

Optimization of 12 Chiral Analytes with 8 Polymeric Surfactants

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Abstract

This manuscript discusses the results of studies that were performed to determine optimum capillary electrophoresis (CE) conditions for the enantiomeric resolution of twelve chiral analytes with eight amino acid based polymeric surfactants. The parameters that were optimized include pH, buffer type, and concentration of surfactant. The results indicated that the optimum conditions for enantiomeric separations with the amino acid based polymeric surfactants examined in this study using CE were analyte dependent, not surfactant dependent. In other words, the optimum conditions for a particular analyte were the same for all the amino acid based polymeric surfactants examined in this study. The results of these studies indicate that when using a large group of related amino acid based polymeric surfactants only a few surfactants need to be optimized for each analyte under study. These studies were limited to anionic surfactants that contain the amino acids glycine, L-alanine, L-valine, and L-leucine only. No inference can be necessarily drawn about surfactants containing other types of amino acids such as threonine and serine, which contain extra heteroatoms, or phenylalanine that has an aromatic moiety.

Introduction

Although enantiomers of chiral compounds often exhibit different biological activities, many chiral pharmaceutical drugs are still sold as racemic mixtures (1). Therefore, the need for analytical methods to discriminate between different enantiomeric forms is very important. Developing techniques to separate chiral compounds is one of the most active and difficult areas of analytical chemistry. Chromatographic methods which have typically been used for chiral separations are high-performance liquid chromatography (HPLC) (2–6), gas chromatography (GC) (7–10), and capillary electrophoresis (CE) (11–14). However, the use of GC is limited to volatile/non-thermally labile compounds, and HPLC exhibits much lower separation efficiencies as compared to CE. In addition to lower separation efficiencies, chiral separations with HPLC require the use of very expensive chiral columns that will only work well for a limited number of chiral

compounds. Because of the limitations of HPLC and GC, and the high efficiency of CE, separation of chiral compounds with CE has become increasingly popular in recent years (14).

Two different strategies are employed for chiral separation with CE. The first technique employs capillaries that have been modified using a chiral selector, while the second technique employs chiral additives in the running buffer. Some of the more common chiral additives which have been used in CE are cyclodextrins (15–16), crown ethers (17–20), peptides and proteins (including macrocyclic antibiotics) (20), polysaccharides (22–23), and chiral [monomeric (24–28) and polymeric (29–41)] surfactants. When surfactants are added to the running buffer, the technique is called micellar electrokinetic chromatography (MEKC). Micellar electrokinetic chromatography was introduced by Terabe and co-workers in 1984 (39) in order to simultaneously separate charged and neutral species. Cohen et al. were the first to employ MEKC for the enantiomeric separation of chiral compounds (40).

Unfortunately, the use of surfactants as pseudostationary phases in CE has certain distinct disadvantages for the separation of chiral compounds, as well as achiral compounds (41). Although the separation efficiencies using MEKC are higher than those observed with HPLC, the dynamics of the micellar system cause a decrease in the separation efficiency as compared to capillary zone electrophoresis. In addition, the elution window in MEKC is limited. Therefore, organic modifiers are usually added to increase the elution window. However, micelles can not tolerate high concentrations of organic solvents. High organic solvent concentrations disrupt the formation of micelles. Moreover, high concentrations of ionic surfactants produce excess current, which creates Joule heating. Joule heating causes band dispersion, which leads to a decrease in separation efficiency.

Polymeric surfactants have been employed in CE to minimize some of the disadvantages of MEKC. One advantage of polymeric surfactants is the elimination of the dynamic equilibrium between monomer and micelle. Elimination of the dynamic equilibrium minimizes problems that are often associated with monomers in chromatography. Another advantage is the lack of a critical micelle concentration (CMC). Thus, the polymer can be used over a wider range of concentrations than the monomer (e.g., below the normal CMC of the unpolymerized surfactants).

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In addition, organic modifiers can be used without disrupting the formation of the micelle. Finally, the structural rigidity and purification of the micelle polymer can often improve the mass transfer rate, thus reducing peak broadening.

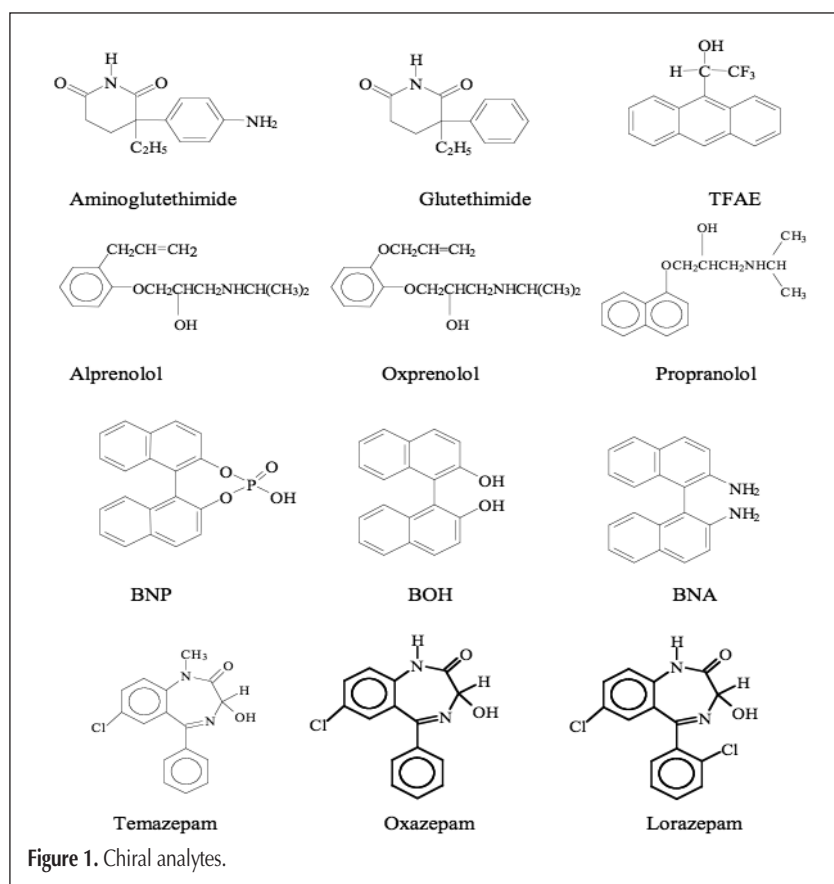
This manuscript discusses the results of studies that were performed to determine optimum CE conditions for the enantiomeric separation of twelve chiral analytes with eight amino acid-based polymeric surfactants. The parameters that were optimized include pH, buffer type, and concentration of surfactant. These results indicate that the optimum conditions for enantiomeric separations of chiral compounds with amino acid based polymeric surfactants containing similar amino acids using CE is analyte dependent, not surfactant dependent. In other words, the optimum conditions for a particular analyte would be the same for other amino acid based polymeric surfactants containing L-glycine, L-alanine, L-valine, and L-leucine. No inference can be necessarily made about surfactants containing other

types of amino acids such as threonine and serine, which contain extra heteroatoms, or phenylalanine which has an aromatic moiety.

Glossary of terms

Abbreviations used for analytes: 1'-bi-2-naphthol (BOH), 1,1'-bi-2-naphthyl-2,2'-diamine (BNA), 1,1'-bi-2-naphthyl-2,2'-diyl hydrogen phosphate (BNP), propranolol (Prop), alprenolol, (Alp), oxprenolol, (Oxp), temazepam (Temaz), lorazepam (Loraz), oxazepam (Oxaz), glutethimide (Glut), aminoglutethimide (Amino), and trifluoranthryl-ethanol (TFAE).

Abbreviations used for polymeric surfactants: poly sodium undecyl L-alanine (poly L-SUA), poly sodium undecyl L-leucine (poly L-SUL), poly sodium undecyl L-glycine-leucine (poly L-SUGL), poly sodium undecyl (L,L) alanine-valine [poly (L,L) SUAV], poly sodium undecyl (L,L) alanine-leucine [poly (L,L) SUAL], poly sodium undecyl (L,L) valine-alanine [poly (L,L) SUVA], poly sodium undecyl L-leucine-glycine (poly L-SULG), and poly sodium undecyl (L,L) leucine-alanine [poly (L,L) SULA].



Experimental

Materials

The racemic mixtures and the pure optical isomers of BOH, BNA, BNP, Prop, Alp, Oxp, Temaz, Loraz, Oxaz, Glut, Amino, and TFAE were purchased from Aldrich (Milwaukee, WI). The tris(hydroxymethyl)aminomethane (TRIS), and sodium borate were obtained from Fisher Scientific Company (Fair Lawn, NJ) and used as received. Chemicals used for the synthesis of surfactants included: *N,N'*-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide, undecylenic acid, various amino acids, and the dipeptides. All were supplied by Sigma (St. Louis, MO) and used as received.

Synthesis of polymeric surfactants

All surfactants in this study were synthesized using the procedure reported by Wang and Warner (31). Surfactant monomers were prepared by mixing the *N*-hydroxysuccinimide ester of undecylenic acid with the amino acid or dipeptide to form the corresponding *N*-undecylenyl chiral surfactant. Polymerization was achieved by ^{60}Co γ -irradiation. All polymers used in this study were found to be 99% pure or better as estimated from elemental analysis. The surfactants used in this study are poly sodium undecyl L-alanine (poly L-SUA), poly sodium undecyl L-leucine (poly L-SUL), poly sodium undecyl L-glycine-leucine (poly L-SUGL), poly sodium undecyl (L,L) alanine-valine [poly (L,L) SUAV], poly sodium undecyl (L,L) alanine-leucine [poly (L,L) SUAL], poly sodium undecyl (L,L)

Table I. Buffer Conditions Used for pH Optimization Studies

pH	Buffer
7.0	100mM boric acid, 25mM sodium diphosphate
8.0	100mM boric acid
8.6	50mM sodium borate
9.1	50mM sodium borate
9.2	50mM TRIS
10.0	30mM sodium borate
10.2	100mM TRIS

valine-alanine [poly (L,L) SUVA], poly sodium undecyl L-leucine-glycine (poly L-SULG), and poly sodium undecyl (L,L) leucine-alanine (poly (L,L) SULA).

Capillary electrophoresis procedure

The EKC experiments were conducted on a Hewlett Packard ³DCE model # G1600AX. An untreated fused silica capillary (effective length 55 cm, 50 μ m i.d.) was purchased from Polymicro Technologies (Phoenix, AZ). Separations were performed at +30 kV, with UV detection at 215 nm. The temperature of the capillary was maintained at 25°C for BOH, BNA, and BNP, and 12°C for the rest of the analytes by the instrument thermostating system, which consisted of a Peltier element for forced air cooling and temperature control. The buffer conditions vary and are given in the main body of the text. The concentration of surfactant for all the pH studies was 15mM equivalent monomer concentrations (EMC). All samples were prepared in 1:1 methanol-H₂O. The concentration of some of the analytes (BOH, BNA, BNP, TAFE, Temaz, Loraz, and Oxaz) was 0.1 mg/mL. The concentration of the other analytes was 0.5 mg/mL for Alp, and Oxp, and 0.2 mg/mL for Prop, Amino, and Glut. The samples were injected for 5 s with 10 mbar of pressure. Prior to use, the new capillary was conditioned for 30 min with 1M NaOH followed by 30 min of 0.1 N NaOH. Then, the capillary was rinsed for 15 min with deionized water. Prior to each run, the buffer was pressure injected through the column for 2 min to condition and fill the capillary.

Table II. Effect of pH with Various Surfactants on the Enantiomeric Separation of BOH, BNA, and BNP

Surfactant	pH 7.0	pH 8.0	pH 8.6	pH 9.1	pH 9.2	pH 10.0	pH 10.2
<i>(A) BOH: Enantiomeric resolution at various pHs</i>							
L-SUA	4.2	4.8	8.2	9.1	*	11.1	*
L-SUL	2.9	3.7	7.0	7.1	*	7.9	*
(L,L) SUGL	0	0	0.5	0.5	*	0.6	*
(L,L) SUAV	0	0.5	0.6	1.0	*	1.3	*
(L,L) SUAL	0	0	0.7	0.8	*	0.6	*
(L,L) SUVA	2.0	2.0	3.9	3.9	*	4.1	*
(L,L) SULG	4.0	5.2	5.0	8.1	*	7.9	*
(L,L) SULA	3.2	3.2	7.7	8.1	*	7.9	*
<i>(B) BNA: Enantiomeric resolution at various pHs</i>							
L-SUA	2.5	3.0	4.2	5.1	*	5.3	*
L-SUL	3.1	3.6	6.2	6.9	*	7.7	*
(L,L) SUGL	0	0	0	0	*	0	*
(L,L) SUAV	1.0	0.8	2.0	2.2	*	2.7	*
(L,L) SUAL	0.7	0.7	1.8	1.8	*	2.2	*
(L,L) SUVA	2.0	2.0	3.9	3.9	*	4.1	*
(L,L) SULG	2.5	3.2	3.3	5.3	*	5.4	*
(L,L) SULA	1.8	2.2	4.7	4.7	*	4.8	*
<i>(C) BNP: Enantiomeric resolution at various pHs</i>							
L-SUA	0	0	0	0	*	0	*
L-SUL	0.9	0	0.9	0.7	*	0.7	*
(L,L) SUGL	3.5	2.5	4.4	4.4	*	4.6	*
(L,L) SUAV	0	0	0	0	*	0	*
(L,L) SUAL	1.0	0.7	1.0	1.0	*	1.1	*
(L,L) SUVA	3.7	2.6	4.3	4.4	*	4.4	*
(L,L) SULG	6.5	5.2	6.4	7.6	*	8.1	*
(L,L) SULA	7.8	5.4	8.8	8.5	*	8.8	*

* Buffers not used.

Results and Discussion

BOH, BNA, and BNP

The first set of analytes to be discussed are the binaphthyl derivatives BOH, BNA, and BNP. The structure of these analytes, as well as the other analytes examined, are shown in Figure 1. The buffer conditions used in this study are given in Table I. However, the buffers containing TRIS were not included in the study of BOH, BNA, and BNP because previous studies by our group have already investigated optimum conditions for these analytes using TRIS as the buffer (36,37). The results of the pH optimization studies for BOH, BNA, and BNP are shown in Table II. Examination of the data in Table II indicates that the enantiomeric separation of all three binaphthyl derivatives are separated best at higher pHs, with the best resolution occurring at pH 10. The trends for BOH and BNA can be easily seen with the surfactant poly L-SUA, Table II (part A & B) respectively. As the pH of the buffer increases, so does the enantiomeric resolution of BNA and BOH. The resolution of BOH went from 4.2 at pH 7.0 to a resolution of 11.1 at pH 10.0. The resolution of BNA also increases from 2.5 at pH 7.0 to a resolution of 5.3 at pH 10.0. However, not much of a change in resolution occurs from pH 9.1

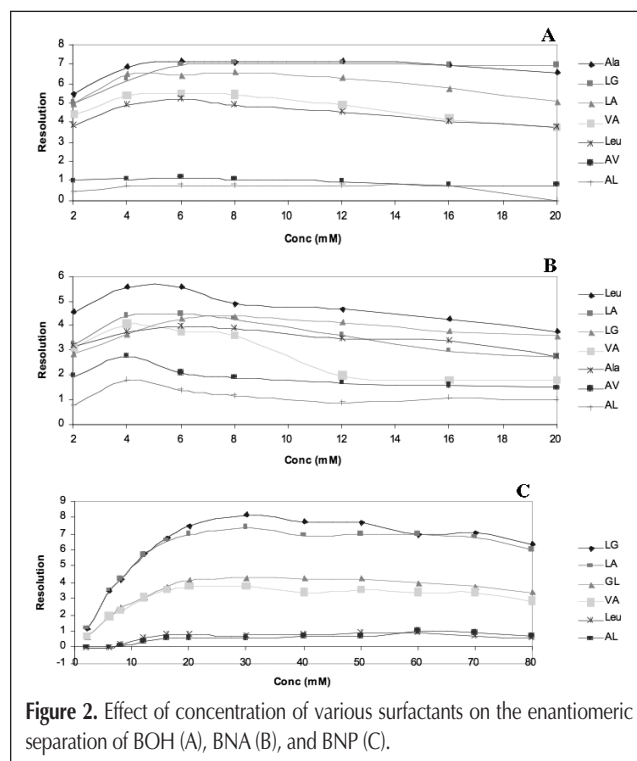


Figure 2. Effect of concentration of various surfactants on the enantiomeric separation of BOH (A), BNA (B), and BNP (C).

to pH 10.0. Similar trends are also observed for BOH and BNA with the rest of the polymeric surfactants.

Because poly L-SUA was not able to resolve the enantiomers of BNP, another surfactant must be examined to observe the effect of pH on the enantiomeric separation of BNP. As shown in Table II, poly L-SUGL was able to separate the enantiomers of BNP but not BNA and only slight resolution of BOH was observed. The resolution of BNP does not change much at pHs of 8.6, 9.1, and 10.0, Table II (part C). The resolutions are all about 4.5 ± 0.1 . However, a drop in resolution at pH 8.0 is observed and an increase in resolution is observed at pH 7.0 as compared to pH 8.0. These same basic trends are observed for the other surfactants which were able to enantiomerically resolve BNP. The results of the pH studies indicate that all three analytes (BOH,

BNP, and BNA) are enantiomerically resolved best at pH 10.0. Therefore, pH 10.0 was chosen to perform concentration studies.

The results of the concentration studies for BOH are shown in Figure 2A. The enantiomeric resolution of BOH appears to reach a plateau at approximately 6mM. All of the surfactants that gave adequate separation of BOH show the same trend. The optimum concentration appears to be analyte dependent, not surfactant dependent. In a similar manner to BOH, the optimum concentration of surfactant for BNA is approximately $5\text{mM} \pm 1$ for all the surfactants examined, Figure 2B.

The concentration studies for BNP are shown in Figure 2C. As with BOH and BNA, the optimum concentration appears to be the same for all the surfactants which gave adequate enantiomeric separation. The optimum concentration of surfactant for the enantiomeric separation of BNP is approximately 30mM. The optimum concentration is significantly higher for BNP than was observed for BOH and BNA. The difference in optimum concentration is believed to be due to the fact that BNP is anionic under the conditions used, while BNA is neutral and BOH is only slightly anionic. Since BNP is anionic, it is less hydrophobic. Therefore, the association constant would be less for BNP as compared to BOH and BNA. Thus, higher concentrations of surfactant are needed to attain optimal resolution.

Propranolol, alprenolol, and oxprenolol

Determination of optimum pH for the three β -blockers (Alp, Prop, and Oxp) was not as straightforward as with the binaphthyls. The results of the

pH studies are shown in Table III. Only two of the eight surfactants gave adequate enantioseparation of these three analytes under the conditions examined for the pH studies. These two surfactants were poly (L,L) SUAL and poly L-SUGL. Since only these two surfactants were able to adequately resolve these analytes, the optimum pH was derived from these two surfactants. Examination of Table III (part A & C) shows that the best enantiomeric separation of Alp and Prop with poly (L,L) SUAL was achieved at pH 9.1. However, no separation of Oxp was observed. In contrast, all three of these chiral compounds were enantiomerically resolved at pH 8.6 with poly L-SUGL. Therefore, the concentration studies were performed at pH 8.6.

Another point of interest to note is that in a comparison of pH 9.1 to 9.2 and pH 10.0 to 10.2, a decrease in resolution is observed with the buffers containing TRIS (pH 9.1 and 10.2). Thus, at these pH values and buffer conditions, borate was shown to be a more effective buffer for the enantiomeric separation of Prop, Alp, and Oxp than TRIS.

The concentration studies for Prop, Alp, and Oxp are shown in Figures 3A–3C. Examination of the concentration studies for Prop indicate that all surfactants follow the same trend (Figure 3A). An increase in resolution is observed from 2mM up to approximately 10 or 12mM for all the surfactants. Not much change in resolution is observed after that. This same behavior is seen in Figures 3B and 3C for Alp and Oxp, respectively. The resolution of Alp increases up to concentrations approximately 16mM, after which the resolution levels off, Figure 3B. The enan-

Table III. Effect of pH with Various Surfactants on the Enantiomeric Separation of Propranolol, Alprenolol, and Oxprenolol

Surfactant	pH 7.0	pH 8.0	pH 8.6	pH 9.1	pH 9.2	pH 10.0	pH 10.2
<i>(A) Alprenolol: Enantiomeric resolution at various pHs</i>							
(L,L) SUGL	0.7	0	1.1	0.7	0.1	0.7	0.5
(L,L) SUAL	0.6	0	0.8	1.1	0	0.8	0.3
<i>(B) Oxprenolol: Enantiomeric resolution at various pHs</i>							
(L,L) SUGL	1.0	0.6	1.6	0	0	0	0
(L,L) SUAL	0.8	0	0	0	0	0	0.4
<i>(C) Propranolol: Enantiomeric resolution at various pHs</i>							
(L,L) SUGL	0.6	0	1.5	1.2	0	0.9	0.6
(L,L) SUAL	0.5	0	1.5	1.6	0	1.3	0.4

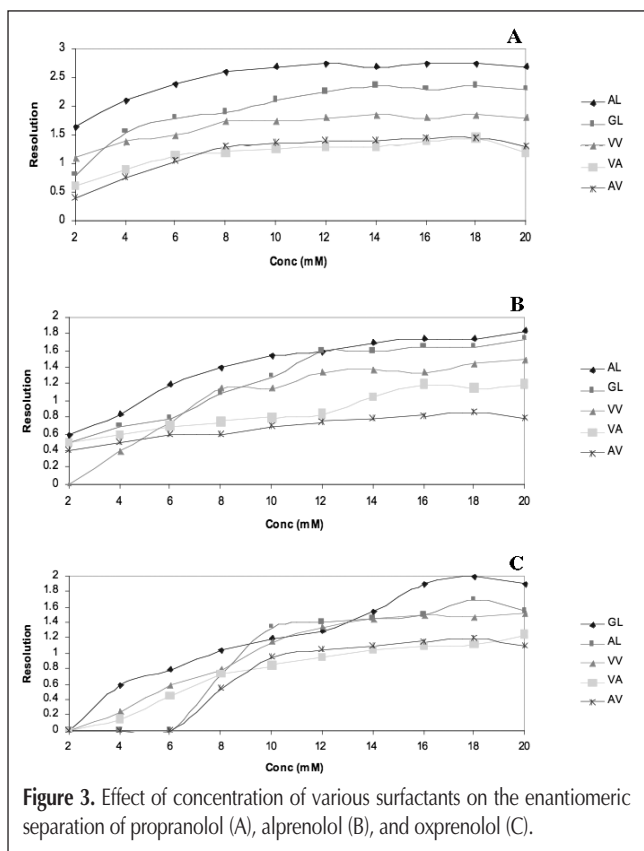


Figure 3. Effect of concentration of various surfactants on the enantiomeric separation of propranolol (A), alprenolol (B), and oxprenolol (C).

tiomeric resolution of Oxp also appears to level off after 16mM for all surfactants (Figure 3C). The optimum concentration of surfactant is again shown to be analyte dependent, not surfactant dependent. This is in agreement with the results observed in the previous section with the binaphthyl derivatives.

TFAE, Amino, and Glut

The results of the pH studies for TFAE, Amino, and Glut are shown in Table IV. An examination of these data indicate that

poly L-SUL separated the enantiomers of TFAE better than the other surfactants examined in this study, Table IV (part A). A steady increase in resolution from pH 7 to pH 10.0 for the buffers that do not contain TRIS can be seen. However, a drop in resolution is observed for the two buffers that contain TRIS (pH 9.2 and pH 10.2). This same trend is observed for the other surfactants where measurable separation of TFAE was achieved. Therefore, it was determined that TRIS was not a good buffer for the separation of TFAE and that the optimum pH for TFAE was ~10.0.

Surfactant	pH 7.0	pH 8.0	pH 8.6	pH 9.1	pH 9.2	pH 10.0	pH 10.2
<i>(A) TFAE: Enantiomeric resolution at various pHs</i>							
L-SUA	0	0	0.5	0.6	0	0.6	0
L-SUL	0	1.3	1.3	1.6	1.0	2.1	1.4
(L,L) SUAV	0	0.6	0.6	0.7	0	0.9	0
(L,L) SUAL	0	0	0.6	0.6	0	0.8	0.6
(L,L) SUVA	0	0.5	0.7	1.0	0.6	1.0	0
<i>(B) Aminoglutethimide: Enantiomeric resolution at various pHs</i>							
L-SUA	0.6	0.6	0.7	0.6	0.7	0.6	0.7
L-SUL	0.7	0.5	0.7	0.7	1.0	0.7	1.0
(L,L) SUAV	0	0	0.5	0	0.7	0	0.6
(L,L) SUAL	0.6	0.4	0.6	0.5	0.8	0.6	0.7
(L,L) SUVA	0.7	0.6	0.7	0.7	1.1	0.7	0.9
(L,L) SULA	0.7	0.5	0.5	0	0.9	0.6	0.8
<i>(C) Glutethimide: Enantiomeric resolution at various pHs</i>							
(L,L) SUVA	0.7	0.7	0.7	0.7	0.7	0.7	0.7

The concentration studies for TFAE show that the optimum concentration is the same for all of the surfactants which gave adequate separation (Figure 4A). Four of the surfactants examined (poly L-SUA, poly L-SUGL, poly L-SULG, and poly (L,L) SULA) did not adequately resolve TFAE and are therefore not shown in the figure. The optimum concentration of TFAE was approximately 6 mM for all four of the surfactants shown.

An examination of the data in Table IV (part B & C) indicate that the pH of the buffer did not affect the resolution of Amino or Glut significantly. However, in all cases where one buffer performed better than the others for the enantiomeric separation of Amino and Glut, pH 9.2 was the best condition. The best separation of Amino and Glut was achieved with poly (L,L) SUVA. As can be seen, no difference was observed in the resolution of Glut at various pH values. However, the enantiomeric resolution of Amino is slightly higher at pH 9.2 than the other buffer conditions examined. Also, the type of buffer did not seem to be much of a factor in

the enantiomeric separation of Amino and Glut. The TRIS buffers seem to perform about as well or better than the borate buffers. The same trends are observed with the other surfactants which gave adequate resolution. Therefore, pH 9.2 was chosen for the concentration studies.

Figures 4B and 4C show the results of the concentration studies for Amino and Glut, respectively. The resolution of Amino appears to be increasing beyond the highest concentration of surfactants examined in this study. At 100mM surfactant the current started to become excessive and the baseline became very noisy. Therefore, higher concentrations were not used. However, as before, all surfactants followed the same trends indicating that the optimum concentration of surfactant is analyte dependent not surfactant dependent. The same general trends are observed for Glut in Figure 4C. The optimum concentration for Glut appears to be approximately 70mM, with all surfactants following the same trend.

Temazepam, Oxazepam, and Lorazepam

The enantiomeric separation of the benzodiazepams (Temaz, Lorax, and Oxaz) was also not greatly affected by pH. The results of the pH studies are shown in Table V (note, only those surfactants which showed enantioselectivity towards these analytes are shown in this table). The best overall surfactant for the enantiomeric separation of the benzodiazepams examined in this study was poly (L,L) SUAL. Examination of the data shows that at low pH (pH 7 and 8) the resolutions were about the same as the

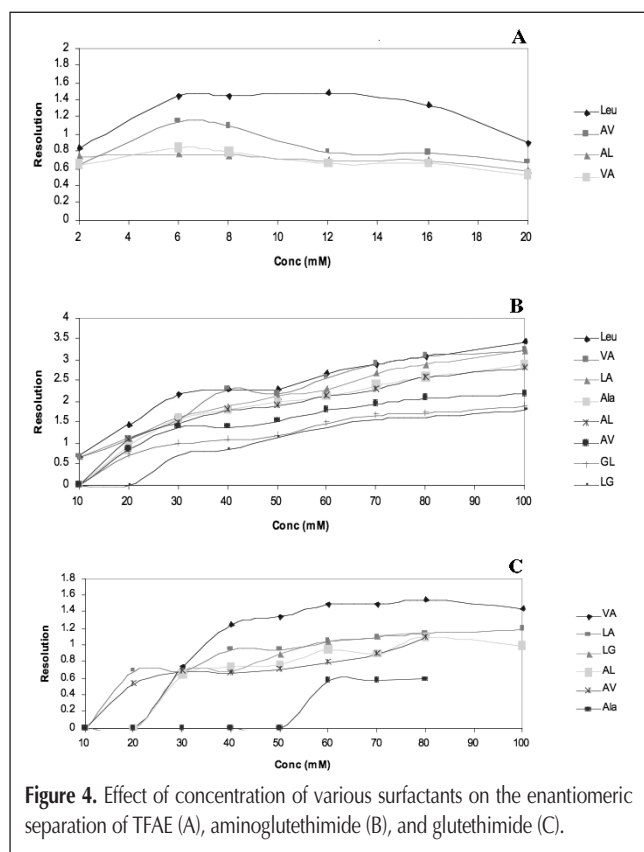


Figure 4. Effect of concentration of various surfactants on the enantiomeric separation of TFAE (A), aminoglutethimide (B), and glutethimide (C).

Table V. Effect of pH with Various Surfactants on the Enantiomeric Separation of Temazepam, Oxazepam, and Lorazepam

Surfactant	pH 7.0	pH 8.0	pH 8.6	pH 9.1	pH 9.2	pH 10.0	pH 10.2
<i>(A) Temazepam: Enantiomeric resolution at various pHs</i>							
L-SUL	2.2	1.9	1.7	2.5	1.9	1.9	2.0
(L,L) SUGL	3.0	2.4	2.4	3.2	2.6	3.0	2.7
(L,L) SUAV	0.8	0.7	0.6	0.7	0.8	0.7	0.7
(L,L) SUAL	2.9	2.6	1.9	3.1	2.5	3.0	2.7
(L,L) SUVA	0.7	0.7	0.6	0.7	0.7	0.7	0.1
(L,L) SULG	0.6	0.7	0	0	0.7	0	0
(L,L) SULA	2.0	1.8	1.7	2.4	1.6	2.3	1.7
<i>(B) Oxazepam: Enantiomeric resolution at various pHs</i>							
L-SUA	0.6	0.7	0	0	0.7	0	0.7
L-SUL	0.7	0.6	0	0	0.8	0	0.8
(L,L) SUGL	0.7	0.7	0	0	0.7	0	0.7
(L,L) SUAV	0	0	0	0	0	0	0
(L,L) SUAL	1.1	1.4	0	0	1.3	0	1.3
(L,L) SUVA	0.5	0.6	0	0	0	0	0
(L,L) SULG	0	0	0	0	0	0	0
(L,L) SULA	0	0	0	0	0	0	0
<i>(C) Lorazepam: Enantiomeric resolution at various pHs</i>							
L-SUA	0	0	0	0	0	0	0
L-SUL	0.6	0.6	0	0	0.7	0	0.7
(L,L) SUGL	0	0	0	0	0	0	0
(L,L) SUAV	0.7	0.7	0	0	0.7	0	0.6
(L,L) SUAL	1.8	1.7	0.8	0.9	1.7	0	1.8
(L,L) SUVA	1.7	1.6	0.8	0	1.6	0	1.2
(L,L) SULG	0.8	1.2	0	0	1.1	0	0.9
(L,L) SULA	0.9	1.1	0	0	0.8	0	1.0

buffers containing TRIS at the higher pH values (pH 9.2 and 10.2). The other surfactants appear to behave in a similar manner. Since no buffer appeared to be necessarily better than another, a combination of borate and TRIS (25mM TRIS and 25mM borate) at intermediate pH 8.5 was used for the concentration studies.

The results of the concentration studies for Temaz, Loraz, and Oxaz are shown in Figures 5A–5C. As with all of the other analytes examined in this study, the optimum concentration is the same for all surfactants. The optimum concentration of surfactant appears to be approximately 20mM for Temaz, (Figure 5A) and around 8mM for Oxaz and Loraz, Figures 5B and 5C, respectively.

Conclusion

The results of these studies demonstrated that the optimum CE conditions for the enantiomeric separation of the chiral compounds examined in this study using amino acid based polymeric surfactants are analyte dependent, not surfactant dependent. Therefore, it is reasonable to assume that this would also be true for other anionic single amino acid or dipeptide surfactants that contain only glycine, alanine, valine, or leucine as part of the hydrophilic moiety of the polar head group. The results of these studies indicate that when using a large group of related amino acid based polymeric

surfactants only a few surfactants need to be optimized for each analyte under study.

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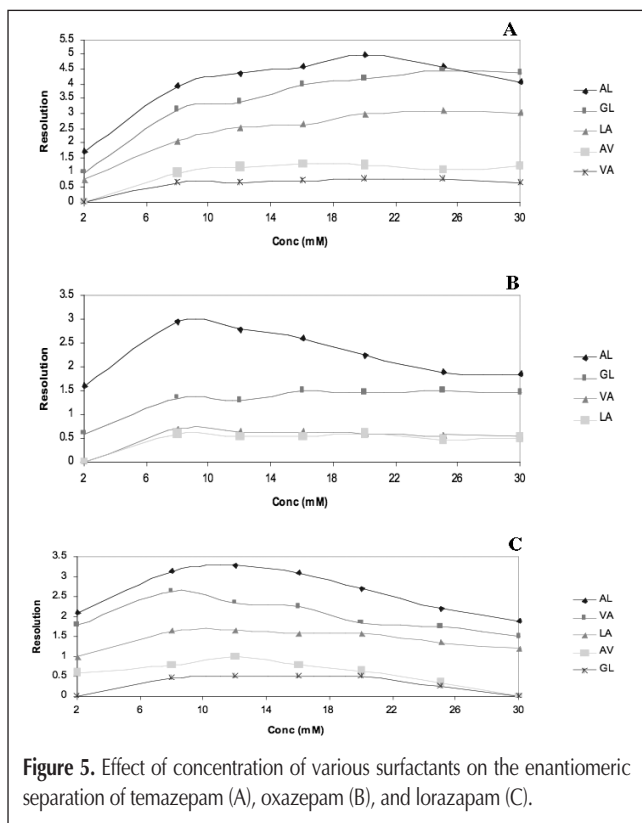


Figure 5. Effect of concentration of various surfactants on the enantiomeric separation of temazepam (A), oxazepam (B), and lorazepam (C).

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