A Comparative Study of the Enantiomeric Separation of Labeled Amino Acids with Cyclodextrins and Mixed Micelles in Capillary Electrophoresis

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Abstract

Enantiomeric separations of fluorescently labeled amino acids are studied by capillary electrophoresis (CE) under a novel variety of experimental conditions. Three different labels are evaluated using two different additives: cyclodextrins (β- and γ-) and a dual surfactant system of sodium dodecyl sulfate and sodium taurodeoxycholate. Fluorescein-5-isothiocyanate is the best label to use in this cyclodextrin-based system, and dansyl chloride is the best label to use in this dual surfactant system. Possible limitations for separation of the enantiomers using the mixed micelle system include the fact that there is little interaction of the solute with the surfactants, the negative charge of the solute is limiting the separation window of the system, and the amount of the chiral phase available for partitioning is limited. The separations using cyclodextrins as a chiral selector show that the label affects migration order of the enantiomers, and the cyclodextrins are very effective in separating numerous enantiomers. Overall, cyclodextrins are the better buffer additive for CE use, and the dual surfactant systems, including sodium taurodeoxycholate, offer future promise.

Introduction

The separation of chiral molecules, including amino acids, has historically been a challenging task. Han and Blomberg have reviewed various analytical techniques that have been used with varying degrees of success for the enantiomeric separation of compounds (1). The goals of this project are to compare the effect of a label's interaction with buffer modifiers and to develop a method for the enantiomeric separation of amino acids within a reasonable analysis time. Capillary electrophoresis (CE) was chosen because of the technique's high efficiency, selectivity, and ability to accommodate a wide analyte variety. Although capillary zone electrophoresis (CZE) can separate charged solutes, CE's ability to separate solutes can be enhanced with the modification of the running buffer by the addition of reagents that can affect the migration rates of the compounds being analyzed. Two common additives are surfactants and cyclodextrins (CDs).

Micellar electrokinetic chromatography (MEKC) was developed by Terabe to separate neutral molecules using surfactants as a run buffer additive (2). The use of bile salts as a surfactant in MEKC can be traced back to Terabe's group in 1989, where they separated dansylated (DNS) amino acids with sodium taurodeoxycholate (STDC) with low resolution and very long run times (3). Discussion over the formation of STDC micelles has been debated recently. STDC can either form a trimer (4–6) or a dimer (7,8), depending on the mathematical model used. However, as the concentration of STDC or ionic strength increases, the aggregation of the trimers or dimers into a cylindrical micelle occurs (6). All of the proposed STDC structures indicate that the polar head groups are located near the center of the structure. This means that the chiral portion of the surfactant is also near the interior of the micelle. For the cylindrical micelle, the chiral portion of the surfactant may be inaccessible to the chiral solutes. However, the structure of the trimer is fairly open in dilute solutions, thus accessible to any chiral solute (6).

To increase efficiency and decrease analysis times, adding another surfactant or additive to the chiral selector was tried. Lu and Chen separated fluorescein-5-isothiocyanate (FITC) labeled amino acids using a mixture of sodium taurocholate and β-CDs. They were able to obtain very good resolution between each enantiomer, but the analysis times were on the order of 50 min (9). Examples of other mixtures include short chain surfactants with bile salts to separate corticosteroids (10) as well as the separation of chiral polychlorinated biphenyls (11), all with reduced analysis times. To improve the resolution in the separation of the amino acids enantiomers using MEKC, a mixed surfactant system containing sodium taurodeoxycholate and sodium dodecylsulfate was reviewed. To characterize the effectiveness of the labels using this buffer additive, the results to the separation of amino acids using the CD phases were compared.

CDs were first reported to be used for the enhancement of

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separations by Terabe et al. in 1985 (12). Many studies since then have been performed showing the benefits of CDs and their derivatives in achieving separations of a variety of analytes (13–18). CDs are macrocyclic oligosaccharides composed of a number of D(+)-glucose units connected by α-(1,4)-linkages. CDs are named according to the number of units connected together: α-CD, β-CD, and γ-CD have 6, 7, and 8 units, respectively. They are torus-shaped with all the glucose units in a largely undistorted chair conformation. The inside cavity of the CD is relatively hydrophobic, which allows for many different types of compounds to be included and fit into the cavity. The inclusion complex formation between compound and CD is influenced by the physical and chemical properties of both. Slight differences in binding constants between the compounds and the CD will aid in the separation. In enantiomeric separations, the difference in the binding constants of two enantiomers with the CD will be very small, but an appropriate CD composition can hopefully be found to give slight changes in binding constants that will allow for resolution of the enantiomers.

For the CD additives, 10mM β- and 10mM γ-CDs were chosen as they represent conventional concentrations used in CE. Two composite CD running buffers, 6mM β/1mM γ and 7.5mM β/19mM γ, were also used based on prior work (19). These two compositions were shown to enhance the enantiomeric separation of some of the amino acids studied in this work with the DNS label. It was decided to see how well these CD combinations worked with different labels as well as changes in pH using the amino acids selected for this study. Previous work has shown how effective dual CD systems can be in achieving enantiomeric separations (20–22). Systems that incorporate more than one type of CD do not necessarily need CDs that are of similar charge. For instance, systems using a neutral and a charged CD have been shown to be ideal for some chiral separations, (23,24), but this study used two basic neutral CDs as the dual CD system.

Because native amino acids are difficult to optically detect, a label was added to the amino acid to enhance its detection. The chosen labels are fluorescent, but they can also be used in absorption spectroscopy. To assess the effect of the label, three labels [4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD), DNS, and FITC] were compared. DNS is a slightly polar label, NBD is more polar than DNS, and bound FITC is negatively charged (25). The labeled amino acids are easy to purchase or synthesize and easily detectable at the wavelength of excitation using a UV–vis detector. DNS-labeled amino acids are available commercially and have been extensively used in this research group (19,26–28). NBD-labeled amino acids can be obtained in a short reaction time, and its long wavelength of fluorescence results in the elimination of competing fluorescent interferences inherent to the analytes in question (29). FITC-labeled amino acids are commonly used in order to achieve extremely low limits of detection, down to the subattomole range (30). Because bound FITC is negatively charged, its applicability in CE techniques will also be assessed.

To assess the effect of the run buffer pH on the separation of the enantiomers, two buffers of similar ionic strength (20mM

Table I. Average Resolution of Separated Enantiomers in Dual Surfactant Mixtures and Cyclodextrin Mixtures

	10 β	6 β/1 γ	7.5 β/19 γ	10 γ
Cyclodextrin mixtures in phosphate buffer at pH 7.0				
rac-DNS-Asp	1.056	1.057	0.728	0.574
rac-DNS-Val	0.469	0.509	NA	0.442
rac-NBD-Phe	NA*	0.465	NA	NA
rac-NBD-Val	NA	0.686	0.469	NA
Cyclodextrin mixtures in borate buffer at pH 9.5				
rac-DNS-Asp	1.355	1.172	0.951	0.482
rac-DNS-Val	NA	0.453	0.712	NA
rac-NBD-Asp	0.541	NA	0.223	0.209
rac-NBD-Phe	0.217	0.364	NA	NA
rac-NBD-Ser	0.332	0.247	0.480	NA
rac-NBD-Val	0.426	0.334	0.266	NA
rac-FITC-Asp	1.347	2.955	NA	2.337
rac-FITC-Phe	0.736	1.225	0.839	NA
rac-FITC-Ser	0.813	1.260	0.589	0.843
rac-FITC-Val	NA	0.319	NA	NA

Surfactant mixtures in borate buffer at pH 9.5	50% SDS–50% SDTC	33% SDS–67% STDC	0% SDS–100% STDC
rac-DNS-Phe	0.516	0.866	0.652
rac-DNS-Asp	NA	0.588	NA

*NA = not applicable.

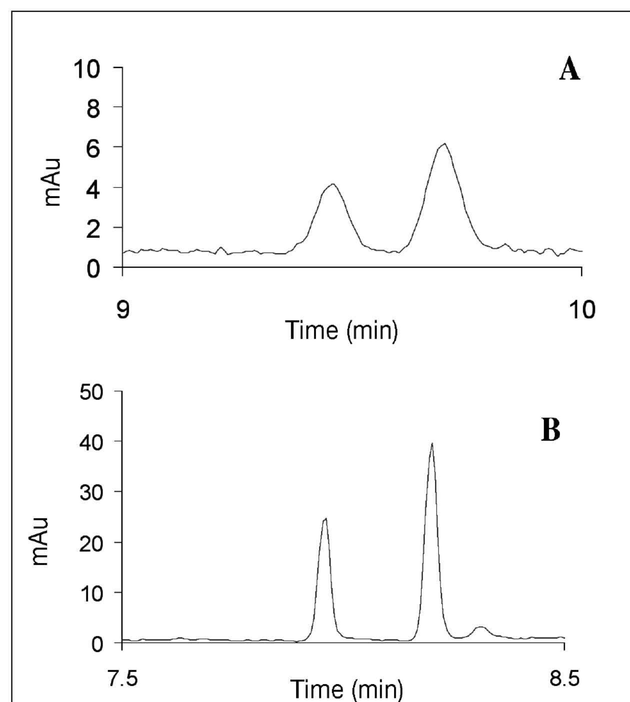

Figure 1. Separation of the enantiomers of FITC-Asp at pH 9.5 with 10mM β-CD (A) and 6mM β:1mM γ-CD (B). Injections were of the ratio 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

phosphate buffer at pH 7 and 50mM borate buffer at pH 9.5) were used. With the labeled amino acids, the charge of each solute was negative in both buffers. Changing the composition of the additives, altering a detectable label, or changing the composition of the run buffer can be employed to achieve an enhancement in resolution. The goal of this study is to create a methodology in which all of these variables are adjusted in order to deduce their effects on a particular enantiomeric separation.

Experimental

Reagents and solutions

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and were used without further purification: the D and L forms of aspartic acid, phenylalanine, valine, and serine, the racemic (rac-) and L forms of DNS-phenylalanine, DNS-aspartic acid, DNS-valine, and DNS-serine; and Sudan 3, FITC, NBD, β-CD hydrate, STDC, and SDS. γ-CD was purchased from Cyclodextrin Technologies Development (High Springs, FL). Methanol, sodium chloride, sodium hydroxide, acetone, and water were purchased from Fisher Scientific (Pittsburgh, PA) and were used without further purification. An Agilent (Palo Alto, CA) HP 3 D CE system with a diode array detector was used. The column was a 50 µm i.d., 50-cm long fused silica column from Polymicro Technologies (Phoenix, AZ) with the detection window burned 8.5 cm away from the outlet end of the column. The column was rinsed with 1M NaOH solution followed by the run buffer at the start of each analytical sequence. The temperature of the column was kept constant at 30°C and a voltage of 16 KV was applied. The detector was programmed to monitor five wavelengths: 205 nm for methanol (EOF), 254 nm for aromatic groups, 350 nm for DNS-amino acids, 475 nm for NBD-amino acids, and 488 nm for FITC-amino acids. The solutes were chosen as representatives of different classes of amino acids (aromatic, acidic, and aliphatic).

Synthesis of the derivatized amino acids

The synthesis of the NBD derivatized amino acids was adapted from the protocol published by Murray and Sepaniak (31). Five hundred microliters of a 20mM solution of the amino acid dissolved in running buffer (20mM phosphate at pH 7) and 500 µL of a 20mM methanolic solution of NBD were added to a vial. The mixture was heated at 70°C for 1 h, then cooled. One hundred-and-twenty-five microliters of a 1M solution of ferric chloride was added to the mixture to precipitate the phosphate. Methanol was added to the vial to create 2 mL of the derivatized standard. The synthesis of the NBD-labeled amino acids in the borate buffer followed the previously described procedure with the omission of the addition of ferric chloride.

The synthesis of the FITC derivatized amino acids was adapted from the protocol published by Takizawa and Nakamura (32). Two hundred microliters of 20mM FITC solution in acetone and 400 µL of 20mM amino acid, dissolved in running buffer, were mixed in a vial and heated at 50°C for 4 h.

For the analysis of the dansylated amino acids, a 1mM solution of each racemic mixture was created in each running buffer. For all analyses involving the determination of D and L forms, the ratio of D to L was set at 1:2.

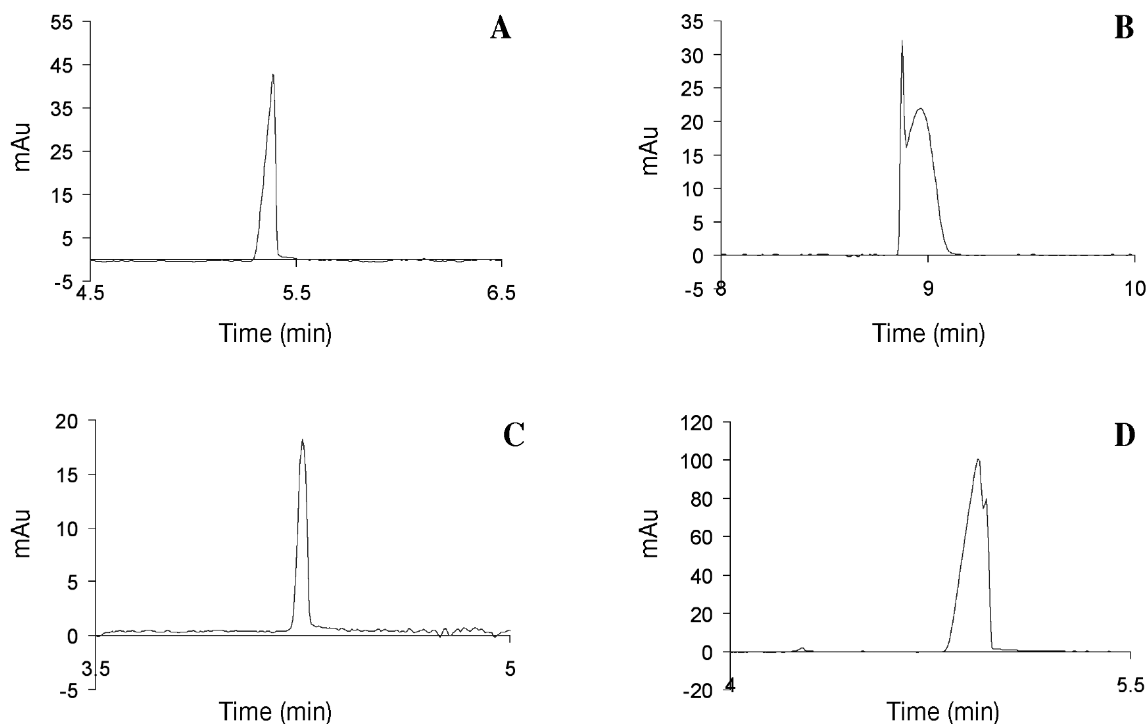

Figure 2. Separation of the enantiomers of NBD-Asp at pH 7.0 (A) and pH 9.5 (B), and NBD-Phe at pH 7.0 (C) and pH 9.5 using 10mM β-CD (D). Injections were of the ratio 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50-cm long, 50 µm i.d., 41.5 cm to the window.

Preparation of run buffer additives

For this study, a series of run buffer additives were produced. For the MEKC experiments, the buffer was either a 20mM phosphate solution at pH 7.0 or a 50mM borate solution at pH 9.5. The CD phases consisted of 10mM β-CD, 6mM β/1mM γ-CD, 7.5mM β/19mM γ-CD, and 10mM γ-CD. The mixed surfactant phases consisted of 60mM SDS (100% SDS), 60mM STDC (0% SDS), 20mM SDS/40mM STDC (33% SDS), 40mM SDS/ 20mM SDTC (66% SDS), and 30mM SDS/30mM STDC (50% SDS). In addition, a series of CZE experiments were conducted using the borate and phosphate buffer as the run buffer. NaCl solution (60mM) in each buffer was utilized to determine the intrinsic electrophoretic mobilities of the solutes.

Table II. Electrophoretic Mobility of NBD-Labeled Amino Acids Under CZE Conditions with no Sodium Chloride*

	pH 9.5	pH 7
rac-NBD-Val	–0.0143	–0.0126
rac-NBD-Phe	–0.0148	–0.0114
rac-NBD-Asp	–0.0236	–0.0200
rac-NBD-Ser	–0.0174	–0.0138

* Units are in $cm^2/Vmin$, the relative standard deviation for all measurements averaged 0.1%.

Results and Discussion

General resolution considerations

In achiral or chiral separations by CE methods, several factors determine the achieved resolution. Efficiency determines the sharpness of the bands and the ease with which a pair of closely migrating solutes can be separated. CE analyses are known to provide very high efficiencies, but without a high enough selectivity, an enantiomeric separation with good resolution will not occur. Efficiency is critical in chiral separations, but is not considered a factor altered within the context of the current study. Selectivity is the difference in net mobilities for two solutes and is inherently difficult to maximize for chiral separations because enantiomers are so similar in properties. Finally, with the inclusion of additives in the run buffer, an elution window is created that is bound by the effective migration time (or mobility) of the free solute and the migration time when it fully associates with the additive. The effect of the magnitude of the elution window on the separation can be dramatic. This work had negatively charged enantiomers with migration times in free form that were greater than t_0 (i.e., they migrated in opposition to EOF). Association with neutral CD additives reduces migration times within the elution window. As the magnitude of the association constant or the concentration of the CD increases, the solute converges on t_0, but will not reach it as the solute-CD complex is still charged, albeit possessing a very small charge-to-mass ratio.

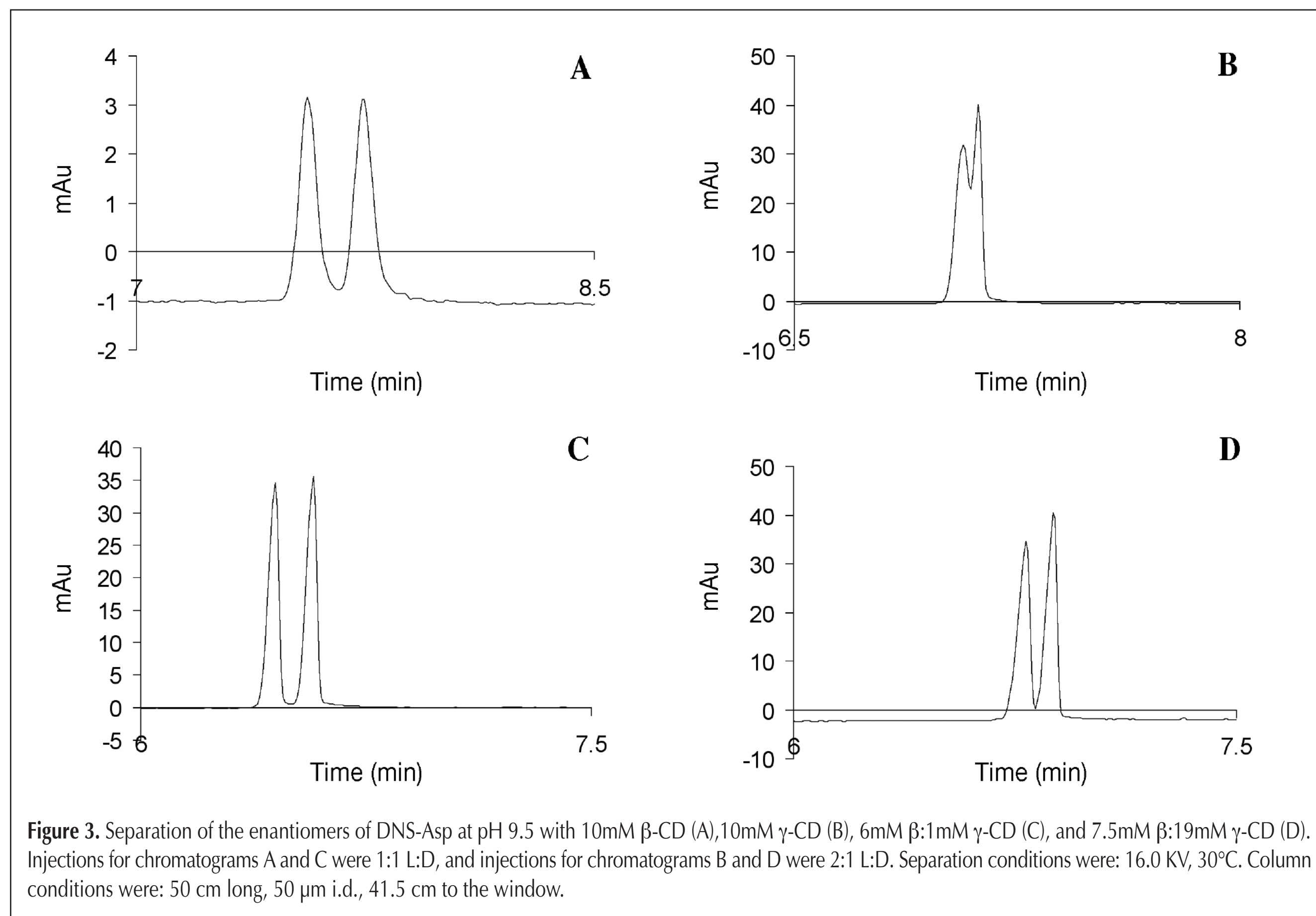

Figure 3. Separation of the enantiomers of DNS-Asp at pH 9.5 with 10mM β-CD (A),10mM γ-CD (B), 6mM β:1mM γ-CD (C), and 7.5mM β:19mM γ-CD (D). Injections for chromatograms A and C were 1:1 L:D, and injections for chromatograms B and D were 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

The situation was more complex with this MEKC work because both the solutes and the surfactant additives were negatively charged. The effect of solute association with the micellar phase is to either decrease or increase migration time, depending if the net mobility of the micelles are smaller or greater than that of the free solute.

This study reports the influence of experimental parameters such as running buffer pH, solute label type, and buffer additives on the resolution of the test labeled amino acid enantiomers. To our knowledge, this work represents a unique perspective, which helps explain the effects of adjusting these parameters simultaneously and their impact on changes in resolution of the enantiomers. The results are presented as observations, and in some cases the expected effects on the aforementioned resolution factors, selectivity, and elution window are discussed.

Synthetic considerations of NBD- and FITC-labeled amino acids in pH 7 phosphate buffer

For the synthesis of the NBD-derivatized solutes in the phosphate buffer, several byproducts were also noticed in the electropherograms. These byproducts do not occur or are not separated in the borate buffer. If a small amount of ferric chloride is added to the sample after heating, the phosphate is precipitated out, and the formation of the byproducts is limited.

The free FITC contains an ester, ether, and two alcohol functional groups. When FITC is bound to a solute of interest, it becomes charged. According to the work by Robeson and Tilton, the bound FITC has a pK_a of 6.2 (25), lower than the pH of both running buffers employed in these studies. Thus the FITC-labeled amino acids add an extra negative charge to the solute, increasing the migration time of the solute and reducing the elution window in MEKC. This increase in negative charge may also interfere with the interaction of the solutes with the surfactants. Also, the derivatization reaction in the phosphate buffer produces many byproducts that render the identification of the racemic amino acids in the surfactant mixtures difficult. The phosphate anion is assumed to interact with the FITC and the NBD label to form various byproducts, which can interfere with the qualification of the solutes. Therefore, the FITC data at pH 7.0 was excluded because of the difficulty in qualifying each enantiomer.

pH Effects on separation of amino acids using CDs

The effects of pH on the separations were studied with equivalent ionic strength buffer solutions at pH 7.0 and 9.5. Table I shows the resolution data obtained for the enantiomers that were separated. The separation with FITC-labeled amino acids at pH 7.0 was not feasible, but good separations were obtained at pH 9.5. Figure 1 shows the separations of FITC-Asp at pH 9.5. For the DNS label, a change in pH did not have an effect on the separation. Regardless of the pH, rac-DNS-Phe and rac-DNS-Ser were not separated, but rac-DNS-Asp and rac-DNS-Val were separated with rac-DNS-Asp being separated in all four CD compositions at both the high and low pH. All four NBD-labeled amino acids were separated at pH 9.5. In contrast, only rac-NBD-Phe and rac-NBD-Val showed enantiomeric separation at pH 7.0. Figure 2 shows the effect of pH on the separation of rac-NBD-Asp and rac-NBD-Phe with 10mM β-CD. The increase in selectivity can be explained by the increase in the

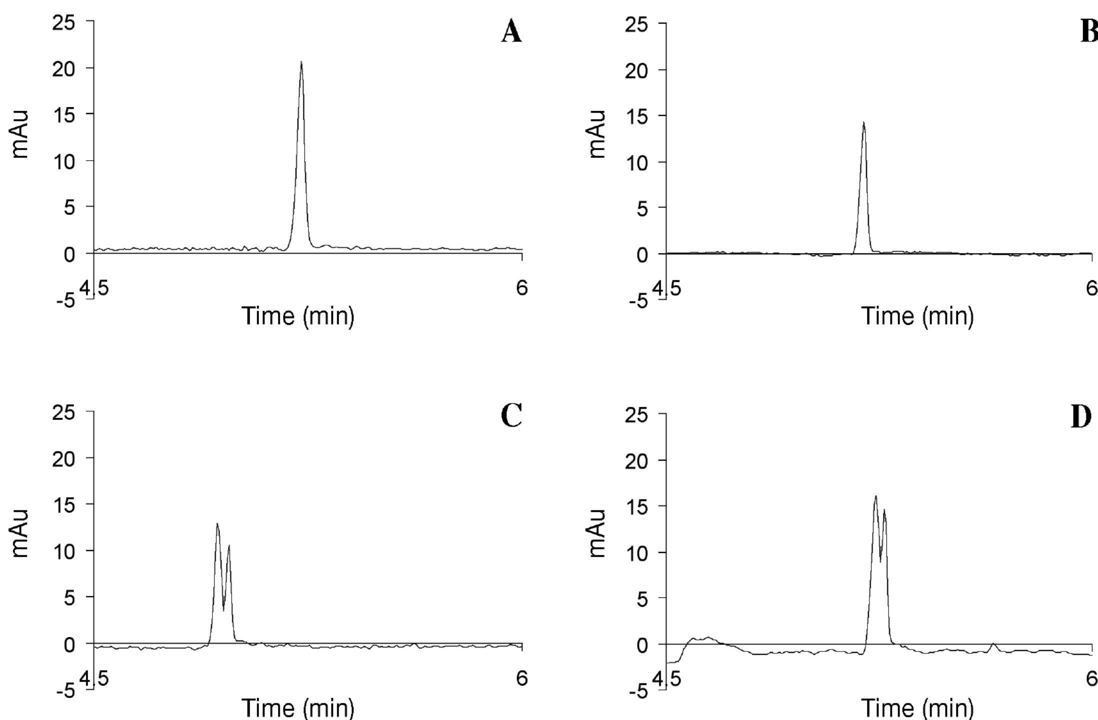

Figure 4. Separation of the enantiomers of NBD-Val at pH 7.0 with 10mM β-CD (A), 10mM γ-CD (B), 6mM β:1mM γ-CD (C), and 7.5mM β:19mM γ-CD (D). Injections were of the ratio 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

intrinsic electrophoretic mobility for NBD-labeled amino acids at pH 9.5 when compared with pH 7.0, as shown in Table II. The pH increase resulted in a difference in mobility between the free and complex enantiomer, which allowed for a better enantiomeric separation.

Effect of different CD phases on the enantiomeric separations

As mentioned previously, four CD compositions were studied in this work. A single CD composition that enantiomerically separates every amino acid in this study with the highest resolution does not exist. The optimal CD composition to use for a separation is dependent on the label and pH. Figure 3 shows rac-DNS-Asp at pH 9.5 at all four CD compositions. It was apparent that 10mM β-CD results in the best resolution (Table I), but with rac-DNS-Val at pH 9.5, the 7.5mM β/19mM γ-CD achieved the best resolution. When lowering the pH to 7.0 for rac-DNS-Val, the 6mM β/1mM γ-CD gave the best resolution out of the four CD compositions. Figure 4 shows rac-NBD-Val at pH 7.0 at all four CD compositions; the two mixed CD compositions show resolution, and the two individual CD compositions do not. The four FITC-labeled amino acids were all able to be separated at pH 9.5. For each amino acid, at least two of the four CD concentrations were useful in obtaining an enantiomeric separation (Table I). It appears that the selectivity of a system can be maximized for a given enantiomeric pair with the correct CD composition, but it does not necessarily have to be the same for all test analytes.

Effect of different labels on enantiomeric separations using CDs

The most noticeable effect of label on the separations in this work is shown in Figure 5. Regardless of the pH, amino acid, or CD concentration in this study, the D-enantiomer migrated first for DNS- and FITC-labeled amino acids, but the L-enantiomer migrated first for NBD-labeled amino acids. This could be because of the effect of the label on mobility of the labeled amino acid/CD complex. For the NBD label, the complex with the L-enantiomer appeared to have a slightly faster mobility, thus resulting in a faster migration.

When performing separations at pH 9.5 with rac-DNS-Ser, no enantiomeric separation was observed. However, when looking at the 10mM β-CD at pH 9.5 for serine (Figure 6), the label appears to affect the resolution of the enantiomers. The NBD (Figure 6B) and FITC (Figure 6C) label give an enantiomeric separation of serine, and the DNS label (Figure 6A) does not give an enantiomeric separation. Similar characteristics can be seen for phenylalanine at pH 7 with the 6mM β/1mM γ-CD concentration and valine at pH 9.5 with the 7.5mM β/19mM γ-CD concentration (see Table I).

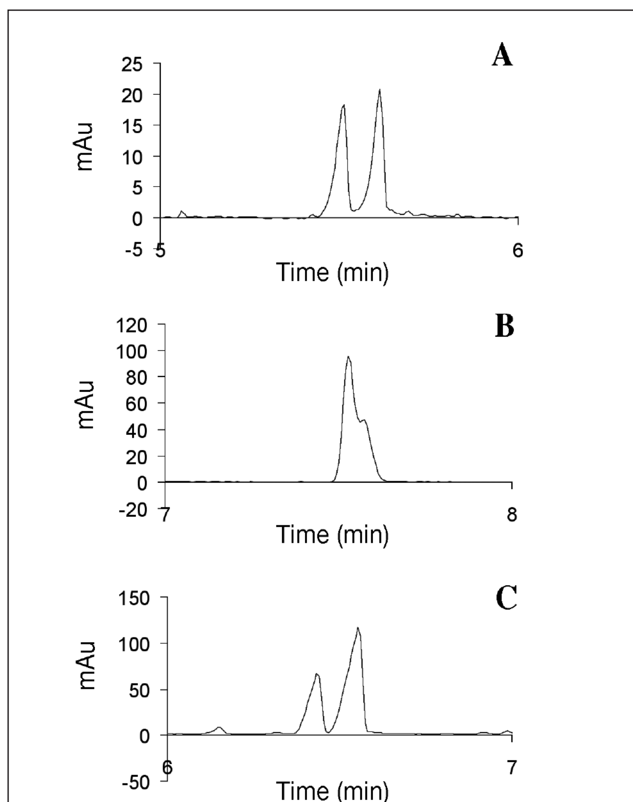

Figure 5. Separation of the enantiomers of DNS-Asp at pH 7.0 with 10mM β-CD (A), NBD-Ser at pH 9.5 with 7.5mM β:19mM γ-CD (B), and FITC-Phe at pH 9.5 with 6mM β:1mM γ-CD (C). Injections were of the ratio 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 μm i.d., 41.5 cm to the window.

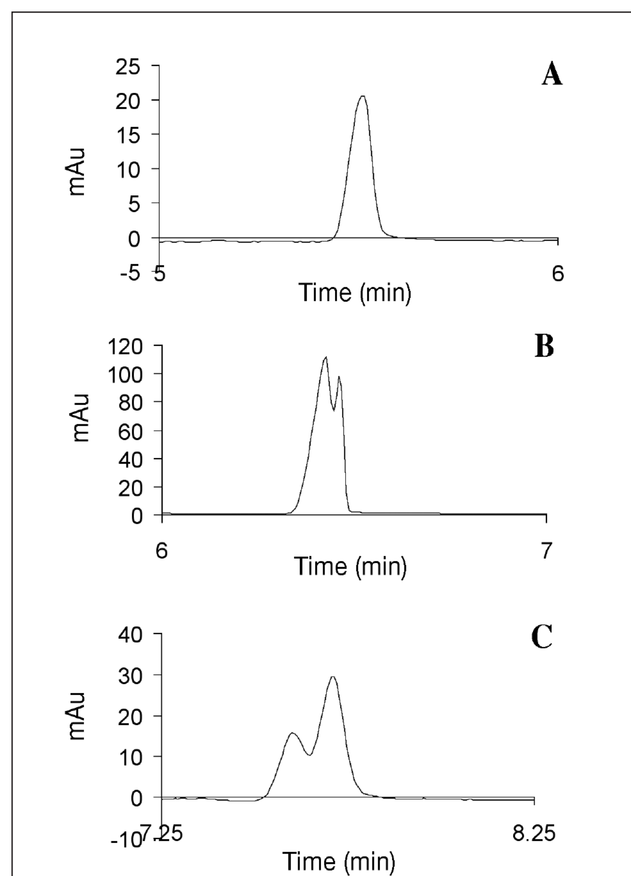

Figure 6. Separation of the enantiomers of serine at pH 9.5 with 10mM β-CD using the DNS (A), NBD (B), and FITC labels (C). Injections were of the ratio 2:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 μm i.d., 41.5 cm to the window.

Effect of surfactant composition on micelle marker

The use of Sudan 3 as a micelle marker was first introduced by Terabe (2). Other markers have been used and several comparative studies have been conducted. (33–35). Although several studies have suggested that dodecanophenone is the best micelle marker, it is dependent on the composition of the surfactant solution (34). However, dodecanophenone has been known to precipitate when exposed to a borate buffer. Therefore, Sudan 3 was chosen to be the micelle marker. Although use of an inappropriate micelle marker can lead to errors and variability in the calculation of the retention factors (33), the purpose of the micelle marker in this study was to obtain an estimate of the electrophoretic mobility of the micelles.

To assess the effect of surfactants as a run buffer additive, comparisons among sodium dodecylsulfate (SDS), STDC, and mixtures of the two surfactants were completed. Work by Haque et al. have shown a decrease in the critical micellar concentration (CMC) of STDC with the addition of SDS, indicating the possible formation of the mixed micelle (36). The effect of SDS in the STDC solution is hypothesized to reduce the number of STDC micelles, increase the number of the trimer, or if a mixed micelle is formed, increase the size of the trimer-based mixed micelles. If mixed micelles contain more than three STDC molecules, the question becomes: will the chiral portion of the molecule be available for interaction with the solute. If a mixed micelle is present, an increase in the charge-to-mass ratio is expected, thus increasing the migration time of the micelle and the micelle marker. There will be a point where the SDS micelle will form and the STDC will incorporate into the SDS micelle. After this point, the magnitude of the electrophoretic mobility of the micelle marker will become constant until the surfactant composition reaches 100% SDS.

Figure 7 contains plots of the electrophoretic mobility of Sudan 3 versus the percent of STDC in the surfactant solution in the borate and phosphate, respectively. As the percent of STDC decreases from 100%, a significant change in the electrophoretic mobility of Sudan 3 was seen. Excluding the error bars associated with each data point, there seemed to be a minimum around 50% SDS–STDC. However, the error bars indicated that the electrophoretic mobility of Sudan III was constant to approximately 33% SDS–67% SDTC.

Effect of solute charge on solute–surfactant interactions and retention factors

At first glance, all solutes have retention in the surfactant systems. However, for a charged species, the mobility of the charged ion must be taken into account. Khaledi et al. published papers regarding the correction of capacity factors of weak acids and bases in MEKC (37,38). In MEKC, the observed migration of a charged solute was because of a weighted average of the electrophoretic mobility of the solute and the mobility of the micelles. There are two published methods to determine the electrophoretic mobility of the solute: one by Khaledi's group, which analyzed the acidic and basic solutes using an unmodified buffer solution (37,38) and the other by Bailey and Dorsey, who used a sodium chloride modified buffer solution as an attempt to reproduce the ionic strength of the micellar solution (39). Both use the same equation to calculate the corrected retention factor (k_c'). The corrected retention factor can be determined by adjusting the observed electrophoretic mobility by subtracting the mobility of the charged solute (equation 1), which is determined by a CZE experiment.

$$k'_c = \frac{\mu - \mu_o}{\mu_{mc} - \mu} \qquad \text{Eq. 1}$$

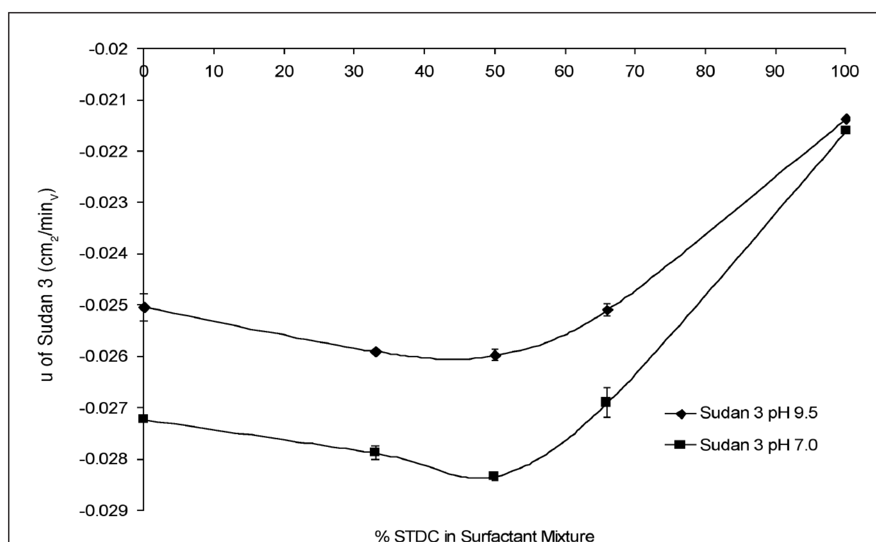

Figure 7. Plot of the electrophoretic mobility of Sudan 3 as a function of STDC concentration in pH 9.5 and 7.0 buffer. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

Table III. Average Electrophoretic Mobilities of Selected Solutes in Various Surfactant Mixtures*

Compounds	100% SDS	66% SDS	50% SDS	33% SDS	0% SDS	Buffer only	60mM NaCl
Electrophoretic mobilities in 50mM borate buffer at pH 9.5							
rac-DNS-Phe	–0.0204	–0.0187	–0.0181	–0.0179	–0.0166	–0.0108	–0.0120
D-DNS-Phe			–0.0181	–0.0181	–0.0167		
rac-DNS-Asp	–0.0196	–0.0196	–0.0196	–0.0193	–0.0182	–0.0196	–0.0224
L-DNS-Asp				–0.0194			
rac-NBD-Val	–0.0147	–0.0147	–0.0151	–0.0153	–0.0149	–0.0143	–0.0175

* Units are in cm²/Vmin, the relative standard deviation for all measurements averaged 0.1%.

where μ is the electrophoretic mobility of the solute in MEKC, μ_{mc} is the electrophoretic mobility of the micelle marker, and μ_o is the electrophoretic mobility of the solute in CZE.

Table III describes the electrophoretic mobility of three labeled amino acids at pH 9.5 in each surfactant mixture, buffer without modifiers, and buffer containing sodium chloride. NBD-Val results were typical for this experiment, and the DNS-Phe and DNS-Asp were the two exceptions. The solutes analyzed in the sodium chloride modified buffers exhibited higher electrophoretic mobilities compared with mobilities in the unmodified buffers. This difference will manifest with calculation of the corrected retention factors, as described in Table IV. Negative retention factors were because of the electrophoretic mobility of the solute in CZE being higher in magnitude than the solute in the surfactant solution. NBD-Val corrected values were all less than one, indicating that this solute had limited interaction with the surfactant mixture. The DNS-Phe corrected values were a little greater or close to one, indicating that the solute–surfactant interaction was enough to result in an enantiomeric separation.

Table IV. Average Uncorrected and Corrected Retention Factors of Selected Solutes in the Surfactant Solutions*

Compounds	100% SDS	66% SDS	50% SDS	33% SDS	0% SDS
Uncorrected retention factors of compounds at pH 9.5					
rac-DNS-Phe	4.36	2.58	2.29	2.51	3.50
D-DNS-Phe			2.31	2.56	3.57
rac-DNS-Asp	3.58	3.14	3.09	3.33	5.74
L-DNS-Asp				3.43	
rac-NBD-Val	1.42	1.32	1.38	1.56	2.31
Retention factors corrected for charge and ionic strength at pH 9.5					
rac-DNS-Phe	1.80	0.93	0.77	0.84	0.98
D-DNS-Phe			0.79	0.87	1.01
rac-DNS-Asp	–0.52	–0.44	–0.44	–0.54	–1.33
L-DNS-Asp				–0.53	
rac-NBD-Val	–0.27	–0.25	–0.26	–0.22	–0.40
Retention factors corrected for charge at pH 9.5					
rac-DNS-Phe	2.04	1.08	0.92	1.00	1.22
D-DNS-Phe			0.93	1.03	1.26
rac-DNS-Asp	–0.01	0.004	0.001	–0.06	–0.45
L-DNS-Asp				–0.03	
rac-NBD-Val	0.04	0.04	0.07	0.10	0.09

* The relative standard deviation for all measurements averaged 0.1%.

Both uncorrected and corrected retention factors tended to decrease as the amount of co-surfactant increased with a minimum around 33% SDS, indicating a reduction in solute–surfactant interactions. This seemed to be counterintuitive, especially in light of the enantiomeric separation in dual surfactant solutions. This phenomenon can be explained by assuming that the majority of the retention of the solute in pure STDC was because of the presence of the cylindrical micelle, which was not enantiomerically selective. In the mixed surfactant solutions, the solutes were interacting specifically with the trimer or the mixed micelle, hence increasing the specificity of the separation. From this data, the solutes were assumed to have a lower affinity to the mixed micelle when compared with the affinity for the cylindrical micelle.

Separation of enantiomers using mixed surfactants

In this work, rac-DNS-Phe was separated in three surfactant mixtures (50% SDS, 33% SDS, and 0% SDS) and rac-DNS-Asp was separated in one surfactant mixture (33% SDS), all under 10 min. Table I shows the average resolution of each additive composition and indicates that the 33% SDS surfactant mixture is optimal in separating the two enantiomers. The partial separation of the rac-DNS-Phe in 0% SDS can be explained by the presence of both trimer and cylindrical micelle being present in solution and rac-DNS-Phe having a higher interaction with the trimer. Figure 8 shows the separation of the rac-DNS-Phe in the 33% SDS surfactant mixture. The L-enantiomer for DNS-Phe was determined to migrate first. However, Figure 9 shows that separation of the rac-DNS-Asp and the L-enantiomer of DNS-Asp were determined to migrate last. This change in the elution order can be explained. The electrophoretic mobility of the uncomplexed rac-DNS-Asp was greater in magnitude than the micelle marker; hence, the negative corrected retention factors. Thus, as the solute interacted with the micelle, it actually sped up with respect to the detector. The broadening of the D-DNS-Asp was also observed for the D-NBD-Asp (Figure 2B). However, the observed band broadening cannot be explained at this time.

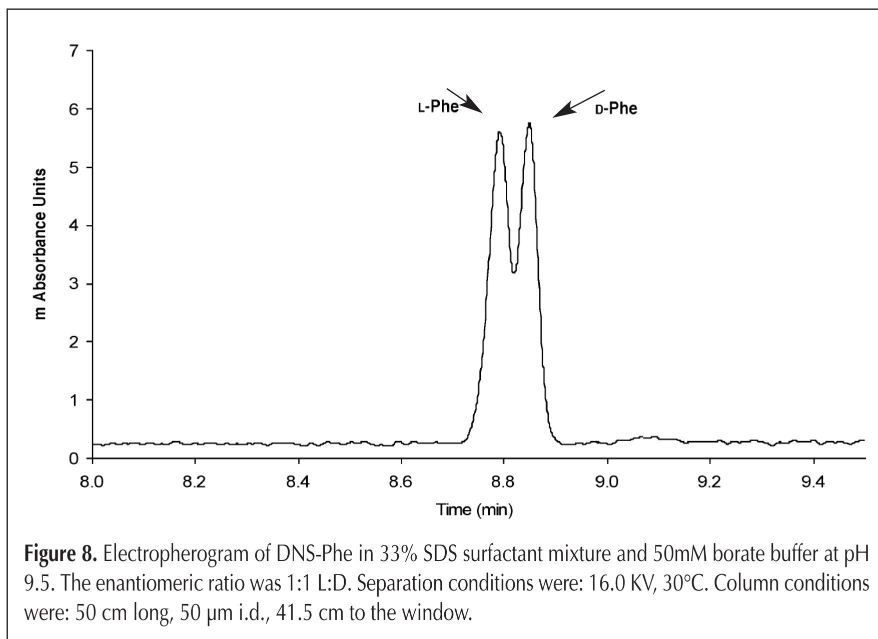

Figure 8. Electropherogram of DNS-Phe in 33% SDS surfactant mixture and 50mM borate buffer at pH 9.5. The enantiomeric ratio was 1:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

Work performed by Amini et al. has shown a concentration dependence of the chiral surfactant on the resolution between two enantiomers with an optimal concentration of the surfactant for max-

imum resolution (40). This work was successfully duplicated by analyzing the rac-DNS-Phe in various concentrations of STDC, as shown in Figure 10. Comparing Figure 8 with Figure 10, the results indicate that the separation of the rac-DNS-Phe improves with the introduction of SDS. This is a clear indication that the presence of the cylindrical micelle actually compromises the enantiomeric separations.

Comparison of dual surfactant system with CDs

In a comparison of the effectiveness of the dual surfactant system with the CD, the CDs have a distinct advantage as an additive for the enhancement of chiral separations. More enantiomers were separated with comparable or higher resolution in the CD systems studied, yet the dual surfactant system exhibited a similar quality of separation when enantiomeric separation was achieved.

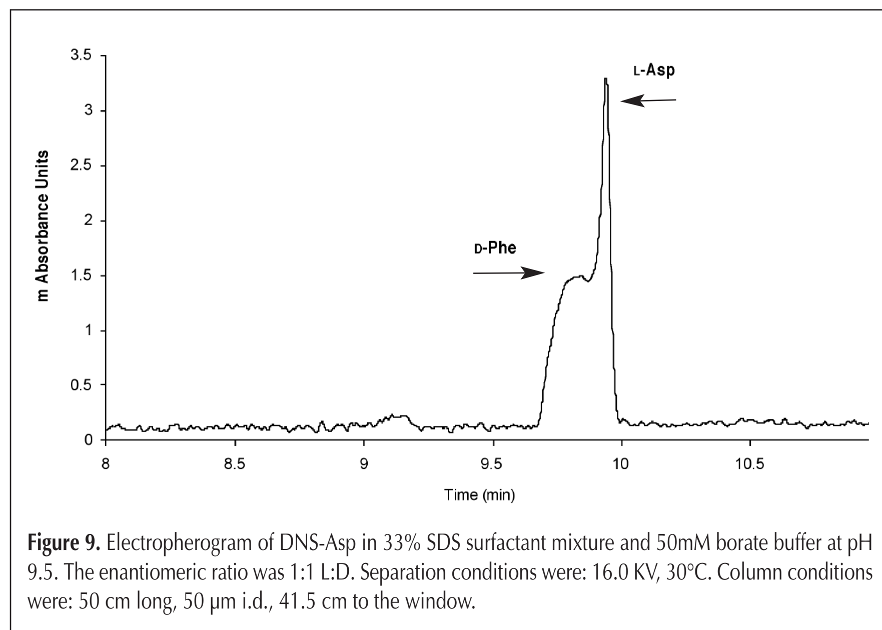

Figure 9. Electropherogram of DNS-Asp in 33% SDS surfactant mixture and 50mM borate buffer at pH 9.5. The enantiomeric ratio was 1:1 L:D. Separation conditions were: 16.0 KV, 30°C. Column conditions were: 50 cm long, 50 µm i.d., 41.5 cm to the window.

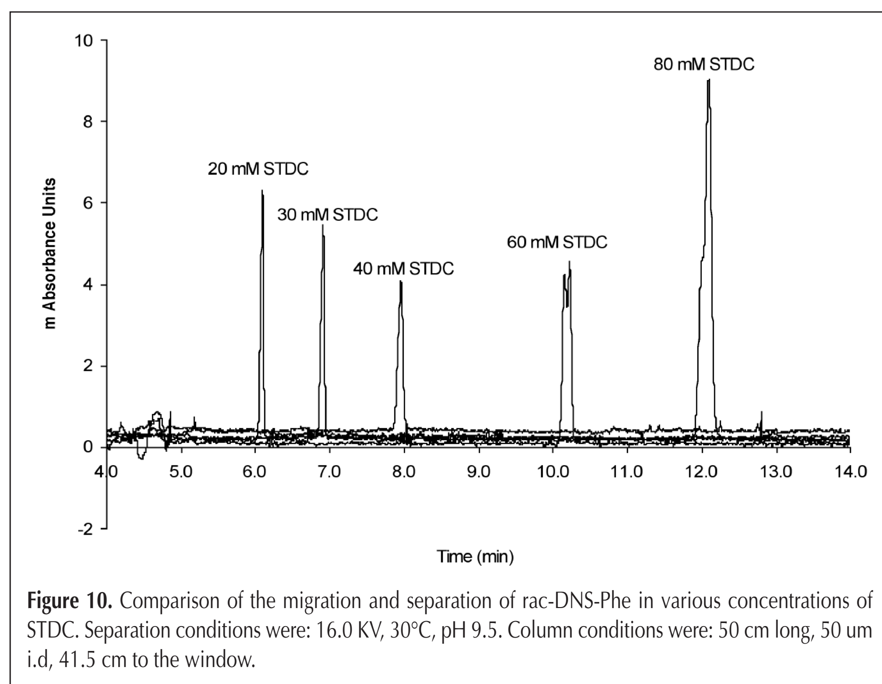

Figure 10. Comparison of the migration and separation of rac-DNS-Phe in various concentrations of STDC. Separation conditions were: 16.0 KV, 30°C, pH 9.5. Column conditions were: 50 cm long, 50 um i.d, 41.5 cm to the window.

Conclusion

Separation of the DNS-labeled phenylalanine and aspartic acid using a mixed surfactant as a run buffer additive was successful. Possible limitations for separation of the enantiomers include the fact that there is little interaction of the solute with the surfactants, the negative charge of the solute is limiting the separation window of the system, and the amount of the chiral phase available for partitioning is limited. There is enough evidence present in this work to demonstrate that using a different chiral selector and/or co-surfactant could improve the number and quality of the separation of the enantiomers. The separations using CDs as a chiral selector showed that the label affected migration order of the enantiomers and the CDs were very effective in separating numerous enantiomers.

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