.2, containing 0.00 to 0.1 % poly-(LSUTBL), and 300 mM CAPS.

Table 16 shows that the migration times as well as the capacity factors increased when the poly-(LSUTBL) concentration increased, as observed with previous surfactants. Also, increasing the pH to 10.2 resulted in both longer migration times as well as higher capacity factors. This additionally indicates that there is more interaction between the polymer and the propoxyphene. We would actually expect the opposite trend with

capacity factors, since at the lower pH the propoxyphene enantiomers will be more positively charged. This positively-charged species would then have more interaction with the negatively-charged surfactant, causing an increase in the migration time as well as the capacity factor. However, as Table 16 shows, the capacity factors are actually lower at pH 8.2 than pH 10.2, indicating less surfactant/propoxyphene interaction at lower pH.

The next table shows the effect on the migration times and capacity factors when the borate buffer (pH 10.2) concentration is increased to 75 mM. Samples were injected n duplicate and the average results are presented. Samples were 0.5 mg/ml d/l-propoxyphene in 30%methanol / 70% water solvent.

Table 17: Table of the Effects of Increasing Buffer Concentration on the Migration Times of d/l-Propoxyphene*

% Poly-(LSUTBL)	Borate Buffer Concentration mM	Average t_m (Propox), min. %RSD	k'
0.00%	25	4.797 (+/-) 0.009 %RSD = 0.1%	0.054
	75	6.524 (+/-) 0.105 %RSD = 1.62%	0.090
0.01%	25	5.559 (+/-) 0.079 %RSD = 1.42%	0.210
	75	6.980 (+/-) 0.026 %RSD = 0.38 %	0.122
0.05%	25	6.066 (+/-) 0.038 %RSD = 0.63%	0.305
	75	8.278 (+/-) 0.004 %RSD = 0.05%	0.332

* The following conditions were employed in all runs in Table 17:

Voltage = 20 kV

Temperature = 25°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 or 75 mM Borate, pH 10.2, containing 0.00 to 0.05 % poly-(LSUTBL), and 300 mM CAPS.

Table 17 shows that the migration times as well as the capacity factors increased when the both the poly-(LSUTBL) concentration increased and the borate buffer (pH 10.2) concentration increased. An increase in the buffer concentration will decrease the electroosmotic flow, causing the propoxyphene ions to migrate more slowly through the capillary.[3]

Throughout our various studies of d/l-propoxyphene, we often noticed non-ideal peak shapes. Tailing peaks indicate that there is adsorption of the analyte species onto the capillary wall. At most of the pHs we employed, the propoxyphene ions were positively charged, while ionization of the silanol groups on the capillary makes the capillary surface negative. Thus, adsorption onto the capillary walls is certainly possible.[3] Based on many of Warner's papers, we decided to add CAPS to minimize this adsorption.[14] The effects of CAPS on peak width is reported in detail in Michelle Nerozzi's Thesis.[10]

Additionally, we observed overload peaks, which are the opposite shape of tailing peaks as shown in Figure 7. Overload peaks occur when the sample concentration is too high. As recommended by the Agilent Technologies literature, reducing the sample concentration can often improve peak shape.[3] Figure 7 shows samples of the non-ideal peak shapes that we have observed.

Tailing Peak
(caused by capillary wall adsorption)

Overload Peak
(caused by high sample concentration)

Figure 7: Figure of Non-ideal Peak Shapes[3]

In our poly-(LSUTBL) analysis, using a d/l-propoxyphene sample with a 0.5 mg/ml concentration resulted in overload peaks. We therefore decided to lower the concentration of propoxyphene. The next table shows the effect on the migration times and peak width when the propoxyphene concentration is decreased. Again, samples were injected in triplicate and the average results are presented.

Table 18: Effects of Decreasing d/l-Propoxyphene Concentration on Peak Widths*

d/l-Propoxyphene Concentration, mg/ml	t_m avg. [d/l-Propoxyphene], min. (+/- Std. Dev.) %RSD	Peak width, min.
0.5	5.366 (+/- 0.002) %RSD = 0.03%	0.278
0.125 (1:4 dilution)	5.280 (+/- 0.010) %RSD = 0.19%	0.261
0.063 (1:8 dilution)	5.395 (+/- 0.017) %RSD = 0.32%	0.192

*The following conditions were employed in all runs in Table 18:

Voltage = 20 kV

Temperature = 25°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 10.2 containing 0.05 % poly-(LSUTBL), and 300 mM CAPS

Table 18 clearly shows that decreasing the concentration of d/l-propoxyphene decreases the peak width. We also observed a more ideal peak shape when the concentration of propoxyphene was reduced. Thus, we concluded that we had been observing sample overload and therefore, decided to used lower concentrations of d/l-propoxyphene in all future studies.

D/L-Propoxyphene Separation Using Methylated β-Cyclodextrin:

After the unsuccessful separation of d/l-propoxyphene with the six different polymer surfactants, we decided to attempt the separation using various chiral cyclodextrin species. Michelle Nerozzi's thesis reports the results using α-cyclodextrin in the separation of d/l-propoxyphene.[10]

The following table shows the results of using various methylated β-cyclodextrin concentrations on the migration times and resolution of d/l-propoxyphene. Since this was just a preliminary investigation, only one injection of each concentration was performed. The migration order was determined by single injections of d- and l-propoxyphene alone.

[redacted]

Table 19: Effects of Changing Methylated β-CD Concentration on d/l-Propoxyphene Separation*

Methylated β-CD Concentration, mM	t_m [l-Propoxyphene], min.	t_m [d-Propoxyphene], min.	Resolution
10	4.892	4.919	0.857
20	5.668	5.691	0.923
30	5.885	5.914	0.818

*The following conditions were employed in all runs in Table 19:

Voltage = 20 kV

Temperature = 25°C

Capillary dimensions = 64.5 cm (L); 50 μm (ID)

Buffer = 25 mM Borate, pH 9.2 containing 10-30 mM Methylated β-CD, and 300 mM CAPS

Propoxyphene concentration = 0.05 mg/ml in 30% methanol / 70% water

Table 19 shows that we achieved chiral separation of d/l-propoxyphene at all concentrations of methylated β-CD tested. The resolution was the best (highest R value) at the 20 mM methylated β-CD concentration. Since baseline resolution was not achieved (R greater than or equal to 1.5), this method can not be used to quantitate propoxyphene. However, the method will be optimized to hopefully achieve baseline resolution and can certainly be used for qualitative analyses.

Additionally, we do see an increase in the migration times of both d- and l-propoxyphene species with an increase in the methylated β-CD concentration. This is expected since a higher cyclodextrin concentration will result in more interaction with the

propoxyphene species, thereby making the migration of the enantiomers through the capillary slower.

DISCUSSION

Diet Pepsi Study:

The Diet Pepsi study was used to familiarize us with Capillary Electrophoresis and also to determine general principles of CE that we could apply to our future research. We mainly analyzed the effects of changing buffer concentration and capillary length on the migration times and reproducibility of those migration times. The tables in the results section illustrate the effects of changing various parameters. We also learned how the preconditioning step between injections can alter the reproducibility. Lastly, we did preliminary investigations of the calibration table feature for quantitation of Diet Pepsi components.

Table 1 provided a detailed analysis of the effect of capillary length on the migration times and reproducibility of caffeine, aspartame, and benzoic acid. The migration times for all components increased when a longer capillary was used. Obviously, under identical conditions of buffer strength and pH, it will take the species longer to migrate through a longer capillary. The shortest capillary (40 cm) certainly had the best reproducibility since it achieved the lowest %RSD for the three components. This is typical for shorter migration times to be more reproducible.

Overall, it is important to note that the %RSDs of the migration times were quite high. The expected range for migration time %RSDs is 0.1 to 0.3%.[3] The CE workshop taught our research group that low reproducibility often occurs when a 1.0 N NaOH wash is done between injections. A NaOH wash is used to condition new columns, but negatively affects the reproducibility when done between every injection.[3] As later data

will show, elimination of this NaOH wash greatly improved migration time reproducibility.

Table 2 illustrates the effect of changing buffer concentration with an 80.5 cm long capillary. Again, it should be noted that the %RSDs are higher than the expected 0.1 to 0.3% range, which was caused by the NaOH wash between injections.[3] However, the 50 mM borate buffer runs achieve the most reproducible migration times as well as the sharpest peaks. These results agree with the Agilent Technologies CE program that recommends higher buffer concentration (within the range of 20-100 mM) for better peak shape and separation. Comparison of the electropherograms for these different buffer concentrations shows that the sharpest peaks are observed with the 50 mM borate buffer. Also, the overall run time increases as the buffer concentration increases. This is expected since a lower buffer concentration produces a higher electroosmotic flow, which in turn will move the analyte components through the capillary faster, making the run time shorter.[3]

In general, Table 2 shows that the %RSD of the migration times increased as the migration time increased from component to component. Thus, the migration times were most repeatable for caffeine, the first component to migrate.

After studying these general trends including the effects of capillary length, buffer strength, and NaOH washing between injections, we wanted to attempt to quantitate the amount of the three components in Diet Pepsi samples. A calibration table was constructed using the determined migration times of the three component standards and the concentrations of these standards. The peak areas of the standards and the Diet Pepsi samples were to be compared in order to quantitate the concentration of the various

components in Diet Pepsi. Table 3 shows the results of this quantitation. The caffeine was the only component that was near the expected value. We were not overly concerned with these results, however, as the goal of this part of the project was to learn basic CE principles and operation of the CE software.

Bi-2-Naphthol Separation Using Poly-(LSUV):

The main goal of this study was to determine if our poly-(LSUV) synthesis was successful, as indicated by the achievement of a chiral separation of the bi-2-naphthol enantiomers. Data in several papers written by Warner's group will be compared with our results in order to assess the quality of our synthesized surfactant.

Our initial experiment was essentially a reproduction of a study done by Warner's group.[7] We used 25 mM borate buffer, pH 9.0, 12 kV for the applied voltage, and detection at 290 and 210 nm. Warner's group looked at poly-(LSUV) surfactant concentrations in the buffer ranging from 0.02% to 0.5%.

In our analysis, we used four different poly-(LSUV) concentrations, 0.05%, 0.1%, 0.2%, and 0.5%. We determined that the "best separation" of enantiomers occurred when we observed the lowest migration times with the best reproducibility as well as when we achieved baseline peak resolution (resolution values of greater than 1.5). Table 9 highlights the effects of surfactant concentration on the migration times of the BOH enantiomers. In general, compared to the Diet Pepsi study results, the %RSDs of migration times are much lower and in the expected 0.1% to 0.3% range.[3] Again, this is due to the elimination of the 1.0 N NaOH wash between injections. The reproducibility of migration times from injection to injection is best at the lowest surfactant concentrations. This is because shorter migration times, in general, are more reproducible.

While each surfactant concentration data set had injection to injection %RSDs within the expected 0.1 to 0.3% range, the overall data given in Table 10 reported

%RSDs that are above the expected range. This illuminates an important CE principle. Table 9 shows the intrasample reproducibility, whereas Table 10 shows intersample reproducibility. Table 9 shows that within a given sample, the %RSDs are generally lower (and in the expected range); however, from sample to sample, the %RSDs are a bit higher. A general increase in migration times from injection to injection as well as sample to sample was observed, accounting for the increased %RSDs between samples. This highlights an inherent problem in CE, whereby the initial conditions are not completely reestablished between injections and samples.

Comparing the overall %RSDs of sample to sample migration times given in Table 10, the lowest %RSDs are observed for the 0.05% poly-(LSUV). We determined that the optimum poly-(LSUV) concentration was 0.05% since this concentration achieved the shortest and most reproducible migration times. Also, chiral separation of the BOH enantiomers indicated we had a successful synthesis of our poly-(LSUV) surfactant, where the two enantiomers interacted differentially with the surfactant.

Comparison of Table 10 and 11 (20°C data) shows the effects of changing the voltage from 12 kV to 20 kV. Clearly, a *significant* improvement (lowering) of the %RSDs from injection to injection as well as run to run was observed with the increase in voltage. The overall %RSDs were about 0.01-0.05% for the 20 kV sequence and 0.25-0.26% for the 12 kV sequence for the 0.05% poly-(LSUV). Also, the migration times for both BOH enantiomers were much less at 20 kV, as predicted. This is desirable since it allows for faster separations with much better reproducibility. Since the improvement was so significant, we decided to change the method for all future runs to 20 kV.

Based on a paper published by Warner's group, we decided to compare the results of a capillary temperature set at 25 °C to a temperature set at 20 °C.[18] Table 11 shows that the higher temperature (25 °C versus 20 °C) results in both lower migration times and lower %RSDs, indicating better reproducibility. While the overall %RSDs are about the same for both temperatures and 0.05% poly-(LSUV), higher surfactant concentrations clearly show better reproducibility at 25 °C. For example, the 0.1% poly-(LSUV) runs have overall %RSDs of 0.35-0.38% at 20 °C and 0.23-0.26% at 25 °C, 0.2% poly-(LSUV) produced overall %RSDs of 0.32-0.54% at 20 °C and 0.12-0.14% at 25 °C, and lastly, the 0.5% poly-(LSUV) had overall migration time %RSDs at 20 °C of about 0.63-0.65% and 0.42-0.44% at 25 °C. Thus, the obvious lowering of migration times as well as improvement in reproducibility for all poly-(LSUV) concentrations indicated increasing the cassette temperature to 25 °C had a positive effect.

Considering the resolution data presented in Table 12 shows the effect of polymer concentration on peak resolution. All concentrations resulted in baseline resolution with all R values reported greater than 1.5. The best peak resolution of enantiomers occurs when the highest resolution value is obtained; therefore, the best resolution of peaks occurred with the 0.1% poly-(LSUV) ($R_{overall}$ = 3.387) and with the 0.05% poly-(LSUV) ($R_{overall}$ = 3.245). Since the lower concentrations of surfactant also had the shortest and most reproducible migration times, this resolution data supported our conclusion that the 0.05% and the 0.1% poly-(LSUV) concentrations produce the best chiral separation of the BOH enantiomers.

Wang and Warner reported that the optimal poly-(LSUV) concentration for the resolution of (+/-)-1,1'-bi-2-naphthol was 0.2%.[7] Our results and determination of the

optimal poly-(LSUV) concentration differ from this since our data indicate that this optimal concentration was 0.05 – 0.1% poly-(LSUV). Furthermore, the maximum resolution value reported by Wang and Warner was about 2.2, whereas we obtained a maximum resolution of 3.387. While we did observe an increase in migration time with increased surfactant concentration as they did, the migration times we observed were overall lower than theirs. They show migration times going from about 15 minutes with 0.05% poly-(LSUV) to about 26 minutes with 0.5% poly-(LSUV), while we observed migration times of about 11 minutes with 0.05% poly-(LSUV) to about 18 minutes with 0.5% poly-(LSUV). This could possibly mean that our surfactant is more pure since we achieve better resolved peaks in a shorter amount of time.

Lastly, Table 13 emphasizes the effects of changing the applied voltage to 20 kV and changing the temperature to 25°C on the peak resolution. Comparing Table 12 and 13 (20°C data), shows that for a given surfactant concentration, the overall resolution is generally improved with an applied voltage of 20 kV. At 0.05% poly-(LSUV), for example, higher overall resolution is obtained with the 20 kV sequence (R = 3.578) than the 12 kV sequence (R = 3.245). The overall resolution data for 0.1% poly-(LSUV) show an improvement in overall resolution from 3.387 at 12 kV to 3.690 at 20 kV. The data for 0.2% poly-(LSUV) also shows that the higher voltage produces higher resolution values (R = 2.823) versus the 12 kV sequence, where R = 2.565. Lastly, the sets of 0.5% poly-(LSUV) data actually show worse resolution at 20 kV in this case, but the resolution values are still relatively close. At 12 kV, the resolution is 1.925, whereas at 20 kV the resolution is 1.755. Since all resolution values calculated are greater than 1.5, the

positive effect on migration times and reproducibility of the higher applied voltage reinforces that using 20 kV results in better peak separation.

Table 13 also looks at the effect of temperature on the resolution of BOH enantiomers with an applied voltage of 20 kV. Table 13 shows that the resolution is generally lower at the higher temperature for all concentrations of poly-(LSUV). For example, the overall resolution data for 0.5% surfactant show a decrease in resolution since it is 1.755 at 20 °C and 1.675 at 25 °C. Although the resolution values are consistently lower, all R values are still above 1.5, indicating baseline resolution.

Compiling all of the data that we collected, the overall best conditions for separation can be determined. There was consistent improvement in the resolution values and %RSDs for injection to injection as well as run to run migration times with the lower surfactant concentrations, namely 0.05% and 0.1% poly-(LSUV). Also, the voltage increase from 12 kV to 20 kV, as recommended by Warner's paper, resulted in much better peak separation as shown by the higher calculated resolution values, lower migration times, and better reproducibility (lower %RSDs).[18] The results of the temperature study, however, were not as conclusive. Analysis of the %RSD data for all surfactant concentrations shows that the 25 °C capillary temperature produces lower migration times and %RSDs. Conversely, the overall resolution values were consistently higher (indicating better peak separation) for the 20 °C capillary temperature. Since the higher temperature resulted in much better migration time reproducibility and lower migration times, the fact that baseline resolution was still achieved at the higher temperature makes 25 °C the better temperature to use. Thus, the overall optimal

conditions for separation of the BOH enantiomers are 20 kV, 0.05-0.1% poly-(LSUV), and 25 °C.

Bi-2-Naphthol Separation Using Poly-(LSULV):

Our first experiment using the poly-(LSULV) involved the attempted chiral separation of BOH enantiomers, varying the concentration of the surfactant used. We employed a 25 mM borate buffer, pH 9.0, 20 kV for the applied voltage, detection at 290 and 210 nm, and poly-(LSULV) concentrations ranging from 0.005% to 1.0%.

Table 14 shows that we were unable to achieve a chiral separation of the BOH enantiomers as only one migration time is recorded. The average migration times increased from 5.403 minutes with 0.005% poly-(LSULV) to 12.915 minutes with 1.0% poly-(LSULV), indicating increased surfactant/solute interaction with greater surfactant concentrations. However, this interaction was not differential for the two enantiomers, causing the BOH enantiomers to migrate at the same rate. The average peak width of the BOH peak was used to determine the concentration where the enantiomers were most nearly separated. The largest peak width was reported with 0.025% poly-(LSULV). Therefore, we concluded that the most differential analyte/surfactant interaction occurred at this surfactant concentration.

Additionally, the %RSDs of the migration times reported were quite high, showing that that migration times were not very reproducible. The %RSDs ranged from 0.11 to 3.62%, which is generally out of the expected 0.1 to 0.3% range.[3] We did not achieve a chiral separation and therefore, were not very concerned with this result. Since Warner's group reported chiral separation of the BOH enantiomers with resolution values of about 5 using their poly-(LSULV) under similar conditions, we determined our poly-(LSULV) synthesis to be unsuccessful, as shown by the lack of chiral separation of the BOH enantiomers.[16]

D/L-Propoxyphene Separation Using Poly-(LSULV):

Despite the failed chiral separation of BOH using poly-(LSULV), we still decided to attempt the separation of d/l-propoxyphene using this surfactant. Table 15 shows that, as we predicted, no chiral separation of the propoxyphene enantiomers was achieved using either 0.01 or 0.025% poly-(LSULV). The increase in migration times as well as average capacity factors with increasing surfactant concentration does indicate increased interaction with the surfactant. However, this interaction is not different for the two enantiomers, resulting in no chiral separation.

D/l-propoxyphene Separation Using Poly-(LSUTBL):

Based on our results of the attempted chiral separation of the BOH enantiomers and d/l-propoxyphene using poly-(LSULV), we determined that our synthesis was not successful. We therefore decided to attempt the chiral separation of d/l-propoxyphene using the poly-(LSUTBL) synthesized in our lab. The pH, surfactant concentration, buffer concentration, and propoxyphene concentration were varied and the results reported.

Table 16 shows the effect on the migration times of d/l-propoxyphene and capacity factors when the surfactant concentration and pH are varied. At both pH 8.2 and pH 10.2, the migration times increased with greater surfactant concentrations. The average capacity factor data supports this trend as higher k' values were reported with greater poly-(LSUTBL) concentrations for a given pH, indicating increased interaction.

Also, comparison of the pH 8.2 data with the 10.2 data shows that there is an increase in the migration times as well as the capacity factors at the higher pH. This indicates that an increase in pH causes more interaction of the propoxyphene with the poly-(LSUTBL). We would actually expect the opposite trend, since at the lower pH the propoxyphene is more positively charged. This more positively-charged propoxyphene is predicted to interact more with the negatively-charged poly-(LSUTBL), which should result in longer migration times and greater k' values. However, as Table 16 illustrates, this is not observed. For example, looking at the data for 0.05% poly-(LSUTBL), at pH 8.2 the average migration time is 5.091 minutes and k' is 0.168, whereas at pH 10.2 the average migration time is 6.066 minutes and k' is 0.305.

Table 17 illustrates the effect of increasing buffer strength and surfactant concentration on the migration times of d/l-propoxyphene and the capacity factors. With an increase in surfactant concentration (from 0.00% to 0.05%), the migration times increase as well. The longer migration times observed with greater buffer strength can be explained since an increase in the buffer concentration will cause the electroosmotic flow to decrease, thus causing propoxyphene ions to migrate more slowly through the capillary.[3] For example, Table 17 shows that with 0.01% poly-(LSUTBL), the average migration time is 5.559 minutes for the 25 mM borate buffer and 6.980 for the 75 mM borate buffer concentration.

Additionally, there is no specific trend observed for the capacity factors with the increase in buffer strength. This is understandable since altering the buffer concentration affects the electroosmotic flow, which controls the migration of all species through the capillary. Since the capacity factor is determined based on both the migration of the methanol and the propoxyphene, a trend is difficult to predict.

As introduced in the results section, we often observed non-ideal peak shapes caused either by sample overload and/or adsorption onto the capillary wall. CAPS was added to minimize peak tailing caused by adsorption of the positively-charged (at most pHs we used) propoxyphene species onto the negatively-charged capillary wall. Using a d/l-propoxyphene sample with 0.5 mg/ml concentration resulted in sample overload peaks. Therefore, we decided to perform a study of the effects of decreasing d/l-propoxyphene concentration on peak width and peak shape.

Table 18 clearly illustrates that the peak width was reduced with decreased propoxyphene concentrations. The peak width was 0.278 minutes with 0.5 mg/ml

propoxyphene concentration and dropped to 0.192 minutes with 0.063 mg/ml propoxyphene concentration. Also, the electropherograms showed more ideal, sharper peaks when the propoxyphene concentration was decreased.

D/L-Propoxyphene Separation Using Methylated β-Cyclodextrins:

After performing several studies using six different polymerized surfactants, we decided to attempt using cyclodextrins for the chiral separation of d/l-propoxyphene. Table 19 shows the effects of changing cyclodextrin concentration on the migration times as well as the resolution values of d/l-propoxyphene. The table shows that successful partial chiral separation of the d/l-propoxyphene enantiomers was achieved.

Table 19 shows that there was an increase in the migration times of both propoxyphene enantiomers when the methylated β-cyclodextrin concentration was increased. This is expected since greater cyclodextrin concentration will allow for more interaction with the propoxyphene species, causing an increase in migration time. When cyclodextrins interact with the sample, the effective mass of the sample is increased. Most importantly, however, is that the interaction of the cyclodextrin with each propoxyphene enantiomer was different, enabling a chiral separation.

The table also shows that the best peak resolution occurred with 20 mM methylated β-cyclodextrin concentration, where $R = 0.923$. Since we did not achieve baseline resolution (R values greater than or equal to 1.5), this method cannot be utilized for quantitative analysis of d/l-propoxyphene. However, qualitative analyses can certainly be performed using this method. For example, caseworkers at the New York State Forensic Investigation Center can detect the presence of d-propoxyphene (the illegal form) in a sample by using this method. If a sample is mixed with an l-propoxyphene standard and two peaks appear, this indicates that the sample does contain the illegal d-form of propoxyphene. Conversely, if a sample only contains the l-form of

propoxyphene and is mixed with the l-propoxyphene standard, only one peak will appear on an electropherogram.

Although the use of cyclodextrins in the chiral separation of d/l-propoxyphene has already been reported, this method has several advantages over the previous methods. Lurie reported chiral separation of d/l-propoxyphene using a mixture of cyclodextrins in about 20-25 minutes.[19] Clearly our method is much faster, as a chiral separation was achieved in about 5 minutes. Additionally, our method only used one cyclodextrin instead of a mixture. Also, comparing our method using cyclodextrins to some of the attempted chiral separations using our polymer surfactants (with migration times up to 14 minutes), shows that the cyclodextrin method is much faster.

In the future, baseline resolution of the propoxyphene enantiomers will hopefully be achieved using the cyclodextrins so that the amounts of d- and l-propoxyphene can be quantitated. In addition to optimization of this cyclodextrin method, a combination of cyclodextrins and surfactant will be used in the attempted chiral separation of d/l-propoxyphene.