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Comparative Study of Anionic and Nonionic/Anionic Surfactant Systems in Micellar Electrokinetic Capillary Chromatography.

Edward Lester Little
Louisiana State University and Agricultural & Mechanical College

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Comparative study of anionic and nonionic/anionic surfactant systems in micellar electrokinetic capillary chromatography

Little, Edward Lester, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1992
COMPARATIVE STUDY OF ANIONIC AND NONIONIC/ANIONIC SURFACTANT SYSTEMS IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

A Dissertation
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by
Edward L. Little
B.S., Northeast Louisiana University, 1985
M.S., Northeast Louisiana University, 1987
August 1992
"Separation is as old as the earth. From the cloud of dust and gases that assembled as protoearth, heavier elements sank inward and condensed to form our planet. Remaining clouds of hydrogen and helium were blown away (separated) by the sun's radiation. The hot, formless core of early earth began to fractionate further. Leaving dense elements like nickel and iron within, the lighter elements floated to the surface and crystallized into the different minerals that make up the crust of the earth. The gas-forming elements emerged from volcanoes and formed the atmosphere. Water condensed out as oceans. Thus the basic layercake of earth—core, mantle, crust, hydrosphere, and atmosphere—is structured according to the driving forces of separation acting on a grand scale."

J. Calvin Giddings

Unified Separation Science
ACKNOWLEDGEMENTS

First and foremost, I express deep gratitude to my family for their love and support throughout the past five years. Special appreciation goes to my mother whose strength and wisdom helped me get through some tremendously difficult times in my life. This accomplishment in my life would not have been possible without her.

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In closing, I thank God for guiding my life and giving me the opportunity to associate with such a wonderful group of people. It has truly been a blessing to work and live in this area for the past five years and I shall always remember these times as some of the best of my life.
DEDICATION

This work is dedicated to Toby. His untimely passing has taught me to live life to its fullest and appreciate the things that come to me until I have the opportunity to be with him again. I am sure that he would have been as proud of this accomplishment in my life as I was of him.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF SYMBOLS AND ABBREVIATIONS</td>
<td>xvi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xxiii</td>
</tr>
<tr>
<td>CHAPTER 1: GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Prelude</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Capillary Zone Electrophoresis</td>
<td>2</td>
</tr>
<tr>
<td>1.2.1 Background</td>
<td>2</td>
</tr>
<tr>
<td>1.2.2 Electroosmosis</td>
<td>5</td>
</tr>
<tr>
<td>1.3 Micellar Electrokinetic Capillary Chromatography</td>
<td>7</td>
</tr>
<tr>
<td>1.3.1 Background on micelles</td>
<td>7</td>
</tr>
<tr>
<td>1.3.2 MECC separation mechanism</td>
<td>9</td>
</tr>
<tr>
<td>1.4 Previous Studies</td>
<td>12</td>
</tr>
<tr>
<td>1.4.1 Different surfactant systems</td>
<td>12</td>
</tr>
<tr>
<td>1.4.2 Extension of elution range</td>
<td>13</td>
</tr>
<tr>
<td>1.4.3 Use of organic modifiers</td>
<td>14</td>
</tr>
<tr>
<td>1.5 Text Organization</td>
<td>14</td>
</tr>
<tr>
<td>Chapter One References</td>
<td>16</td>
</tr>
<tr>
<td>CHAPTER 2: OPTIMIZATION OF THE RESOLUTION OF PTH-AMINO ACIDS</td>
<td>18</td>
</tr>
<tr>
<td>ACIDS THROUGH CONTROL OF SURFACTANT CONCENTRATION IN MICELLAR</td>
<td>18</td>
</tr>
<tr>
<td>ELECTROKINETIC CAPILLARY CHROMATOGRAPHY: SDS AND BRIJ 35/SDS</td>
<td>18</td>
</tr>
<tr>
<td>MICELLAR SYSTEMS</td>
<td>18</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>20</td>
</tr>
<tr>
<td>2.2 Experimental</td>
<td>23</td>
</tr>
<tr>
<td>2.2.1 Materials</td>
<td>23</td>
</tr>
<tr>
<td>2.2.2 Equipment</td>
<td>23</td>
</tr>
<tr>
<td>2.2.3 Methods</td>
<td>24</td>
</tr>
<tr>
<td>2.2.3.1 Capillary treatment</td>
<td>24</td>
</tr>
<tr>
<td>2.2.3.2 Buffer/surfactant systems</td>
<td>24</td>
</tr>
<tr>
<td>2.2.4 Measurement of resolution</td>
<td>26</td>
</tr>
<tr>
<td>2.3 Results and Discussion</td>
<td>27</td>
</tr>
<tr>
<td>2.3.1 Resolution in the SDS system</td>
<td>33</td>
</tr>
<tr>
<td>2.3.2 Resolution in the Brij 35/SDS system</td>
<td>34</td>
</tr>
<tr>
<td>2.3.3 Efficiency</td>
<td>35</td>
</tr>
<tr>
<td>2.3.4 Selectivity</td>
<td>40</td>
</tr>
<tr>
<td>2.3.5 Retention mechanism</td>
<td>41</td>
</tr>
<tr>
<td>2.4 Conclusions</td>
<td>45</td>
</tr>
<tr>
<td>Chapter Two References</td>
<td>47</td>
</tr>
</tbody>
</table>
CHAPTER 3: COMPARISON OF SDS AND BRUJ 35/SDS MICELLAR MEDIA IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY ................................................. 49
3.1 Introduction ......................................................................................... 50
3.2 Experimental ...................................................................................... 52
  3.2.1 Materials ..................................................................................... 52
  3.2.2 Equipment .................................................................................. 52
  3.2.3 Methods ..................................................................................... 53
    3.2.3.1 Capillary treatment ............................................................. 53
    3.2.3.2 Buffer systems ..................................................................... 53
3.3 Results and Discussion ....................................................................... 53
  3.3.1 Effect on retention ......................................................................... 53
  3.3.2 Comparison of partition coefficients .......................................... 56
  3.3.3 Variations in selectivity ............................................................... 62
    3.3.3.1 Functional group selectivity ................................................. 62
    3.3.3.2 Adjacent solute selectivity .................................................. 65
  3.3.4 Efficiency ................................................................................... 70
  3.3.5 Comparison of electroosmotic and micellar electrophoretic velocities and their effect on \( t_{mc}/t_0 \) ......................................................... 74
3.4 Conclusions ...................................................................................... 82
Chapter Three References ........................................................................ 87

CHAPTER 4: EFFECT OF ORGANIC SOLVENT ON THE RETENTION AND SELECTIVITY OF N-ALKYLPHENONE HOMOLOGUES IN SDS-MEDIATED MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY ............................................. 88
4.1 Introduction ....................................................................................... 89
4.2 Experimental ..................................................................................... 90
  4.2.1 Apparatus ................................................................................ 90
  4.2.2 Materials and Methods ............................................................. 91
4.3 Results and Discussion ..................................................................... 92
  4.3.1 Methylene Selectivity ................................................................. 94
    4.3.1.1 General trends ..................................................................... 94
    4.3.1.2 Methylene selectivity between individual solute pairs .......... 98
  4.3.2 Partition coefficients and CMC .................................................... 103
    4.3.2.1 Comparison of partition coefficients ................................. 103
    4.3.2.2 Trends in variation of CMC ................................................. 109
  4.3.3 Electroosmotic and micellar electrophoretic velocities .............. 110
4.4 Conclusions .................................................................................... 113
Chapter Four References ........................................................................ 115

CHAPTER 5: EFFECT OF ORGANIC SOLVENT ON THE RETENTION AND SELECTIVITY OF N-ALKYLPHENONE HOMOLOGUES IN BRUJ 35/SDS-MEDIATED MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY ........................................... 118
5.1 Introduction ..................................................................................... 119
5.2 Experimental ................................................................................... 120
  5.2.1 Apparatus ............................................................................... 120
  5.2.2 Materials and Methods ............................................................ 121
LIST OF TABLES

Table 2.1: Effect of surfactant concentration on separation currents and $t_{m_0}/t_0$ at constant applied voltages........................................................................................................ 25

Table 2.2: Efficiencies for SDS surfactant system.......................................................... 37

Table 2.3: Efficiencies for Brij 35/SDS surfactant system.......................................... 38

Table 2.4: Selectivities for SDS and Brij 35/SDS surfactant systems......................... 42

Table 3.1: Partition coefficients and CMCs for benzene derivatives with varying functionality ........................................................................................................ 59

Table 3.2: Comparison of partition coefficients for benzene derivatives with literature values from MLC .............................................................. 61

Table 3.3: Comparison of functional group selectivity for Brij 35/SDS and SDS micellar systems............................................................................... 64

Table 3.4: Comparison of adjacent solute selectivity for Brij 35/SDS and SDS micellar systems............................................................................... 67

Table 3.5: Efficiencies of nitrobenzene for SDS and Brij 35/SDS systems at different surfactant concentrations ......................................................... 72

Table 3.6: Comparison of electroosmotic and micellar electrophoretic velocities ...................................................................................................................... 77

Table 4.1: Comparison of log ($\alpha_{CH_2}$) and log $\beta$ values.................................. 97

Table 4.2: Partition coefficients and CMC values for members of n-alkylphenone homologous series............................................................................... 104

Table 4.3: Comparison of slopes and intercept values for log $P_{wm}$ vs $N_c$ plots ......................................................................................... 108

Table 4.4: Comparison of electroosmotic and micellar electrophoretic velocities ...................................................................................................................... 111

Table 5.1: Comparison of log ($\alpha_{CH_2}$) and log $\beta$ values.................................. 131

Table 5.2: Partition coefficients for members of n-alkylphenone homologous series ............................................................................... 136

Table 5.3: Comparison of slopes and intercept values for log $P_{wm}$ vs $N_c$ plots ................................................................. 138

Table A.1: Comparison of between-class selectivity, $\alpha_{bc}$, for unimodal (conventional) elution and sequential multimodal elution in RPLC... 174
Table A.2: Comparison of within-class selectivity, $\alpha_{wc}$, for unimodal (conventional) elution and sequential multimodal elution in RPLC... 176

Table A.3: Reproducibility of retention times using sequential multimodal elution in RPLC ................................................................. 184

Table A.4: Area reproducibility using sequential multimodal elution ............. 185
LIST OF FIGURES

Figure 1.1: Schematic diagram of a capillary zone electrophoresis (CZE) apparatus................................................................. 3

Figure 1.2: Pictorial model of the mechanism of electroosmotic flow and its comparison to plug and parabolic capillary flow........... 6

Figure 1.3: Examples of three different types of surfactants used in MECC: SDS (anionic), CTAB (cationic), and Brij 35 (nonionic)........ 8

Figure 1.4: Qualitative comparison of relative and observed velocities in MECC for different surfactant systems: Anionic (top), cationic (middle), and nonionic/anionic mixture (lower). Definition of velocities are given in text ............................................................ 10

Figure 2.1: Representative electrokinetic chromatograms of six PTH-amino acids. Elution order: PTH-gly, PTH-ala, PTH-val, PTH-nva, PTH-ile, PTH-nle, and decanophenone (tmc marker). Capillary: 50 μm i.d., 170 μm o.d., 57.5 cm (50.0 cm to detector). Detection wavelength: 254 nm. Upper chromatogram: 75 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 18 kV; current, 27.2 μA. Lower chromatogram: 40 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 17.1 μA ........ 30

Figure 2.2: Resolution vs [SDS] for 3 pairs of PTH-amino acids: (A) PTH-gly/PTH-ala; (B) PTH-val/PTH-nva; and (C) PTH-ile/PTH-nle... 31

Figure 2.3: Resolution vs [Brij 35] for 3 pairs of PTH-amino acids. Pair identification as in Figure 2.2 .................................................. 32

Figure 2.4: Comparison of retention mechanism for hydrophilic and hydrophobic PTH-amino acids in (A) SDS and (B) Brij-35/SDS micellar systems. The function g(k') is defined in Equation 2.11 ........................................ 43

Figure 3.1: Representative electrokinetic chromatograms of six benzene derivatives. Capillary: 50 μm i.d., 170 μm o.d., 47.5 cm (40.0 cm to detector). Detection wavelength: 254 nm. Solute identity: 1) phenol, 2) benzene, 3) nitrobenzene, 4) acetophenone, 5) anisole, 6) toluene, 7) chlorobenzene, and 8) decanophenone (tmc marker). Upper chromatogram: 100 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 10 kV; current, 21.9 μA. Lower chromatogram: 40 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 28.7 μA ............... 55

Figure 3.2: Solute retention with respect to chlorobenzene (k'/k'B2-Cl) vs [Brij 35] for different benzene derivatives. Figure legend denotes solute identity .................................................................................. 66
Figure 3.3: f(α) vs adjacent solute pairs. Solute identification follows numerical designation in Figure 3.1. The function f(α) is defined in Equation 3.9.............................................................. 69

Figure 3.4: Comparison of peak asymmetry (b/a) variation vs [SDS] for three selected solutes: phenol (■), nitrobenzene (□), and acetophenone (□). ............................................................................................................. 75

Figure 3.5: Peak profile of phenol at different SDS concentrations. Run conditions are given in Figure 3.1 .............................................................................. 76

Figure 3.6: Reciprocal velocity vs [Brij 35] for the electroosmotic velocity (●) and Brij 35/SDS micelle electrophoretic velocity (■). Run conditions are given in Figure 3.1............................................................................ 79

Figure 3.7: Electrophoretic chromatogram of alkylphenone homologues. Peak identity: 1) acetophenone, 2) propiophenone, 3) butyrophenone, 4) valerophenone, and 5) hexanophenone. Capillary: 50 μm i.d., 170 μm o.d., 47.5 cm (40.0 cm to detector). Detection wavelength: 254 nm. Micelle medium: 11.8 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 35.2 μA ...... 81

Figure 3.8: log k' vs carbon number (Nc) for alkylphenones in Brij 35/SDS. Data were extracted from Figure 3.7 (●) and an alkylphenone separation under the conditions described in Figure 3.1 for Brij 35/SDS (○). Figure legend gives tmc/t0 values for each data set ..................... 83

Figure 4.1: Mechanistic comparison of MLC to MECC: (A) the MLC three-phase (pseudophase) model, (B) the MECC retention model. Lowercase letters designate the various solute partitioning processes occurring in each system: (a) micellar phase/aqueous phase, (b) micellar phase/stationary phase, and (c) stationary phase/aqueous phase interactions .................................................................................................. 93

Figure 4.2: Qualitative depiction of the effect of organic solvent on polarity variation in micellar and aqueous phases of MECC. From left to right, 1) polarity difference between the micellar and aqueous phases with no organic modifier, 2) change in polarity difference when organic solvent is excluded from the micellar phase (Type I modifier), and 3) change in polarity difference when organic solvent is solvated in both phases (Type II modifier) .............................................................................. 96

Figure 4.3: α_{modifier}/α_{H2O} vs [SDS] for n-alkylphenone solute pairs in each organic modifier system. The solvent and solute pair identities are given within the figure. For specific mobile phase and run conditions see Experimental Section .................................................. 99
Figure 4.4: Correlation of partition coefficients ($P_{wm}$) of n-alkylphenones with Hildebrand solubility values ($\delta$) of each organic modifier. See figure legend for solute identities. All plots were correlated to $r^2 \geq 0.999$ except $C_{10}$ which gave $r^2 = 0.994$.

Figure 5.1: $k^*$ ratio of organic solvent modified to pure Brij 35/SDS vs carbon number for n-alkylphenones at different Brij 35 concentrations. Headings above each plot denote the organic solvent added. See figure legend for specific Brij 35 concentrations.

Figure 5.2: Ratio of net micellar ($V_{net}$) to electroosmotic ($V_{eo}$) velocities vs [Brij 35] for the Brij 35/SDS mixed surfactant system. Velocities were measured by migration times of acetonitrile ($V_{eo}$) and decanophenone ($V_{net}$).

Figure 5.3: $k^*$ ratio of organic solvent modified to pure SDS vs carbon number for n-alkylphenones at different SDS concentrations. Headings above each plot denote the organic solvent added. See figure legend for specific SDS concentrations.

Figure 5.4: $\alpha_{\text{modifier}/\alpha_{H_2O}}$ vs [Brij 35] for n-alkylphenone solute pairs in each organic modifier system. The solvent and solute pair identities are given within the figure. For specific mobile phase and run conditions see Experimental Section.

Figure A.1: Illustration of a typical sequential multimodal elution separation (A) and a conventional reversed-phase separation (B). Mobile phases are described in the text. The dotted line in A represents the time at which the second elution mode begins.

Figure A.2: Examples of possible elution orders for a one-dimensional separation (A) and a sequential multimodal elution separation (B). Dotted line as in Figure A.1.

Figure A.3: Comparison of the disorder of separation (randomness of elution order) for a one-dimensional elution technique ($r = 1$) and sequential multimodal chromatography ($r = 2-4$). $n$ is the number of sample components, and $r$ is the number of sequential elution modes. Numbers in parentheses for $r = 2$ indicate relative proportion of components that elute in the first and second separation modes. See eqs A.9-A.12 and related text.

Figure A.4: Examples of mobile-phase composition gradients necessary for linear pH gradients. The ordinate represents the percentage of conjugate base. Gradient identification: curve A, $\Delta p\text{H}/\text{min} = 1$; curve B, $\Delta p\text{H}/\text{min} = 0.5$; curve C, $\Delta p\text{H}/\text{min} = 0.25$. Curves represent a pH gradient from 3.50 to 6.00 and were calculated for a 96 mM formic acid/sodium formate buffer with a constant ionic strength and 5% organic solvent.
Figure A.5: Sequential multimodal reversed-phase separation of a sample of acids and neutrals on a C1 column with UV detection at 280 nm (acids) and 260 nm (neutrals). Analyte identification: 2-CBA, 2-chlorobenzoic acid; 3-NBA, 3-nitrobenzoic acid; BA, benzoic acid; 3-CBA, 3-chlorobenzoic acid; 3-BBA, 3-bromobenzoic acid; NB, nitrobenzene; ACP, acetophenone; BB, bromobenzene; 4-dCB, 1,4-dichlorobenzene; 2,2'-dBBP, 2,2'-dibromobiphenyl. Mobile-phase components: reservoir A = 43.5 mM acetic acid, B = 43.5 mM sodium acetate, and C = methanol. The pH gradient, initiated at the time of sample injection, was linear from pH 3.55 to 5.5 at 0.8 pH units/min and held constant at the final pH until all benzoic acid derivatives had eluted. The methanol gradient, shown in the figure, was then applied.

Figure A.6: Sequential multimodal elution for RPLC separations using a C8 (A) and a C18 (B) column. Detection and sample components as in Figure A.5, except that nitrobenzene was omitted.

Figure A.7: Alternative reversed-phase separations (cf. Figure A.5) using (A) simultaneous bimodal elution (pH/methanol gradients) and (B) unimodal elution (methanol gradient at 5%/min). Detection at 260 nm. Sample components as in Figure A.6. pH and methanol gradients of A as in Figure A.5, except that both were started at the time of injection.

Figure A.8: Sequential multimodal elution RPLC separation of a complex sample containing di- and tripeptides and neutral compounds. Detection conditions: λ = 254 nm, sensitivity = 0.2 AUFS. Mobile-phase program: reservoir A = 100 mM formic acid + NaClO4, B = 100 mM sodium formate, and C = methanol. Solutions A and B contained 6% 1-propanol to improve column efficiency. pH gradient profile: initial pH held at 3.20 for 0.5 min, followed by linear pH gradient of 1.1 pH units/min to pH 4.30 and held for 1.5 min. MeOH gradient profile: a step gradient from 0 to 65% MeOH was performed after 3 min and then maintained at 65% for 1 min, followed by a linear gradient at 10%/min to 95% MeOH and then held at 95% for 5 additional min.

Figure A.9: Example of control of solute retention in sequential multimodal elution RPLC. The elution of a second class of compounds during a second separation mode can be delayed (or accelerated) simply by delaying (or accelerating) the start of the second elution mode. Delay of second elution mode (methanol gradient): (A) no delay; (B) 1 min; and (C) 2 min. Sample and other conditions as in Figure A.8.

Figure A.10: Separation of phenolic priority pollutants and polyaromatic hydrocarbons (PAHs) in creosote sludge using sequential multimodal elution RPLC. Mobile-phase program: reservoir A = 50 mM sodium carbonate, B = 50 mM sodium bicarbonate + NaClO4, and C = acetonitrile. The pH was held constant at a pHopt = 9.11 with 30% acetonitrile to accommodate electrochemical detection of phenols.
(alleviate baseline drift). The ACN gradient profile, initiated at 13 min, was 30-95% ACN at a rate of 5%/min with an isocratic hold at 95% ACN for 2 min.
LIST OF SYMBOLS AND ABBREVIATIONS

1-PrOH 1-propanol

a micelle radius

ala alanine

\[
\begin{align*}
\text{HOOC} & \quad \text{C} \\
\text{H}_2 & \quad \text{CH}_3
\end{align*}
\]

b/a peak asymmetry factor measured at 10\% peak height

Brij® 35 polyoxyethylated nonionic surfactant, polyoxyethylene(23)dodecanol

[Brij 35] molar concentration of Brij 35 surfactant monomer

[Brij 35]$_{opt}$ Brij 35 surfactant concentration for optimum solute resolution

C analyte concentration

C$_8$ acetophenone, C$_8$H$_8$O

\[
\begin{align*}
\text{C} & \quad \text{O} \\
\text{C} & \quad \text{CH}_3
\end{align*}
\]

All of the following compounds to C$_{24}$ are homologues of acetophenone with methylene group addition between the carbonyl and methyl groups.

C$_9$ propiophenone, C$_9$H$_{10}$O

C$_{10}$ butyrophenone, C$_{10}$H$_{12}$O

C$_{11}$ valerophenone, C$_{11}$H$_{14}$O

C$_{12}$ hexanophenone, C$_{12}$H$_{16}$O
C_{13} \text{ heptanophenone, } C_{13}H_{18}O
C_{14} \text{ octanophenone, } C_{14}H_{20}O
C_{16} \text{ decanophenone, } C_{16}H_{22}O
C_{18} \text{ dodecanophenone, } C_{18}H_{24}O
C_{24} \text{ octadecanophenone, } C_{24}H_{30}O
cm \text{ centimeter}
CMC \text{ critical micelle concentration}
CMC_{Brij \text{ 35}} \text{ critical micelle concentration of pure Brij 35}
CMC_{mix} \text{ critical micelle concentration of Brij 35/SDS surfactant mixture}
CMC_{SDS} \text{ critical micelle concentration of pure SDS}
CTAC \text{ cetyltrimethylammonium chloride, cationic surfactant}
CZE \text{ capillary zone electrophoresis}
DTAB \text{ dodecyltrimethylammonium bromide, cationic surfactant}
DTAC \text{ dodecyltrimethylammonium chloride, cationic surfactant}
E \text{ electrical field strength, defined in terms of volts per unit length}
f(\alpha) \text{ function of adjacent solute selectivity relating selectivity differences between Brij 35 and SDS}
f(k') \text{ retention term in the MECC fundamental resolution equation, equivalent to } \left(\frac{k'}{1 + k'}\right)\left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'}\right)
f(\kappa_2) \text{ function related to micellar size and shape}
g \text{ gravitational force constant}
g(k') \text{ function defining solute retention in Brij 35/SDS with respect to solute retention in 20 mM SDS}
gly cline

[Chemical structure of glycine]

HPCE  high-performance capillary electrophoresis
HPLC  high-performance liquid chromatography
i.d.  inner diameter
ile  isoleucine

$k'$  solute capacity factor
$k'_{20 \text{ mM SDS}}$  solute capacity factor in 20 mM SDS
$k'_{\text{micelle}}$  solute capacity factor at some surfactant concentration in an arbitrary surfactant system
$k'_{Nc}$  solute capacity factor for an alkylphenone homologue
$k'_{Nc+1}$  solute capacity factor for an alkylphenone homologue containing an additional methylene group to the previously defined term
$k'_{\text{opt}}$  solute capacity factor for optimum resolution
$k'_{\text{BZ}}$  solute capacity factor of benzene
$k'_{\text{BZ-X}}$  solute capacity factor of benzene analogue where $X$ can be a variety of functionalities
$k'_{\text{mod}}$  solute capacity factor in organic solvent modified surfactant system
$k'_o$ solute capacity factor at zero current, determined from intercept of $k'$ versus current. Used to negate effect of temperature on retention.

$k'_{pure}$ solute capacity factor in pure surfactant systems, i.e., no organic solvent is present

$K_{mw}$ solute-micelle binding constant

LC liquid chromatography

$L_{col}$ column length

MECC micellar electrokinetic capillary chromatography

MLC micellar liquid chromatography

$N$ separation efficiency

$N_c$ carbon number

nle norleucine

\[
\begin{align*}
\text{HOOC} & \quad \text{(CH}_2\text{)}_3\text{CH}_3 \\
\text{C} & \\
\text{H} & \\
\text{NH}_2 & \\
\end{align*}
\]

nva norvaline

\[
\begin{align*}
\text{HOOC} & \quad \text{(CH}_2\text{)}_2\text{CH}_3 \\
\text{C} & \\
\text{H} & \\
\text{NH}_2 & \\
\end{align*}
\]

o.d. outer diameter

ODS octadecylsilane

POE polyoxyethylene

PTH phenylthiohydantoin, amino acid derivative prepared by reaction of acid with phenyl isothiocyanate, where $R$ represents the amino acid side chain
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{wm}$</td>
<td>water-micelle partition coefficient</td>
</tr>
<tr>
<td>$Q$</td>
<td>quantity of analyte injected</td>
</tr>
<tr>
<td>$r$</td>
<td>column radius</td>
</tr>
<tr>
<td>$r^2$</td>
<td>correlation coefficient</td>
</tr>
<tr>
<td>RPLC</td>
<td>reversed-phase liquid chromatography</td>
</tr>
<tr>
<td>$R_s$</td>
<td>solute resolution</td>
</tr>
<tr>
<td>RSD</td>
<td>relative standard deviation</td>
</tr>
<tr>
<td>SCE</td>
<td>secondary chemical equilibria</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate, anionic surfactant</td>
</tr>
<tr>
<td>$[SDS]_{opt}$</td>
<td>optimum SDS concentration for solute resolution</td>
</tr>
<tr>
<td>sec</td>
<td>second</td>
</tr>
<tr>
<td>STS</td>
<td>sodium decyl sulfate, anionic surfactant</td>
</tr>
<tr>
<td>$[SURF]$</td>
<td>surfactant concentration</td>
</tr>
<tr>
<td>$[SURF]_{total}$</td>
<td>total surfactant concentration, used in mixed surfactant systems</td>
</tr>
<tr>
<td>$t_i$</td>
<td>elapsed injection time</td>
</tr>
<tr>
<td>$t_{mc}$</td>
<td>retention time of a solute permanently retained in the micelles</td>
</tr>
<tr>
<td>$t_{mx}/t_o$</td>
<td>ratio used to define the “window” where solute elution can occur</td>
</tr>
<tr>
<td>$t_o$</td>
<td>elution time of an unretained solute</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>t_R</td>
<td>solute retention time</td>
</tr>
<tr>
<td>V</td>
<td>partial molar volume of surfactant monomer</td>
</tr>
<tr>
<td>val</td>
<td>valine</td>
</tr>
<tr>
<td>V_{aq}</td>
<td>volume of aqueous phase</td>
</tr>
<tr>
<td>V_{Brij 35}</td>
<td>partial molar volume of Brij 35 surfactant monomer</td>
</tr>
<tr>
<td>V_{mc}</td>
<td>volume of micellar phase</td>
</tr>
<tr>
<td>V_{mix}</td>
<td>partial molar volume of surfactant mixture</td>
</tr>
<tr>
<td>V_{org}</td>
<td>partial molar volume of surfactant in the presence of organic solvent</td>
</tr>
<tr>
<td>V_{SDS}</td>
<td>partial molar volume of SDS surfactant monomer</td>
</tr>
<tr>
<td>w_{0.5}</td>
<td>peak width at half-height</td>
</tr>
<tr>
<td>w_1</td>
<td>baseline peak width of solute 1</td>
</tr>
<tr>
<td>w_2</td>
<td>baseline peak width of solute 2</td>
</tr>
</tbody>
</table>

**Greek Symbols and Abbreviations**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>solute selectivity</td>
</tr>
<tr>
<td>α-CH₂-</td>
<td>methylene selectivity</td>
</tr>
<tr>
<td>α_{Brij 35}</td>
<td>solute selectivity in Brij 35/SDS</td>
</tr>
<tr>
<td>α_{func}</td>
<td>functional group selectivity</td>
</tr>
<tr>
<td>α_{modifier/α_{H2O}}</td>
<td>ratio for comparison of methylene selectivity in the presence and absence of organic modifier</td>
</tr>
<tr>
<td>α_{SDS}</td>
<td>solute selectivity in pure SDS</td>
</tr>
<tr>
<td>α_{SDS/α_{Brij 35/SDS}}</td>
<td>ratio for comparison of solute selectivity in pure SDS and Brij 35/SDS</td>
</tr>
</tbody>
</table>
\( \beta \)  
phase ratio

\( \chi_{\text{Brij 35}} \)  
surfactant mole fraction of Brij 35, defined as moles of Brij 35 per total moles of surfactant

\( \chi_{\text{SDS}} \)  
surfactant mole fraction of SDS, defined as moles of SDS per total moles of surfactant

\( \Delta h \)  
height difference between injection and run positions for hydrostatic injection

\( \Delta t_R \)  
difference in solute retention times

\( \varepsilon \)  
permittivity of solution

\( \gamma_{\text{Brij 35}} \)  
activity coefficient of Brij 35 in mixed micelle formation

\( \gamma_{\text{SDS}} \)  
activity coefficient of SDS in mixed micelle formation

\( \eta \)  
solution viscosity

\( \kappa \)  
Debye reciprocal length, related to the ionic atmosphere surrounding the micelle whose reciprocal may be regarded as the thickness of an electrical double layer

\( v_{\text{eo}} \)  
electroosmotic velocity

\( v_{\text{ep}} \)  
micellar electrophoretic velocity

\( v_{\text{net}} \)  
net micellar velocity, equivalent to \( v_{\text{eo}} + v_{\text{ep}} \) where \( v_{\text{ep}} \) may be either positive (with \( v_{\text{eo}} \)) or negative (against \( v_{\text{eo}} \))

\( \rho \)  
solution density

\( \zeta \)  
electrokinetic potential
ABSTRACT

Micellar electrokinetic capillary chromatography (MECC) is a rapidly growing technique allowing separation of electrically neutral compounds with the high separation efficiencies typically observed in capillary zone electrophoresis. This technique is still in its infancy and subsequently suffers from several inherent problems which must be addressed such as the limited range of solute elution and clear protocol for optimization of separations. This work addresses these problems with primary emphasis on using mixed nonionic/anionic surfactant systems as opposed to pure surfactants.

In Chapter One, the fundamental theory behind MECC is discussed along with a brief synopsis of previous studies involving different surfactant systems, modification of micellar media with organic solvents, and the effect of mobile phase variation on the solute elution range. Chapter Two addresses resolution optimization by adjusting surfactant concentration. Two surfactant systems, pure SDS and Brij 35/SDS, were used in this study. The Brij 35/SDS system was advantageous due to higher separation efficiency, more constant elution ranges (tme/t0), and resolution optimization between hydrophilic analytes; SDS found favor for optimizing the resolution between moderately hydrophobic solutes.

Chapter Three is a more in-depth comparison of SDS and Brij 35/SDS surfactant systems. Results indicate that in the Brij 35/SDS system, solute/micellar surface interactions play a key role in retention of certain compounds. Also, changes in electroosmotic and micellar electrophoretic velocities with varying Brij 35 concentration indicate the possibility of an infinite elution range in this system.

Chapters Four and Five discuss the effects of three organic modifiers; acetonitrile, methanol, and 1-propanol; upon the retention characteristics of an n-alkylphenone homologous series for the SDS and Brij 35/SDS systems, respectively. Our results point to greater structural stability for Brij 35/SDS micelles. Also, the polyoxyethylene surface layer of Brij 35/SDS micelles may influence solute partitioning via reduced solute/micellar surface interactions. Finally, the appendix reports our work on the combination of secondary chemical equilibrium (SCE)-LC sequentially with reversed-phase HPLC to provide multimodal separation of complex samples on a single column.
CHAPTER 1

GENERAL INTRODUCTION
1.1 PRELUDE

The art of separation science has evolved tremendously since the pioneering work of Tswett and exhaustive labor by key researchers such as Martin and Synge in an attempt to understand clearly chromatographic processes (1.1). The continually increasing demand for better ways to separate complex mixtures has stimulated the development of chromatographic methods capable of separating compounds based on a variety of physical properties, such as volatility, hydrophobicity, size, charge, and chirality. Along with improved versatility, miniaturization has also evolved in chromatography, allowing analyses of nanoliter to picoliter sample volumes.

1.2 CAPILLARY ZONE ELECTROPHORESIS

1.2.1 Background

Electrophoresis, a separation technique based on differences in solute electrophoretic mobilities, has adapted well to this trend of miniaturization. One variation of electrophoresis, capillary zone electrophoresis (CZE), has become particularly useful in the biological sciences which in some cases require analysis and characterization of specific solutes in exceedingly small sample volumes (1.2). Several laboratories have demonstrated the superiority of CZE over traditional electrophoretic techniques. Jorgenson and Lukacs (1.3) were first to introduce capillary zone electrophoresis (CZE) in small inner diameter (75 μm) open-tubular glass capillaries. Efficiencies in excess of 400,000 theoretical plates were demonstrated. Several other groups have shown the high efficiency and variability of selectivity attainable from varying experimental conditions (1.4-1.8).

A schematic diagram of the CZE instrumentation is shown in Figure 1.1. A fused-silica capillary with 50-100 μm inner diameter and 25-100 cm length is filled with operating buffer and each end is immersed in a separate vial of electrolyte.
Figure 1.1  Schematic diagram of a capillary zone electrophoresis (CZE) apparatus.
Sample may be injected into the capillary by either hydrostatic or electromigration injection. Hydrostatic injection is performed by inserting one end of the capillary into the sample solution and raising this solution above the other electrolyte vial for a set period of time. A siphoning effect occurs which delivers a volume of sample solution into the capillary. With electromigration injection, a voltage is applied across the capillary after its immersion into the sample solution. Injection occurs via electromigration of the sample solution into the capillary.

After injecting the sample, a high voltage (up to 30 kV) is applied across the capillary under conditions of fairly low current (<100 μA) using platinum electrodes immersed in the electrolyte solutions. The small internal capillary diameters provide efficient dissipation of heat generated from these high powers, thereby reducing convective forces which would degrade separation efficiency. Sample components migrate at different rates toward either end of the capillary, depending on their charge. Simultaneously, the bulk solution flows via electroosmosis toward the cathode. These combined motions carry the sample components through the detection window at rates dependent on the analytes' net velocities.

Light absorption or fluorescence, either direct or indirect, is most commonly employed for on-column detection of analytes. The capability of performing on-column detection in electrophoresis was demonstrated by Catsimpooolas (1.9) and Hjerten (1.10) where, in both cases, the entire length of the column was scanned. Everaerts et al. (1.11) first introduced the currently employed technique where on-column detection is performed at a fixed region along the column. A small section of a polyimide layer, typically coated on fused-silica capillaries to increase their resistance, is thermally removed to expose the fused-silica. Light directed through this "window" perpendicular to the capillary permits detection of solutes. Although absorbance is the primary source for detection, several laboratories have shown the
power of laser-induced fluorescence (1.12, 1.13) and thermo-optical techniques (1.14, 1.15) for attomolar quantities of compounds.

1.2.2 Electroosmosis

Bulk flow within the capillary occurs via electroosmosis. Figure 1.2 illustrates this phenomenon which is detailed in many standard texts (1.16, 1.17). Upon base activation of the fused silica capillary, negatively charged silanols cover its inner surface. These surface ions attract and preferentially adsorb oppositely charged ions from the solution. The formation of an electrical double layer occurs which can be visualized as a relatively immobile layer of ions on the solid surface and a diffuse layer extending into the solution. The application of an electrical field tangential to the solid surface normal imparts an electroosmotic flow with an essentially flat profile. The high separation efficiencies in CZE are a direct result of this “plug” flow since axial dispersion, which is prevalent in laminar flow systems, is minimized.

The electroosmotic velocity, $v_{eo}$, is given by

$$v_{eo} = \frac{\varepsilon \zeta}{\eta} E$$

where $\varepsilon$ and $\eta$ are the dielectric constant and viscosity of the solution, respectively; $\zeta$ is the zeta-potential at the solid-liquid interface; and $E$ is the electrical field strength, equivalent to applied voltage per unit length. Obviously, the electroosmotic velocity is most easily controlled by adjustment of the applied voltage. However, changes in solution composition, e.g., variation of ionic strength, pH, or addition of
Figure 1.2  Pictorial model of the mechanism of electroosmotic flow and its comparison to plug and parabolic capillary flow.
organic modifiers, can also be used to alter electroosmotic velocity via their effects on
dielectric constant, solution viscosity, and zeta-potential.

1.3 MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

Although CZE is an extremely powerful technique for separating complex samples, it is unable to resolve electrically neutral compounds. Terabe et al. first addressed this shortcoming in 1984 (1.18) followed by a more thorough study in 1985 (1.19). Their solution involved the addition of a surfactant above its critical micelle concentration (CMC) to provide a medium through which neutral solutes could differentially partition. Termed micellar electrokinetic capillary chromatography (MECC), this technique permits separation of electrically neutral compounds while also maintaining the high separation efficiencies typically observed in CZE.

1.3.1 Background on micelles

Surfactants are amphiphilic molecules bearing both hydrophilic and lipophilic moieties. Some common surfactants are shown in Figure 1.3. Surfactants are typically classified by their hydrophilic or "head" groups, being anionic, cationic, nonionic, or zwitterionic. The lipophilic or "tail" group consists of a straight- or branched-chain alkyl group usually containing more than seven carbons. In dilute solutions, these molecules are generally observed as discrete monomers. However, an increase in surfactant concentration will ultimately induce aggregation of the monomers to form micelles. The concentration at which micellization occurs is referred to as the critical micelle concentration (CMC). Simply stated, a micelle is a structure consisting of a hydrophilic surface and lipophilic core. Although micelle formation occurs above the CMC, the monomer form of the surfactant is still present
Figure 1.3 Examples of three different types of surfactants used in MECC: SDS (anionic), CTAB (cationic), and Brij 35 (nonionic).
at a concentration equivalent to the CMC. Several structural models for micelles have emerged (1.20-1.23) based on various micellar properties such as viscosity, polarity, and kinetics. Although each model has its merits, their lack of universality warrants further study in this area.

1.3.2 MECC separation mechanism

Separation processes in MECC are rather simple to describe qualitatively. A concentration of charged surfactant above its CMC is added to the electrolyte solution typically used in CZE. Aggregation of an ionic surfactant yields micelles with a charged surface and lipophilic core. Since surface charge is present, the micelle will migrate in a direction contingent on its surface charge when a potential is applied. For example, a micelle formed from an anionic surfactant will migrate toward the anode.

The micellar electrophoretic velocity, $v_{ep}$, is given by

$$v_{ep} = \frac{2\varepsilon \zeta_m}{3\eta} f(\kappa a) E$$

(1.2)

where $\zeta_m$ is the zeta-potential at the micellar surface and $f(\kappa a)$ is a function dependent on the micellar size and shape. Note that $\zeta$ given in this formula is different from that in eq 1.1 due to the differences in charge surface density of the silica surface and the micelles. Consequently, the bulk flow and micellar electrophoretic velocities will be different. The net micellar velocity, $v_{net}$, is expressed by

$$v_{net} = v_{eo} + v_{ep}$$

(1.3)

where $v_{ep}$ is positive or negative depending on whether it follows or opposes electroosmotic flow. Figure 1.4 qualitatively illustrates the relationship between
Figure 1.4 Qualitative comparison of relative and observed velocities in MECC for different surfactant systems: Anionic (top), cationic (middle), and nonionic/anionic mixture (lower). Definitions of velocities are given in text.
velocities for different micellar systems. Assuming electroosmosis toward the
cathode in all cases, net micellar velocities less than, equal to, and greater than the
electroosmotic velocity are observed for anionic, nonionic, and cationic micellar
systems, respectively. Separation occurs through differential partitioning of neutral
compounds between the micellar and aqueous phases. Most applications in MECC
typically use anionic micellar systems since the micelles remain in the capillary for an
extended time. This permits solutes to partition more frequently so that adequate
separation can occur.

The above model for cationic micellar systems would be accurate if ion-pairing
between surfactant monomers and the silica surface were not expected. However,
surfactant monomers do adsorb to the silica surface via ion-pairing. This adsorption
is followed by the formation of a cationic surfactant bilayer which results in a net
positive surface charge. Reversal of electroosmosis occurs which, along with the net
positive surface charge of the micelles, yields electroosmotic and micellar
electrophoretic velocities opposite to those observed with anionic surfactant systems.
However, in order to utilize the same instrumentation shown in Figure 1.1, the
polarity of the voltage supply must be reversed.

A primary limitation of MECC is the limited range where solute elution can
occur. If the micellar electrophoretic velocity were equivalent to the electroosmotic
velocity, then the micellar phase would display true stationary phase behavior,
permitting infinite retention of extremely lipophilic solutes. However, unlike
reversed-phase separations in HPLC, elution of the micelles limits the time in which a
separation can occur. Therefore, solute retention is usually described in terms of a
capacity factor, \( k' \), which is expressed by

\[
k' = \frac{t_R - t_0}{t_0(1 - (t_R/t_{mc}))}
\]  

(1.4)
where $t_R$ is the solute retention time, $t_0$ is the time required for an unretained solute to elute (electroosmotic velocity marker), and $t_{mc}$ is the elution time of the micelles (net micellar velocity marker). Organic solvents such as methanol and acetonitrile have low solubility in micelles and therefore make suitable $t_0$ markers. Appropriate $t_{mc}$ markers must be permanently retained by the micelles. Several water insoluble dyes such as Sudan III, Yellow OB, and Coumarin 153 have been used for this purpose (1.24, 1.25). The micelle's elution time may also be determined from a homologous solute series by an iterative scheme involving goodness-of-fit to a plot of log $k'$ vs carbon number (1.26, 1.27).

1.4 PREVIOUS STUDIES

1.4.1 Different surfactant systems

Several studies have pondered the applicability of different surfactant systems in MECC. Burton et al. (1.28) investigated some common anionic and cationic surfactant systems. In comparing sodium decyl sulfate (STS) and sodium dodecyl sulfate (SDS), their study indicated that SDS behaved analogously to octadecyl (ODS) stationary phases in HPLC. STS was unsuitable due to poor retention reproducibility and increasingly asymmetric peaks for late eluting solutes. Of the two cationic surfactant systems, dodecyltrimethylammonium chloride (DTAC) and cetyltrimethylammonium chloride (CTAC), CTAC was very useful for moderately large molecular weight solutes with limited solubility in the aqueous phase. Also, separation selectivities differed with surfactant charge, indicating effects of the polar head group on relative retention. Otsuka et al. (1.24) investigated the effect of SDS and dodecyltrimethylammonium bromide (DTAB) on the retention of phenylthiohydantoin (PTH)-derivatized amino acids and found significant differences.
in retention characteristics between the two systems. Bile salt micelles formed from compounds such as sodium taurocholate and sodium taurodeoxycholate have received attention for the separation of enantiomeric mixtures (1.29, 1.30). The use of anionic and nonionic micellar mixtures has been previously documented in MECC. Otsuka and Terabe (1.31) illustrated the usefulness of digitonin-SDS surfactant systems for optical resolution of PTH-amino acid mixtures. Rasmussen et al. (1.32) first introduced Brij® 35, polyoxyethylene(23)dodecanol, as a possible micellar phase in MECC. When mixed micelles were formed from Brij 35 and SDS, a significant difference in net micellar velocity and, moreover, separation selectivity was observed in comparison to pure SDS. However, no additional work has been published to date with regard to the novel properties of this mixed surfactant system.

1.4.2 Extension of elution range

As was mentioned earlier, the elution range in which solute separation can occur in MECC is sometimes small in comparison the other chromatographic techniques. Extension of the elution range has been the topic of several studies. Otsuka and Terabe (1.33) illustrated the effects of pH on electrokinetic velocities. Electroosmosis decreased regularly below pH 5.5 while the electrophoretic velocity of the micelles remained constant over the investigated pH range. Consequently, the electroosmotic and SDS micellar electrophoretic velocity were nearly equivalent at pH 5.0 providing a substantial increase in elution range. Balchunas and Sepaniak (1.34) investigated the use of surface-silanated fused silica capillaries to reduce electroosmotic flow. This technique was successful in extending the elution range, yet a loss in column efficiency due to solute-wall interactions may significantly hinder the widespread use of this approach. Note that each of the previous studies solely involved reduction of electroosmotic flow. Mixed micellar systems may provide
additional control of the elution range since both electroosmosis and micellar electrophoretic velocity may be modified simultaneously.

1.4.3 Use of organic modifiers

Organic modifiers can serve to increase the solubility of extremely lipophilic compounds in the aqueous phase and moderately affect the elution range. Gorse et al. (1.35) studied the effect of 1-20% v/v of acetonitrile and methanol on retention and elution range. They observed significant extension of elution range with each modifier and decreased retention of hydrophobic solutes. Modifier addition also affected selectivity and efficiency. The addition of methanol has also aided in the separation of isotopically substituted compounds (1.27). Finally, various studies employ propanol to increase solute solubilization, enhance solute-micelle mass transfer, and decrease solute-capillary wall interactions (1.34, 1.36). However, further studies are necessary to ascertain how organic solvents can modify micellar structure and how these structural variations can affect solute retention.

1.5 TEXT ORGANIZATION

Subsequent chapters in this work deal primarily with applications of two surfactant systems, SDS and Brij 35/SDS, to MECC and the influence of organic modifiers on retention and other parameters for these particular micellar phases. Chapter Two addresses resolution optimization between PTH-amino acids by adjusting surfactant concentration in both surfactant systems. Differences in column efficiency and selectivity between the two systems are also discussed. Chapter Three is a more in-depth comparison of these surfactant systems with primary importance placed upon differences in retention, selectivity, efficiency, and the possibility of infinite elution ranges using mixed micellar systems. Chapters Four and Five discuss
the effects of three organic modifiers; acetonitrile, methanol, and 1-propanol; upon the retention characteristics of an alkylphenone homologous series for the SDS and Brij 35/SDS surfactant systems, respectively. Finally, the appendix reports previous work on the combination of secondary chemical equilibrium (SCE)-LC sequentially with reversed-phase HPLC to provide multimodal separation of complex samples on a single column.
CHAPTER ONE REFERENCES


CHAPTER 2

OPTIMIZATION OF THE RESOLUTION OF PTH-AMINO ACIDS THROUGH CONTROL OF SURFACTANT CONCENTRATION IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY: SDS VS BRIJ 35/SDS MICELLAR SYSTEMS
March 9, 1992

Dr. Milton Lee
Department of Chemistry
106 ESC
Brigham Young University
Provo, Utah 84602

Dear Dr. Lee,

I am writing to you in reference to the article entitled “Optimization of the Resolution of PTH-Amino Acids Through Control of Surfactant Concentration in Micellar Electrokinetic Capillary Chromatography: SDS vs Brij 35/SDS Micellar Systems”, recently submitted to the Journal of Microcolumn Separations for publication, of which I am an author. I would like to include this manuscript as part of my Ph.D. dissertation. The completed dissertation will be submitted to University Microfilms Incorporated. Please forward permission for the reprint of this manuscript.

Thank you for your prompt reply on this matter by mail or by FAX.

Sincerely,

Edward L. Little
Principal author

Approved as requested

Milton Lee
3/25/92
2.1 INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) is a rapidly growing technique which utilizes the attributes of capillary zone electrophoresis (CZE) for the separation of neutral compounds. This technique employs a surfactant, most commonly sodium dodecyl sulfate (SDS), at concentrations above its critical micelle concentration (CMC) to serve as a pseudo-stationary phase. Due to the charge density surrounding the micelle, it tends to migrate toward an electrode (the anode for the case of SDS) at some electrophoretic velocity, $v_{ep}$. The buffer in which the micelle is solubilized, typically phosphate or phosphate/borate buffer, is responsible for the flow of the bulk solution toward the cathode via electroosmosis, $v_{eo}$. The net velocity of the micelle, $v_{mc}$, is then defined as

$$v_{mc} = v_{eo} + v_{ep}$$

(2.1)

where $v_{ep}$ is usually a negative velocity. Separation of neutral solutes is accomplished through their differential partitioning between the micellar and aqueous phases. The order of solute elution generally follows the same trend as in reversed-phase HPLC (RP-HPLC); that is, the greater the hydrophobicity of a compound, the longer it is retained. To date, MECC has been applied to a variety of solute systems such as phenolic compounds (2.1, 2.2), derivatized amino acids (2.3), hop bitter acids (2.4), metabolites of vitamin B₆ (2.5), nucleic acid constituents (2.6, 2.7), and derivatized amines (2.8). An important distinction between MECC and RP-HPLC, however, is the fact that the stationary phase of the former (the micellar phase), is not truly stationary.

The basic equations that define retention and resolution in MECC are modified versions of those used in conventional chromatography (2.9). The modifications
reflect the slow, but non-zero migration of the pseudo-stationary micellar phase through the capillary and the finite (limited) elution range that this implies. The capacity factor, $k'$, is defined in MECC as

$$k' = \frac{t_R - t_0}{t_0 \left(1 - \frac{t_R}{t_{mc}}\right)} \quad (2.2)$$

where $t_R$, $t_0$, and $t_{mc}$ are the retention times of the neutral solute, an unretained compound (typically methanol or acetonitrile), and the migration time of the micelle, respectively. The parenthetical term in the denominator of Equation 2.2 accounts for the migration of the micelle; note that it is physically unrealistic for the retention time of a neutral analyte to exceed the migration time of the micelle ($t_R > t_{mc}$), since this would result in a negative retention factor ($k' < 0$).

The fundamental resolution equation in MECC is

$$R_s = \left(\frac{\sqrt{N}}{4}\right) \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k'_2}{1 + k'_2}\right) \left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'_1}\right) \quad (2.3)$$

where $N$ is the efficiency of the column, and $\alpha$ is the selectivity. If one assumes that elution times of the two solutes are almost identical, i.e., $k'_1 = k'_2 = k'$, Equation 2.3 can be further simplified to

$$R_s = \left(\frac{\sqrt{N}}{4}\right) \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k'}{1 + k'}\right) \left(\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'}\right) \quad (2.4)$$
Whereas the first three terms in Equations 2.3 and 2.4 are identical to those found in conventional chromatography, the fourth term is an additional one that accounts for finite elution range.

Several studies have been implemented to determine the effects of various experimental parameters involved in MECC upon solute resolution, i.e., applied voltage (2.10-2.12), buffer concentration (2.10), pH (2.13, 2.14), surfactant concentration (2.6, 2.13, 2.14), surfactant identity (2.3, 2.11, 2.12), and organic modifier content (2.15, 2.16), to name a few. In each case, these variables were investigated with regard to their effect on efficiency and/or selectivity. Unfortunately, less attention has been given to the fundamental effect of solute retention on resolution. Terabe et al. were the first to recognize that an optimum $k'$ should exist from the point-of-view of resolution, for a given value of $N$ and $\alpha$ (2.9). More recently, Foley developed a theoretical basis for the retention-based optimization of resolution and resolution per unit time via surfactant concentration or other variables such as organic modifier that have an effect on $k'$ (2.17). He found that the optimum $k'$ value was solely dependent on the ratio $t_{mc}/t_0$, an easily measurable value; and that this optimum $k'$ can then be combined with a solute's water/micelle partition coefficient ($P_{wm}$) and selected parameters of the surfactant system to predict the optimum surfactant concentration for the separation of two analytes.

The purpose of this paper is to convey our experimental findings concerning the effect of surfactant concentration on the resolution of six PTH-amino acids by MECC, using two micellar systems: (i) pure anionic surfactant (SDS); and (ii) a mixture of nonionic and anionic surfactants (Brij 35/SDS). We will also discuss the effect of surfactant concentration and identity on other parameters affecting resolution such as efficiency, selectivity, and $t_{mc}/t_0$; and the practical aspects of this approach to optimization in MECC.
2.2 EXPERIMENTAL

2.2.1 Materials

Electrophoresis-grade SDS was purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA) while Brij® 35 (polyoxyethylene(23)dodecanol) was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). The phenylthiohydantoin derivatized (PTH) amino acids (PTH-glycine (gly), PTH-alanine (ala), PTH-valine (val), PTH-norvaline (nva), PTH-isoleucine (ile), and PTH-norleucine (nle)) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Nanograde® acetonitrile was obtained from Mallinkrodt, Inc. (Paris, KY, USA) and decanophenone, used as the t\text{me} marker, was purchased from Aldrich. Distilled water was deionized and redistilled with a Corning Mega-Pure™ Water Purification System (Corning, Inc., Corning, NY, USA).

Untreated fused-silica capillary tubing with dimensions of 50 mm i.d. and 170 mm o.d. was purchased from Alltech Associates, Inc. (Deerfield, IL, USA) and cut to lengths of 57.5 cm (SDS system) and 42.5 cm (Brij 35/SDS system). Windows were burned through the polyimide coating at a distance of 7.5 cm from the outlet end of each column yielding columns with injector-to-detector lengths of 50.0 (SDS) and 35.0 cm (Brij 35/SDS).

2.2.2 Equipment

A Quanta 4000 Capillary Electrophoresis System was provided by Millipore Corporation, Waters Chromatography Division (Milford, MA, USA). This instrument was equipped with hydrostatic injection used for 1 sec intervals and a fixed-wavelength UV absorbance detector operated at 254 nm. Data were acquired on an IBM Personal Computer AT (Boca Raton, FL, USA) using a PE-Nelson Omega-2
chromatography application package obtained from Perkin-Elmer Corporation (Milford, CT, USA).

2.2.3 Methods

2.2.3.1 Capillary treatment

Activation of the capillaries was performed using a modification of a procedure described previously (2.18). The capillary was initially rinsed with 1 M KOH for 15 min followed by subsequent rinses of 0.1 M-KOH and water for 15 min each. The capillary was finally rinsed for 20 min with the operating buffer. Purges with the operating buffer were performed after each run for 5 min using a vacuum of ~14 inches Hg at the detector reservoir.

2.2.3.2 Buffer/surfactant systems

Stock buffer solution was prepared with NaH$_2$PO$_4$ • H$_2$O and NaOH to give a 100 mM phosphate buffer at pH 6.8. This solution was diluted to a concentration of 10 mM which was used for preparation of surfactant solutions. SDS solutions were prepared at concentrations ranging from 15 to 150 mM. Brij 35/SDS solutions were prepared with a constant concentration of 20 mM SDS and a Brij 35 concentration ranging from 10 to 60 mM. Table 2.1 gives the resultant currents from applied voltages for the range of each surfactant system. These results point out one advantage of using Brij 35 as a micellar system in MECC; since Brij 35 is a nonionic surfactant, it does not contribute to the current as is observed with ionic surfactants, thus it may be used in high concentrations without an increase in Joule heating (2.19). However, since Brij 35 is not ionic, it cannot migrate electrophoretically. Therefore, a modifier or co-surfactant must be added to incorporate with the Brij 35 micelles which gives each micelle a charge density on its outer surface. SDS is a logical additive due
Table 2.1  Effect of surfactant concentration on separation currents and \( t_{mc}/t_0 \) at constant applied voltages.

<table>
<thead>
<tr>
<th>[SURF], mM</th>
<th>SDS (^a) current, (\mu A)</th>
<th>[SURF], mM</th>
<th>Brij 35 (^{b,c}) current, (\mu A)</th>
<th>( t_{mc}/t_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>17.2</td>
<td>10</td>
<td>17.5</td>
<td>2.67</td>
</tr>
<tr>
<td>25</td>
<td>19.5</td>
<td>20</td>
<td>16.6</td>
<td>2.64</td>
</tr>
<tr>
<td>35</td>
<td>23.3</td>
<td>30</td>
<td>17.7</td>
<td>2.56</td>
</tr>
<tr>
<td>50</td>
<td>26.2</td>
<td>40</td>
<td>17.1</td>
<td>2.37</td>
</tr>
<tr>
<td>100</td>
<td>35.3</td>
<td>60</td>
<td>16.6</td>
<td>2.31</td>
</tr>
</tbody>
</table>

\(^a\)applied voltage of 18.00 kV  
\(^b\)applied voltage of 25.00 kV  
\(^c\)mixed surfactant system containing stated Brij 35 concentration along with 20 mM SDS
to its availability and widespread use in MECC. Other aspects of this mixed micellar system will be discussed later.

2.2.4 Measurement of resolution

When measuring resolution between two compounds, a choice must be made as to whether direct or indirect methods of measurement are to be used. The direct method of measurement is accomplished using

\[ R_s = \frac{2\Delta t_R}{w_1 + w_2} \]  

(2.5)

where \( \Delta t_R \) is the difference between retention times of the two solutes and \( w_1 \) and \( w_2 \) is the baseline width of the first and second peaks, respectively. Indirect measurement is performed using Equation 2.3 or 2.4 where the efficiency, selectivity, and \( t_{mc}/t_0 \) must be determined prior to calculation of \( R_s \) on the basis of \( k' \) values. Although Equation 2.5 is typically the standard for measuring experimental resolution, the high efficiencies often observed in MECC limit the accuracy of resolution measurements calculated by Equation 2.5. This limitation is caused by the narrowness of peak widths and the subsequent error introduced by attempting to measure these widths. Manual width measurement could possibly yield errors in excess of 10% for each measurement due to limitations in the data system. This would significantly impair our ability to compare trends in resolution with increasing surfactant concentration. Therefore, \( R_s \) values were calculated using Equation 2.3. Although more tedious, this method of calculation should provide us with a better means for observing trends in solute resolution. We chose to calculate \( R_s \) via Equation 2.3 rather than Equation 2.4 so that individual \( k' \) values rather than averages could be utilized for each pair of solutes.
For calculating $R_s$ via Equation 2.3, the efficiency, selectivity, $t_{mc}/t_o$, and $k'$ values for the solute pair must be measured. Efficiencies for each solute were calculated from

$$N = 5.54 \left( \frac{t_R}{w_{0.5}} \right)^2$$

(2.6)

where $w_{0.5}$ is the peak width at half-height, and averaged over the surfactant concentration range to yield a mean efficiency. An average of mean efficiencies for individual solute pairs was used for the determination of resolution between these solutes. For the theoretical resolution curves, arbitrary values for efficiency slightly less than experimental values ($N=25,000$ for SDS, $N=100,000$ for Brij 35/SDS) were used to offset these theoretical curves from the experimental data. Selectivities used in resolution calculations were averaged over the entire range of surfactant concentration, and the same values were used for both the theoretical curves and experimental data.

Resolution data were plotted versus surfactant concentration in two ways: (i) using the assumption that the ratio $t_{mc}/t_o$ was constant (an average of this ratio over all investigated surfactant concentrations), and (ii) using the experimental values of $t_{mc}/t_o$ for each surfactant concentration. Theoretical curves were generated only for constant $t_{mc}/t_o$ conditions. Justification for using efficiency and selectivity values averaged over the range of surfactant concentrations employed will be given later.

### 2.3 RESULTS AND DISCUSSION

Solute resolution in MECC is determined by the same general parameters found in conventional chromatography; however, the fundamental resolution equation, see Equations 2.3 and 2.4, is complicated by the limited time in which a solute must elute. The retention term of the resolution equation,
\[ t'(k') = \left( \frac{k'}{1 + k'} \right) \left( \frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})k'} \right) \]  

(2.7)

is important in that it is dependent not only on the retention of the compounds of interest, but also reliant on the elution range, as expressed by the ratio \( t_{mc}/t_0 \). Foley (2.17) has shown that the capacity factor for optimum resolution is exclusively dependent on the ratio \( t_{mc}/t_0 \) under conditions of constant efficiency and selectivity as described by

\[ k'_{opt} = \sqrt{t_{mc}/t_0} \]  

(2.8)

This value can be used to determine the surfactant concentration necessary for optimum resolution of two analytes. The capacity factor of a solute in MECC can be defined by

\[ k' = P_{wm} \beta \]  

(2.9)

where \( P_{wm} \) is the partition coefficient of the solute between the aqueous and micellar phases and \( \beta \) is the phase ratio. The phase ratio, which is generally defined as a ratio of volume of micellar phase to volume of aqueous phase, can be expressed explicitly as

\[ \beta = \frac{V([SURF] - CMC)}{1 - V([SURF] - CMC)} \]  

(2.10)

where \( V \) is the partial molar volume of the surfactant, \([SURF]\) is the total molar concentration of surfactant, and \( CMC \) is its critical micelle concentration. Substitution of Equation 2.10 into Equation 2.9 yields an expression for the surfactant
concentration based on easily measurable or predetermined values for the solute and surfactant system, namely,

\[
[SURF] = \frac{k' + V	ext{CMC}(k' + P_{wm})}{V(k' + P_{wm})}
\]  

(2.11)

This equation allows one to ascertain the surfactant concentration necessary to obtain a specific capacity factor for a solute. A useful approximation of Equation 2.11 can be derived by the assumption that \( P_{wm} \gg k' \) (except in the case of very hydrophilic compounds), giving

\[
[SURF] = \frac{k'}{P_{wm}V} + \text{CMC}
\]  

(2.12)

Using Equation 2.8 and 2.12, the surfactant concentration for optimum resolution of two solutes can be determined via experimental and accepted values of \( t_{mc}/t_0 \), \( V \), CMC, and the average partition coefficient of the two solutes of interest, \( P_{wm} \).

Figure 2.1 illustrates typical electrokinetic chromatograms of PTH-amino acids using SDS (upper chromatogram) and Brij 35/SDS (lower chromatogram) micellar systems. In order to examine the effect of surfactant concentration upon solute resolution, we will concentrate on the variation of resolution between closest pairs of solutes, i.e., gly/ala, val/nva, and ile/nle. This will illustrate the effect that surfactant concentration has upon solute resolution over a fairly wide range of solute hydrophobicity.

Figures 2.2 and 2.3 show the effect of surfactant concentration on the resolution of each pair of solutes using SDS and Brij 35/SDS, respectively. Note that theory predicts a different optimum surfactant concentration for each pair of solutes via differences in average partition coefficients for each solute pair (see Equation 2.12).
Figure 2.1 Representative electrokinetic chromatograms of six PTI-amine acids.

**Elution order:** PTH-gly, PTH-ala, PTH-val, PTH-nva, PTH-ile, PTH-nle, and decanophenone (time marker). **Capillary:** 50 µm i.d., 170 µm o.d., 57.5 cm (50.0 cm to detector). **Detection wavelength:** 254 nm. **Upper chromatogram:** 75 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 18 kV; current, 27.2 µA.

**Lower chromatogram:** 40 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 17.1 µA.
Figure 2.2  Resolution vs [SDS] for 3 pairs of PTI1-amino acids: (A) PTI1-gly/PTI1-ala; (B) PTI1-val/PTI1-nva; and (C) PTI1-ile/PTI1-nle.
Figure 2.3  Resolution vs [Brij 35] for 3 pairs of P111-amino acids. Pair identification as in Figure 2.2.
2.3.1 Resolution in the SDS system

As shown in Figures 2.2B and 2.2C, the theoretical resolution curves reach a maximum within the experimental limits of surfactant concentration, although the maximum for ile/nle occurs at a very low surfactant concentration (~25 mM) which may degrade solute resolution via poor column efficiency (2.10). The theoretical curve for the gly/ala solute pair (Figure 2.2A) shows no optimum within the experimental limits of SDS concentration, indicating a significant disadvantage of using SDS when separating fairly hydrophilic compounds.

As was previously described, resolution data were plotted assuming either a constant $t_{mc}/t_o$ or using the actual values of this ratio for each investigated surfactant concentration (variable $t_{mc}/t_o$). Experimental data for gly/ala and ile/nle follow the trend described by the theoretical curve fairly well using either set of values for $t_{mc}/t_o$. However, the dependence of $t_{mc}/t_o$ on surfactant concentration may occasionally have a significant effect on the range of surfactant concentration that is optimum for a given pair to be separated. For example, in Figure 2.2B the optimum range of SDS concentration is much broader for val/nva than would otherwise be expected.

For optimization of $k'$ via surfactant concentration, a slight preference should be given to surfactant systems in which $t_{mc}/t_o$ does not change appreciably with surfactant concentration, assuming other factors such as efficiency and selectivity are equal. Optimization of surfactant concentration at constant $t_{mc}/t_o$ is slightly more straightforward, although systems in which $t_{mc}/t_o$ varies can generally be optimized through an iterative scheme (2.17). With such an approach, an accurate value for [$SDS]_{opt}$ can be determined by remeasuring $t_{mc}/t_o$ and recalculating [$SDS]_{opt}$ after each run until convergence to [$SDS]_{opt}$ is reached. Typically, this convergence should require two iterations at most.
2.3.2 Resolution in the Brij 35/SDS system

With regard to Figure 2.2A, it is evident that [SDS]_{opt} is relatively large (>100 mM), hence posing the possibility of higher currents which may ultimately degrade column efficiency via temperature gradient effects (2.20). We therefore considered an alternate surfactant system consisting of a mixture of nonionic and anionic surfactants, Brij 35 and SDS. Using the criterion of the sensitivity of the elution range \((\text{t}_{\text{mc}}/\text{t}_0)\) to surfactant concentration, a mixture of SDS and Brij 35 may prove to be a better surfactant system than pure SDS since \(\text{t}_{\text{mc}}\) could be controlled by using a constant SDS concentration in the mixed surfactant system. The use of Brij 35 as one component of a mixed surfactant system has been previously explored, although a rigorous examination of its properties as a micellar system in MECC was not attempted (2.19). Although we intend to show the usefulness of Brij 35/SDS as a micellar system in this paper, a more in-depth study of this system by our laboratory will be published elsewhere (2.21).

Figure 2.3 illustrates the effect of Brij 35/SDS concentration upon resolution of all solute pairs. As stated previously, accurate values for \(P_{\text{wn}}\) were impossible to determine at this time due to the lack of information on certain intrinsic parameters of the Brij 35/SDS mixed micellar system, i.e., partial molar volume \((V)\), CMC, and the effect of Brij 35/SDS mole ratio upon these values. Figure 2.3A shows the effect of Brij 35 concentration upon the resolution of the gly/ala solute pair. In contrast to SDS, the retention of the gly/ala pair is sufficient to permit adjustment of [Brij 35] for optimum resolution. Also, the variation of \(\text{t}_{\text{mc}}/\text{t}_0\) is much less in Brij 35/SDS than in pure SDS, virtually eliminating the need for the iterative procedure. Table 2.1 compares the variation of \(\text{t}_{\text{mc}}/\text{t}_0\) with an increase in surfactant concentration for the two micellar systems. Whereas \(\text{t}_{\text{mc}}/\text{t}_0\) decreases only marginally (15%) in the Brij 35/SDS
system, it increases by nearly three-fold (2.97) in the pure SDS system. Note, however, that the Brij 35/SDS system has a narrower elution range than SDS due to the lower micellar electrophoretic mobility of the former. (The elution range of the Brij 35/SDS system could presumably be increased by adjustment of pH or other operational parameter, although it was not done for the present study.)

As shown in Figures 2.3B and 2.3C, the performance of the Brij 35/SDS system is not satisfactory for more hydrophobic compounds due to their excessively high retention. Although a maximum is present for the val/nva solute pair in Figure 2.3B, separations for which the mole ratio of Brij 35 to SDS drops below 1:1 are generally less desirable because of the tendency of SDS to dominate the retention mechanism under those conditions. SDS-dominated separations are less desirable for reasons discussed earlier and subsequently. We have witnessed significantly different retention behavior for Brij 35/SDS surfactant systems, depending on whether the mole ratio is above or below unity. For example, the relationship between \( k' \) and \([\text{Brij 35}]\) is linear at Brij 35:SDS mole ratios greater than 1:1, but nonlinear at ratios less than 1:1; such trends suggest a retention mechanism that is consistent and changing, respectively. Although the difficulty in separating hydrophobic compounds via the Brij 35/SDS systems may have been alleviated by using a lower concentration of SDS than in the present study or by the addition of organic modifier to the mobile phase, these possibilities were beyond the scope of the present investigation.

2.3.3 Efficiency

As previously stated, the surfactant concentration approach to optimization in MECC which we have introduced (2.17) relies on the assumption of nearly constant efficiency and selectivity over the practical range of surfactant concentration. A variety
of intracolumn (2.11, 2.20) and extracolumn phenomena (2.22, 2.23) can affect the separation efficiency in MECC. Five sources of intracolumn band broadening have been proposed: (i) longitudinal diffusion, (ii) sorption-desorption kinetics, (iii) intermicellar mass transfer, (iv) radial temperature gradients, and (v) variation of micellar mobilities due to the polydispersity of the micelles.

Tables 2.2 and 2.3 give approximate efficiencies for the SDS and Brij 35/SDS systems, respectively. These values were measured using the half-height equation which may introduce some error resulting from nonideal peak profiles (2.24, 2.25). Furthermore, imprecision of these data may be as high as 10% due to manual measurement of the peak widths. Nonetheless, we believe that these data accurately illustrate the trends in column efficiency for both surfactant systems.

For the SDS micellar system, large discrepancies in efficiency were observed for the less retained, more hydrophilic compounds as the SDS concentration was varied from 15 to 100 mM; relatively consistent results were obtained for moderately to highly retained (hydrophobic) compounds. In contrast, reasonably constant column efficiencies were observed for each solute in the Brij 35/SDS mixed micellar system over the entire concentration range of Brij 35.

Although some variation in N with surfactant concentration is evident for both surfactant systems, its effect on resolution ($R_s$) is reduced by the square root dependence of $R_s$ on N (see Equation 2.3). Therefore, with the exception of two hydrophilic (poorly retained) solutes in the SDS system, variations in N with surfactant concentration are responsible for no more than an 8% change in resolution for all solutes and both surfactant systems of the present study. Since no exceptions were observed for the Brij 35/SDS system compared to two for the pure SDS system, the former is a significantly better system according to this criterion. Nevertheless, even in situations where variations in $\sqrt{N}$ with surfactant concentration are significant,
Table 2.2 Efficiencies for SDS surfactant system.$^a$

<table>
<thead>
<tr>
<th>PTH-amino acid</th>
<th>k' range</th>
<th>$\bar{N}$</th>
<th>% RSD</th>
<th>$\sqrt{\bar{N}}$</th>
<th>% RSD (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycine</td>
<td>0.29-1.40</td>
<td>62,000</td>
<td>64</td>
<td>239</td>
<td>34</td>
</tr>
<tr>
<td>alanine</td>
<td>0.51-2.38</td>
<td>67,700</td>
<td>49</td>
<td>253</td>
<td>28</td>
</tr>
<tr>
<td>valine</td>
<td>1.58-7.37</td>
<td>48,800</td>
<td>10</td>
<td>221</td>
<td>5.2</td>
</tr>
<tr>
<td>norvaline</td>
<td>2.07-9.30</td>
<td>46,300</td>
<td>4.6</td>
<td>215</td>
<td>2.3</td>
</tr>
<tr>
<td>isoleucine</td>
<td>3.48-14.4</td>
<td>30,600</td>
<td>16</td>
<td>174</td>
<td>8.0</td>
</tr>
<tr>
<td>norleucine</td>
<td>4.82-22.1</td>
<td>29,800</td>
<td>5.8</td>
<td>173</td>
<td>2.9</td>
</tr>
</tbody>
</table>

$^a$SDS concentration range from 25 to 100 mM.
<table>
<thead>
<tr>
<th>PTH-amino acid</th>
<th>k' range</th>
<th>$\overline{N}$</th>
<th>% RSD</th>
<th>$\sqrt{N}$</th>
<th>% RSD (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycine</td>
<td>0.96-3.00</td>
<td>151,000</td>
<td>13</td>
<td>388</td>
<td>6.6</td>
</tr>
<tr>
<td>alanine</td>
<td>1.40-4.33</td>
<td>165,000</td>
<td>4.0</td>
<td>406</td>
<td>2.0</td>
</tr>
<tr>
<td>valine</td>
<td>3.25-9.99</td>
<td>140,000</td>
<td>12</td>
<td>374</td>
<td>5.9</td>
</tr>
<tr>
<td>norvaline</td>
<td>4.08-12.6</td>
<td>126,000</td>
<td>6.6</td>
<td>355</td>
<td>3.3</td>
</tr>
<tr>
<td>isoleucine</td>
<td>6.63-19.8</td>
<td>94,500</td>
<td>10</td>
<td>307</td>
<td>5.2</td>
</tr>
<tr>
<td>norleucine</td>
<td>9.08-26.1</td>
<td>106,000</td>
<td>3.9</td>
<td>326</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*a* Brij 35 concentration range from 20 to 60 mM with constant [SDS] of 20 mM.
optimization can still be achieved simply by performing one or two additional iterations (2.17). Note that since the surfactant concentration will typically be within 10-20 mM of the actual optimum after the first iteration, only minimal changes in column efficiency are likely as additional iterations (if necessary) are made.

Importantly, efficiencies for the Brij 35/SDS micellar system were significantly greater (by factors of 2.5 to 3.5) than those observed for pure SDS. Although a small portion of these differences can be attributed to minor differences in operating voltages and currents, the bulk of the differences in separation efficiency between SDS and Brij 35/SDS systems must be attributed to differences in the micellar systems themselves, perhaps in the kinetics of solute sorption and/or desorption. Assuming that SDS micelles are more highly ordered and/or charged than their poorly characterized mixed Brij 35/SDS counterparts, we speculate that the activation barriers to sorption/desorption may be greater in the SDS system due to greater steric and/or electrostatic hindrance to mass transfer. With regard to the latter, a profound effect may be possible for highly hydrophobic compounds in their desorption from the SDS micelle since such solutes may tend to be "trapped" in the micelles.

Another trend not shown in Tables 2.2 and 2.3 is the reduction in column efficiency with decreasing surfactant concentration, particularly for the pure SDS system. As observed by Sepaniak (2.10), we found much lower column efficiencies at low concentrations in the SDS system. Operation at the low surfactant concentrations produced more asymmetric peaks (peak fronting) than at higher concentrations of SDS, suggesting that the separation may have been complicated by sample overloading or a high solute to micelle concentration ratio. This observation supports the hypothesis of Terabe et al. that micelle overloading and not intermicellar mass transfer was largely responsible for poor efficiency at low SDS concentrations (2.11).
Although not as strong, a similar trend in efficiency with surfactant concentration was also observed for the Brij 35/SDS system. This was surprising since the concentration of mixed micelles was presumably much larger than the SDS micelles under the conditions of comparison, and presumably sufficient to preclude poor efficiency due to micelle overloading or intermicellar mass transfer. To illustrate, let us assume that a 10 mM Brij 35/20 mM SDS mixed micellar system has (i) a critical micelle concentration equivalent to pure Brij 35 (CMC = 0.1 mM); (ii) an aggregation number of ~50 (intermediate between pure SDS (62) and Brij 35 (40)); and (iii) an SDS monomer concentration in the aqueous phase equal to the CMC of pure SDS (8.1 mM). With these assumptions, the micelle concentration in the Brij 35/SDS solution would be $4 \times 10^{-4}$ M. In contrast, the micelle concentration of a 25 mM SDS solution is $2.7 \times 10^{-4}$ M, approximately 40% smaller. An alternative explanation for the lower efficiencies observed with the Brij 35/SDS system at surfactant mole ratios less than 1:1 is the greater SDS-like character of the mixed micelles, and thus a more SDS-like efficiency.

2.3.4 Selectivity

The invariable nature of separation selectivity in MECC for neutral compounds is fairly well documented. Selectivity variations resulting from changes in concentration of a specific surfactant have been investigated by Row (2.6) and Fujiwara (2.13, 2.14). Their results indicate that separation selectivity in MECC for neutral solutes is independent of surfactant concentration. Also, moderate changes in selectivity have been reported (2.3) when surfactant identity is varied. Rasmussen et al. (2.19) have shown that modification of an SDS system with Brij 35 has a significant effect on selectivity, permitting the separation of compounds which may not otherwise be separated in MECC. In light of these studies, the selectivities of the
micellar systems used in this study were determined for adjacent solutes to confirm their lack of dependence on surfactant concentration and to allow comparison of the selectivities of the two systems.

Table 2.4 gives the selectivity of each surfactant system over the range of applied surfactant concentrations. Deviations of less than 3.3% were found for all solute pairs indicating that selectivity changes as a result of surfactant concentration were minimal. Equally as important, Table 2.4 also shows that the selectivity of hydrophilic solutes varies much more with surfactant identity than the selectivities observed for the more hydrophobic compounds. A significant change in selectivity ratio of SDS to Brij 35/SDS is observed for the gly/ala and ala/val pairs while the change in selectivity ratio for other solute pairs was minimal. We believe that this supports our hypothesis that surface interactions with Brij 35/SDS micelles play a role in the retention of hydrophilic solutes (vide infra).

2.3.5 Retention mechanism

Since oxyethylene groups predominate over the overall character of the Brij 35/SDS micelle surface, it is possible that the surface may play a significant role in the retention of polar compounds. Figure 2.4 illustrates this effect. In this case, the PTH-amino acids were used to model the retention characteristics of each micellar system. The function, \( g(k') \), was used to relate the retention of the solutes in a specific micellar system to their retention in 20 mM SDS

\[
g(k') = \frac{k_{\text{micelle}}' - k'_{20\text{mM SDS}}}{k'_{20\text{mM SDS}}} \quad (2.11)
\]

where \( k_{\text{micelle}}' \) represents the capacity factor of a compound in a given micellar system and \( k'_{20\text{mM SDS}} \) is the capacity factor of the compound in 20 mM SDS. A difference
Table 2.4 *Selectivities for SDS and Brij 35/SDS surfactant systems.*

<table>
<thead>
<tr>
<th>PTH-amino acid pair</th>
<th>SDS α</th>
<th>% RSD (n=4)</th>
<th>Brij 35/SDS α</th>
<th>% RSD (n=5)</th>
<th>α&lt;sub&gt;SDS&lt;/sub&gt;/α&lt;sub&gt;Brij 35/SDS&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>gly/ala</td>
<td>1.716</td>
<td>1.73</td>
<td>1.460</td>
<td>0.74</td>
<td>1.175</td>
</tr>
<tr>
<td>ala/val</td>
<td>3.068</td>
<td>1.26</td>
<td>2.326</td>
<td>2.05</td>
<td>1.319</td>
</tr>
<tr>
<td>val/nva</td>
<td>1.296</td>
<td>1.66</td>
<td>1.268</td>
<td>0.70</td>
<td>1.022</td>
</tr>
<tr>
<td>nva/ile</td>
<td>1.695</td>
<td>3.22</td>
<td>1.601</td>
<td>1.19</td>
<td>1.059</td>
</tr>
<tr>
<td>ile/nle</td>
<td>1.375</td>
<td>1.60</td>
<td>1.350</td>
<td>1.77</td>
<td>1.019</td>
</tr>
</tbody>
</table>
Figure 2.4  Comparison of retention mechanism for hydrophilic and hydrophobic PTH-amino acids in (A) SDS and (B) Brij-35/SDS micellar systems. The function $g(k')$ is defined in Equation 2.11.
in slope between different solutes reflects a difference in their retention in the given micellar system and in 20 mM SDS and, accordingly, a different retention mechanism. Figures 2.4A and 2.4B illustrate the variation of solute retention from 20 mM SDS for the SDS and Brij 35/SDS micellar systems, respectively. Figure 2.4A reveals that, as one would expect, the mechanism of retention is common for each solute and does not vary with SDS concentration as evidenced by overlapping plots of equivalent slope. When the Brij 35/SDS micellar system is used (Figure 2.4B), the slopes of PTH-gly and PTH-ala are much steeper than those of the more hydrophobic compounds, which are nearly equivalent. This illustrates that the Brij 35/SDS system shows different retention characteristics for the more hydrophilic compounds, indicating that their retention may be governed by interactions with micellar regions other than the inner hydrophobic region, i.e., interactions with the polar oxyethylene surface. It is expected that this interaction would be a type of inclusion with the polyoxyethylene groups of the micelle as described by Shinoda et al. (2.26) rather than a surface adsorption process typically observed for polar compounds in SDS.

Since the more hydrophobic compounds are expected to experience a similar retention mechanism in both micellar systems (Figure 2.4 and related text), there should be very little difference in the hydrophobic selectivity between the two systems. That is, if the retention of hydrophobic compounds was due solely to their interaction with the hydrophobic interior of the Brij 35/SDS micelle, then we would not expect much difference in the selectivity of hydrophobic compounds between Brij 35/SDS and pure SDS micelles since their interior hydrophobicity is expected to be similar. Table 2.4 gives both absolute selectivities and relative selectivities ($\alpha_{\text{SDS}}/\alpha_{\text{Brij 35/SDS}}$) for each pair of PTH-amino acids examined. The nearness to unity of the relative selectivities for the more hydrophobic solute pairs (val/nva,
nva/ile, and ile/nle) suggests a common retention mechanism for hydrophobic solutes in the micellar systems of the present study.

2.4 CONCLUSIONS

Surfactant concentration is an important factor for solute resolution in MECC, primarily through its effect on retention and, to a lesser extent, efficiency. It is clearly beneficial to optimize surfactant concentration for best resolution, especially for extremely complex separations; however, surfactant concentration restrictions from lack of micelle formation (lower limit) to temperature considerations resulting from extremely high currents (upper limit) may sometimes hinder or preclude the optimization of surfactant concentration for some compounds. Such problems were evident for SDS, leading to practical monomer concentration limits from 25 to 150 mM. The Brij 35/SDS mixed micellar system extended both limits significantly due to its unique properties. It was found, however, that the optimization of separations involving moderately to highly hydrophobic compounds may be more difficult to accomplish in pure aqueous buffers with Brij 35/SDS micelles due to the solutes' greater affinity for them. Excessive solute-micelle binding may of course be reduced by the addition of a small amount of organic solvent to the buffer.

Several advantages of the Brij 35/SDS micellar system were observed, including a 2.5 to 3.5-fold increase in column efficiency, unique selectivity for hydrophilic solutes, more uniform $t_{me}/t_0$ ratios, and a greatly reduced risk of capillary overheating. These advantages merit a more detailed study of the Brij 35/SDS micellar system as an alternative and possibly more appropriate pseudo-stationary phase than pure SDS. Measurements of certain micellar parameters such as partial molar volume and critical micelle concentration and their variation, or lack thereof, over varying mole
ratios will facilitate additional fundamental studies of this mixed Brij 35/SDS micellar system and its acceptance as a substitute for pure SDS.
CHAPTER TWO REFERENCES


2.21. E.L. Little and J.P. Foley, manuscript in preparation.


CHAPTER 3

COMPARISON OF SDS AND BRIJ 35/SDS MICELLAR MEDIA IN MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY
3.1 INTRODUCTION

The use of micellar electrokinetic capillary chromatography (MECC) has grown significantly since its inception due to its ability to provide high efficiency separations for a wide variety of neutral solute systems. The mechanism behind separations in MECC is the differential partitioning of solutes between an aqueous phase and a micellar "pseudostationary" phase. Anionic surfactant systems are typically used in MECC since the resultant micelles electrophoretically migrate in the opposite direction of electroosmotic flow and do not interact appreciably with the negatively charged walls of the fused silica capillary columns. Sodium dodecyl sulfate (SDS) is by far the most popular micellar system in MECC due to its low cost, availability at high purity, UV absorption characteristics, and intrinsic micellar properties.

Several other surfactant systems have been investigated to ascertain their applicability to MECC. Terabe et al. (3.1) examined three surfactant systems: SDS, sodium decyl sulfate (STS), and sodium dodecylsulfonate. Changes in solute retention and distribution coefficients suggested that retention of more hydrophilic compounds was affected by the polar moiety present on the micellar surface and that smaller micellar size increased solute retention. A later study by Burton et al. (3.2) revealed that greater elution ranges were observed for STS due to smaller micellar size, resulting in greater electrophoretic velocities; several problems were also encountered for STS such as poor retention time reproducibility and increased peak asymmetry with increasing solute retention. Problems also arose when alkyl sulfates with tail lengths greater than fourteen carbons were considered due to their limited solubility in water. In addition to alkyl sulfate and sulfonate systems, other anionic surfactant systems such as bile salts (3.3, 3.4) have been studied and shown effective in separating enantiomers, although improvements in enantiomeric resolution and separation efficiencies are necessary.
Although anionic surfactants, primarily SDS, have received the bulk of attention to date, their usefulness is limited by the concentrations which may be utilized in MECC. Due to its charge, high concentrations of SDS result in increased Joule heating and, consequently, degradation of separation efficiency. The use of nonionic surfactants in MECC may be advantageous since micellar concentration can be increased dramatically with no change in operating currents at constant voltage. However, the lack of surface charge on nonionic micelles necessitates the addition of anionic surfactant to form mixed micelles. This permits the micelles to migrate electrophoretically in opposition to the bulk electroosmotic flow.

Polyoxyethylene(23)dodecanol (Brij® 35) has been demonstrated in previous studies to be useful as a pseudostationary phase in MECC. Initial studies of this micellar system showed particular promise in alteration of selectivity; benzene and benzaldehyde, inseparable in SDS or STS, were easily separated using a Brij 35/SDS micellar phase (3.5). Our group has further demonstrated the usefulness of this micellar system in the optimization of solute resolution via changes in surfactant concentration (3.6). We reported significant improvement in separation efficiency (2.5-3.5 fold) and unique hydrophilic solute selectivity along with more uniform \( t_{me}/t_{0} \) ratios.

The favorable findings for Brij 35/SDS mixed micellar systems merits a more in-depth study of this novel system. In this Chapter, we intend to illustrate various findings for this micellar system including further studies on column efficiencies, variation in selectivity for a variety of solute systems, and differences in retention characteristics.
3.2 EXPERIMENTAL

3.2.1 Materials

Electrophoresis-grade SDS was purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA) while Brij® 35 (polyoxyethylene(23)dodecanol) was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Phenol and chlorobenzene were purchased from Aldrich, benzene from EM Science (Gibbstown, NJ, USA), nitrobenzene from Mallinckrodt, Inc. (Paris, KY, USA), toluene from Fisher Scientific Co. (Fair Lawn, NJ, USA), anisole (methoxybenzene) from Matheson Coleman & Bell (Norwood, OH, USA) and were used as received. Nanograde® acetonitrile (t₀ marker) was obtained from Mallinckrodt. Distilled water was deionized and redistilled with a Corning Mega-Pure™ Water Purification System (Corning, Inc., Corning, NY, USA).

Untreated fused-silica capillary tubing with dimensions of 50 μm i.d. and 170 μm o.d. was purchased from Alltech Associates, Inc. (Deerfield, IL, USA) and cut to a length of 47.5 cm. A window was burned through the polyimide coating at a distance of 7.5 cm from the outlet end of each column yielding a column with injector-to-detector length of 40.0 cm.

3.2.2 Equipment

A Quanta 4000 Capillary Electrophoresis System was provided by Millipore Corporation, Waters Chromatography Division (Milford, MA, USA). This instrument was equipped with hydrostatic injection used for 1 sec intervals and a fixed-wavelength UV absorbance detector operated at 254 nm. Data were acquired on an Apple MacintoshPlus computer (Cupertino, CA, USA) equipped with a Rainin Dynamax® Method Manager data acquisition package (Woburn, MA, USA).
3.2.3 Methods

3.2.3.1 Capillary treatment

Activation of the capillaries was performed using a modification of a procedure described previously (3.7). The capillary was initially rinsed with 1 M KOH for 15 min followed by subsequent rinses of 0.1 M KOH and water for 15 min each. The capillary was then rinsed for 20 min with the operating buffer. Purges with the operating buffer were performed after each run for 5 min using a vacuum of ~14 inches Hg at the detector reservoir. SDS separations were performed with a 10 kV applied voltage and 25 kV was used for Brij 35/SDS separations. These voltages yielded currents of <35 μA for all micellar systems.

3.2.3.2 Buffer systems

Stock buffer solution was prepared with NaH₂PO₄ • H₂O and NaOH to give a 100 mM phosphate buffer at pH 6.8. This solution was diluted to a concentration of 10 mM which was used for preparation of surfactant solutions. SDS solutions were prepared from a 100 mM stock solution at concentrations ranging from 40 to 100 mM in 20 mM increments. Brij 35/SDS solutions were prepared with a constant concentration of 20 mM SDS and dilution of a 100 mM Brij 35 stock solution to give Brij 35 concentrations ranging from 30 to 60 mM in 10 mM increments.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect on retention

Solute separation in MECC stems from differential partitioning of solutes between aqueous and micellar phases. Although retention is similar to conventional reversed-phase chromatography, solute interaction with the micellar surface may play an integral role in its retention characteristics.
Solute retention in MECC is fundamentally described by

$$k' = P_{wm} \beta$$  \hspace{1cm} (3.1)

where \( k' \) is the solute capacity factor, \( P_{wm} \) is the solute's water-micelle partition coefficient, and \( \beta \) is the phase ratio. The phase ratio can be defined in terms of surfactant concentration by

$$\beta = \frac{V([SURF] - CMC)}{1 - V([SURF] - CMC)}$$  \hspace{1cm} (3.2)

where \( V \) is the partial molar volume of the surfactant monomer, \([SURF]\) is the surfactant concentration, and \( CMC \) is the surfactant concentration required for micellization to occur (critical micelle concentration).

Trends in retention characteristics between pure SDS and Brij 35/SDS systems were observed using benzene derivatives of different functionality. Figure 3.1 shows representative chromatograms from each micellar system. In general, \( k' \) values were greater in Brij 35/SDS which suggests greater solute solubilization. However, since retention is dependent on micelle concentration (see eqs 3.1 and 3.2) and the CMC of the Brij 35/SDS micellar systems are not specifically reported in the literature, it is necessary to develop an expression which can reasonably predict the CMC at any Brij 35/SDS mole fraction.

Through the use of the phase separation approximation, the mixture CMC of a binary surfactant system can be written in terms of monomer composition and pure component CMCs (3.8). This relationship is

$$CMC_{mix} = y_{Brij \ 35} \times CMC_{Brij \ 35} + y_{SDS} \times CMC_{SDS}$$  \hspace{1cm} (3.3)
Figure 3.1 Representative electrophoretic chromatograms of six benzene derivatives.  
**Capillary**: 50 μm i.d., 170 μm o.d., 47.5 cm (40.0 cm to detector).  
**Detection wavelength**: 254 nm.  
**Solute identity**: 1) phenol, 2) benzene, 3) nitrobenzene, 4) acetophenone, 5) anisole, 6) toluene, 7) chlorobenzene, and 8) decanophenone (line marker).  
**Upper chromatogram**: 100 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 10 kV; current, 21.9 μA.  
**Lower chromatogram**: 40 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 28.7 μA.
where $\gamma_{\text{Brij 35}}$ and $\gamma_{\text{SDS}}$ are activity coefficients, $X_{\text{Brij 35}}$ and $X_{\text{SDS}}$ are surfactant mole fractions where $X$ equals the ratio of moles of a particular surfactant to the total moles of surfactant ($n_{\text{SDS}} + n_{\text{Brij 35}}$), and $\text{CMC}_{\text{Brij 35}}$ and $\text{CMC}_{\text{SDS}}$ are the critical micelle concentrations of Brij 35 and SDS, respectively. Using unity for activity coefficients and assuming an aggregation number of 50, the Brij 35/SDS micelle concentrations fall from ~1 to 1.5 mM whereas SDS micelle concentrations range from 0.5 to 1.5 mM. In addition, the SDS and Brij 35/SDS micelle concentrations are almost identical at 80 mM SDS ($[\text{SDS micelle}] = 1.16$ mM) and 40 mM Brij 35 ($[\text{Brij 35/SDS micelle}] = 1.14$ mM). At these particular monomer concentrations, retention in the Brij 35/SDS system was significantly greater for all compounds except acetophenone (which, as will be discussed later, undergoes dramatic retention variation in Brij 35/SDS in comparison to pure SDS). For example, the capacity factors of nitrobenzene and toluene in 80 mM SDS were 2.18 and 4.65, respectively, whereas the capacity factors of these same compounds in 40 mM Brij 35/SDS were 2.60 and 6.16. These differences reflect 19% and 32% greater retention in the Brij 35/SDS system for nitrobenzene and toluene, respectively. In fact, the Brij 35/SDS micellar system solubilizes most of the solutes (except acetophenone) more effectively than SDS. The literature supports these findings via reports that mixtures of nonionic and anionic surfactants exhibit excellent powers of detergency in contrast to pure anionic surfactants (3.9). Accordingly, these systems have been applied in commercial processes due to their enhanced solubilizing power (3.10).

### 3.3.2 Comparison of partition coefficients

Determination of partition coefficients is easily accomplished using the relationship between retention and surfactant concentration described by Terabe et al.
which was developed by combination of eqs 3.1 and 3.2 under the assumption that the denominator in eq 3.2 was unity.

\[ k' = P_{wm}V([\text{SURF}] - \text{CMC}) \]  

(3.4)

However, in a binary surfactant system one would expect differences in partial molar volumes and, as previously mentioned, CMCs with varying nonionic/anionic surfactant mole fractions. Therefore, modification of eq 3.4 is necessary to measure accurately partition coefficients in mixed micellar systems. For a binary surfactant system, the following equation can be written to yield the same basic relationship between \( k' \) and \([\text{SURF}]\) yet include parameters unique to binary surfactant mixtures.

\[ k' = P_{wm}V_{\text{mix}}([\text{SURF}]_{\text{total}} - \text{CMC}_{\text{mix}}) \]  

(3.5)

A relationship defining \( \text{CMC}_{\text{mix}} \) has been previously shown in eq 3.3. The term \( V_{\text{mix}} \) is expressed by

\[ V_{\text{mix}} = \chi_{\text{Brij 35}}V_{\text{Brij 35}} + \chi_{\text{SDS}}V_{\text{SDS}} \]  

(3.6)

where \( V_{\text{Brij 35}} \) and \( V_{\text{SDS}} \) are the partial molar volumes of Brij 35 and SDS monomers, and \( \chi_{\text{Brij 35}} \) and \( \chi_{\text{SDS}} \) are, as previously defined, the surfactant mole fractions of Brij 35 and SDS, respectively. \( V_{\text{mix}} \) must be expressed in terms of partial molar volumes of the surfactant monomers since surfactant concentration rather than micelle concentration is used in eq 3.5. Expressions have been developed to describe variation in micellar volume at different nonionic/anionic surfactant mole fractions.
(3.11) but they cannot be used unless solute retention is viewed with regard to micellar rather than surfactant monomer concentration.

Note that, as addressed in eq 3.5, the total surfactant concentration should be used rather than the concentration of surfactant which is varied. However, use of Brij 35 concentration is adequate for determination of partition coefficients as long as [SDS] is constant and $V_{mix}$ does not vary significantly over the investigated concentration range. In our case, the [Brij 35] range reflects $V_{mix}$ variation of 6.7% RSD. This variation illustrates a significant disadvantage to changing Brij 35/SDS surfactant mole fractions for increasing surfactant concentration. However, the choice of using [Brij 35] or [SURF]$_{total}$ is arbitrary for determination of partition coefficients since this determination is slope dependent and neither concentration expression decreases the error produced by changes in $V_{mix}$.

Table 3.1 gives the partition coefficients of benzene derivatives for the pure SDS and Brij 35/SDS micellar systems. The value of $V_{mix}$ used for $P_{win}$ determination in the Brij 35/SDS system was 0.832 L/mole. Notable differences between the two surfactant systems were evident. Most notably, acetophenone partitioned much less into Brij 35 micelles than all other solutes, an occurrence not observed in the SDS system. Its reduced retention may be due to greater interaction with the polyoxyethylene (POE) surface layer of these micelles. Preferential solvation of acetophenone in the POE layer, which is fairly disperse and significantly hydrated (3.12), would probably make partitioning of this solute into the aqueous phase much more favorable and, hence, decrease its retention in comparison to pure SDS. Of the other solutes, phenol may also undergo similar favorable interaction with this layer; however, greater retention of this solute by the POE layer was probably achieved through hydrogen bonding between the phenolic hydroxide group and the oxygen atoms of the oxyethylene units. If this is the case, then its relative retention with
Table 3.1  Partition coefficients and CMCs for benzene derivatives with varying functionality

<table>
<thead>
<tr>
<th>compound</th>
<th>slope</th>
<th>$P_{wm}$</th>
<th>intercept</th>
<th>CMC, mM</th>
<th>$r^2$ (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenol</td>
<td>12.51 (±0.43)</td>
<td>50.85</td>
<td>-0.135 (±0.031)</td>
<td>10.8</td>
<td>0.998</td>
</tr>
<tr>
<td>benzene</td>
<td>25.51 (±0.87)</td>
<td>103.7</td>
<td>-0.292 (±0.064)</td>
<td>11.4</td>
<td>0.998</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td>31.55 (±0.84)</td>
<td>128.3</td>
<td>-0.326 (±0.062)</td>
<td>10.3</td>
<td>0.999</td>
</tr>
<tr>
<td>acetophenone</td>
<td>39.51 (±1.20)</td>
<td>160.6</td>
<td>-0.407 (±0.088)</td>
<td>10.3</td>
<td>0.998</td>
</tr>
<tr>
<td>anisole</td>
<td>39.51 (±1.20)</td>
<td>160.6</td>
<td>-0.407 (±0.088)</td>
<td>10.3</td>
<td>0.998</td>
</tr>
<tr>
<td>toluene</td>
<td>67.33 (±2.37)</td>
<td>273.7</td>
<td>-0.692 (±0.174)</td>
<td>10.3</td>
<td>0.998</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td>86.13 (±3.33)</td>
<td>350.1</td>
<td>-0.863 (±0.245)</td>
<td>10.0</td>
<td>0.997</td>
</tr>
<tr>
<td><strong>Brij 35/SDS</strong>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetophenone</td>
<td>19.20 (±0.23)</td>
<td>23.08</td>
<td>0.227 (±0.016)</td>
<td>-11.8</td>
<td>1.000</td>
</tr>
<tr>
<td>phenol</td>
<td>33.35 (±1.18)</td>
<td>40.08</td>
<td>-0.143 (±0.083)</td>
<td>4.29</td>
<td>0.999</td>
</tr>
<tr>
<td>benzene</td>
<td>32.05 (±0.26)</td>
<td>38.52</td>
<td>0.388 (±0.018)</td>
<td>-12.1</td>
<td>1.000</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td>36.25 (±0.14)</td>
<td>43.57</td>
<td>0.426 (±0.010)</td>
<td>-11.8</td>
<td>1.000</td>
</tr>
<tr>
<td>anisole</td>
<td>41.35 (±1.24)</td>
<td>49.70</td>
<td>0.673 (±0.088)</td>
<td>-16.3</td>
<td>0.999</td>
</tr>
<tr>
<td>toluene</td>
<td>77.85 (±2.92)</td>
<td>93.57</td>
<td>1.504 (±0.206)</td>
<td>-19.3</td>
<td>0.999</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td>115.0 (±4.76)</td>
<td>138.2</td>
<td>3.308 (±0.336)</td>
<td>-28.8</td>
<td>0.998</td>
</tr>
</tbody>
</table>

*aThe negative values for CMC will be discussed later in the text.
respect to other solutes may be affected by changes in Brij 35 concentration. This possibility will be discussed later. All other solutes follow the same trend in partition coefficients as in SDS, suggesting comparable micellar interactions.

In comparing partition coefficients of solutes in Brij 35/SDS and SDS, the values for the SDS system were much higher. This was unexpected since solute solubilization by the nonionic/anionic micellar systems should be enhanced (vide supra). Thus, we have compared these partition coefficients to those observed in micellar liquid chromatography (MLC). Kord et al. (3.13) have compared solute-micelle binding constants (K_{nmw}) for a number of solutes determined by MECC and MLC. They observed that, in general, values measured by MECC were higher than observed in MLC. Poor temperature control and higher ionic strengths in MECC were assumed to be the major contributors to this deviation.

Table 3.2 shows P_{wm} values for both micellar systems in comparison to MLC literature values from an available data base (3.14). Partition coefficients for the Brij 35/SDS system were compared to literature values for pure Brij 35. Partition coefficients from our results in the SDS system were from 1.35 to 1.58 times greater than values determined from MLC data. On the other hand, MECC partition coefficients in the Brij 35/SDS system compared extremely well with MLC data with MECC values being generally lower than observed in MLC. Lower partition coefficients for mixed micellar systems in comparison to pure nonionic systems have been documented (3.15) and were found to be linearly related to differences in ζ-potential at the micellar surface. It has been proposed that the POE chains of the mixed micelle are more extended into the aqueous phase due to electrostatic repulsion of the anionic head groups present at the micellar surface. Extension of the POE groups into the aqueous phase results in a less compact micellar structure and, consequently, less solubilizing power, i.e., smaller partition coefficients.
<table>
<thead>
<tr>
<th>Compound</th>
<th>$P_{wm}$ (MECC)</th>
<th>$P_{wm}$ (MLC)</th>
<th>Ratio (MECC/MLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>50.85</td>
<td>36.05</td>
<td>1.411</td>
</tr>
<tr>
<td>Benzene</td>
<td>103.7</td>
<td>75.30</td>
<td>1.377</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>128.3</td>
<td>86.45</td>
<td>1.484</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>160.6</td>
<td>101.6</td>
<td>1.581</td>
</tr>
<tr>
<td>Anisole</td>
<td>160.6</td>
<td>48.62</td>
<td>3.303</td>
</tr>
<tr>
<td>Toluene</td>
<td>273.7</td>
<td>202.6</td>
<td>1.351</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>350.1</td>
<td>$b$</td>
<td></td>
</tr>
</tbody>
</table>

| **Brij 35/SDS**  |                 |                |                  |
| Phenol           | 23.08           | 23.69          | 0.974            |
| Acetophenone     | 40.08           | $b$            |                  |
| Benzene          | 38.52           | 37.55          | 1.026            |
| Nitrobenzene     | 43.57           | 44.11          | 0.988            |
| Anisole          | 49.70           | 51.83          | 0.959            |
| Toluene          | 93.57           | 105.8          | 0.884            |
| Chlorobenzene    | 138.2           | $b$            |                  |

*Values used for conversion of $K_{mw}$ to $P_{wm}$. SDS: $V$, 0.246 L/mol; CMC, 0.0081 M; aggregation number, 62. Brij 35: $V$, 1.105 L/mol; CMC, 0.0001 M, aggregation number, 40.*

*BMLC literature values unavailable.
As shown in eq 3.5, the CMC of a surfactant system may be obtained from a plot of $k'$ versus $[\text{SURF}]_{\text{total}}$. Terabe et al. (3.1) have previously shown that the CMC values obtained by this method seem unreliable. As shown in Table 3.1, the intercepts of these plots produce overestimated CMC values for the SDS system (8.1 mM at 25°C). This problem can be overcome to a degree by using $k'_0$ values, where $k'_0$ is the intercept of a plot of $k'$ versus operating current for a given solute, which negate the effects of temperature on the retention of analytes (3.1). Yet, as was previously mentioned, the accuracy of CMC measurements still remains questionable.

For the Brij 35/SDS system, the intercept values were all positive except for phenol. Therefore, investigation of $\text{CMC}_{\text{mix}}$ for the Brij 35/SDS system was impossible. Variation of $\text{CMC}_{\text{mix}}$ at different Brij 35/SDS surfactant mole fractions may explain the positive deviation in intercept values. This problem may be alleviated by performing separations under constant mole fraction. In this particular study, we chose to vary the surfactant mole fraction to investigate the constancy of $t_{\text{me}}/t_0$ and the relative variation of electroosmotic and micellar electrophoretic velocities. Our findings on these topics will be discussed later in this Chapter.

3.3.3 Variations in selectivity

3.3.3.1 Functional group selectivity

In simple terms, separation selectivity, $\alpha$, is the capability of some separation technique to differentiate between two unlike solutes. For extremely hydrophobic solutes, retention is primarily based on interaction with the micellar core. However, the particular solutes we have chosen range from moderately to highly hydrophilic. Therefore, surface interactions with the micelles should play a significant role in solute retention.
Table 3.3 displays functional group selectivities for the investigated solutes. This selectivity, \( \alpha_{\text{func}} \), is based on solute retention with respect to benzene

\[
\alpha_{\text{func}} = \frac{k'_{\text{BZ-X}}}{k'_{\text{BZ}}}
\]  

(3.7)

where \( k'_{\text{BZ-X}} \) and \( k'_{\text{BZ}} \) are the capacity factors for the benzene derivative and benzene, respectively. In most cases, variation in selectivity with increasing [SURF] was less than 1% RSD for both surfactant systems. However, variation in phenol selectivity in the Brij 35/SDS system stood out among the other data. Generally, we observed a slight decrease in selectivity with increasing surfactant concentration. This was probably due to changes in micellar composition and error associated with the measurement of \( t_{\text{inc}} \). Phenol marginally followed this trend in the SDS-mediated separations. However, in Brij 35/SDS, selectivity between phenol and benzene actually increased with increasing surfactant concentration. We have previously surmised that phenol probably interacts significantly with the micellar POE layer. However, this does not explain the observed change in selectivity since acetophenone, which may also interact significantly with this micellar region, generally followed the same trend in selectivity as the more hydrophobic solutes. We propose that this increase in functional group selectivity of phenol with increasing Brij 35 concentration is due to its interaction not only with the micellar POE layer but also the nonionic head groups of Brij 35 surfactant monomers present in solution. When dissociated, Brij 35 monomers should migrate at a velocity equivalent to \( v_{eo} \). If the retention of phenol is partially due to hydrogen-bonding, then its retention may be dependent on interaction with the nearest available Brij 35 molecule whether it be in the micellar or monomer state. The increase in phenol retention with increasing [Brij 35] may be simply due to
### Table 3.3 Comparison of functional group selectivity for Brij 35/SDS and SDS micellar systems

<table>
<thead>
<tr>
<th>micellar medium</th>
<th>X-COCH₃</th>
<th>X-OH</th>
<th>X-NO₂</th>
<th>X-OCH₃</th>
<th>X-CH₃</th>
<th>X-Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brij 35/SDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM</td>
<td>0.598</td>
<td>0.773</td>
<td>1.122</td>
<td>1.366</td>
<td>2.725</td>
<td>4.519</td>
</tr>
<tr>
<td>40 mM</td>
<td>0.598</td>
<td>0.808</td>
<td>1.127</td>
<td>1.363</td>
<td>2.667</td>
<td>4.408</td>
</tr>
<tr>
<td>50 mM</td>
<td>0.595</td>
<td>0.827</td>
<td>1.125</td>
<td>1.360</td>
<td>2.653</td>
<td>4.331</td>
</tr>
<tr>
<td>60 mM</td>
<td>0.598</td>
<td>0.858</td>
<td>1.128</td>
<td>1.347</td>
<td>2.615</td>
<td>4.229</td>
</tr>
<tr>
<td>mean</td>
<td>0.597</td>
<td>0.816</td>
<td>1.126</td>
<td>1.359</td>
<td>2.665</td>
<td>4.372</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.251</td>
<td>4.36</td>
<td>0.235</td>
<td>0.616</td>
<td>1.71</td>
<td>2.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SDS</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mM</td>
<td>1.601</td>
<td>0.501</td>
<td>1.274</td>
<td>1.601</td>
<td>2.750</td>
<td>3.560</td>
</tr>
<tr>
<td>60 mM</td>
<td>1.593</td>
<td>0.500</td>
<td>1.271</td>
<td>1.593</td>
<td>2.694</td>
<td>3.458</td>
</tr>
<tr>
<td>80 mM</td>
<td>1.575</td>
<td>0.494</td>
<td>1.262</td>
<td>1.575</td>
<td>2.691</td>
<td>3.450</td>
</tr>
<tr>
<td>100 mM</td>
<td>1.567</td>
<td>0.495</td>
<td>1.249</td>
<td>1.567</td>
<td>2.673</td>
<td>3.433</td>
</tr>
<tr>
<td>mean</td>
<td>1.584</td>
<td>0.498</td>
<td>1.264</td>
<td>1.584</td>
<td>2.702</td>
<td>3.475</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.992</td>
<td>0.706</td>
<td>0.888</td>
<td>0.992</td>
<td>1.23</td>
<td>1.65</td>
</tr>
</tbody>
</table>

<sup>a</sup>Identical selectivities are reported for acetophenone and anisole due to their co-elution at all SDS concentrations.
an increase in phase ratio which produces greater probability that phenol/micelle interaction will occur.

Figure 3.2 shows the retention of solutes relative to chlorobenzene plotted versus [Brij 35]. Solutes retained in the same manner as chlorobenzene should undergo comparable k' changes as chlorobenzene with variation of [Brij 35]. The slopes of these plots should therefore be equivalent. Figure 3.2 shows small, nearly congruent slopes for all solutes except phenol, whose relative retention increases with increasing mole fraction. This trend supports our hypothesis since the difference in retention of phenol is significantly affected by differences in Brij 35 micelle/monomer concentrations rather than simply differences between micellar concentrations. In other words, since phenol interacts with Brij 35 monomer and micellar states, its retention is influenced differently by [Brij 35] variation than the other solutes as reflected by a steeper slope in Figure 3.2.

In conclusion, we had previously postulated that retention of acetophenone was primarily due to POE layer interactions. The congruency of its slope in Figure 3.2 with other solutes indicates that, unlike phenol, its retention is primarily affected by interaction with the micellar surface rather than with free Brij 35 monomer.

3.3.3.2 Adjacent solute selectivity

Selectivity between adjacent solutes is determined by their retention relative to one another

\[ \alpha = \frac{k'_{2}}{k'_{1}} \]  \hspace{1cm} (3.8)

where \( k'_{1} \) and \( k'_{2} \) are capacity factors of the first and second eluting compound, respectively. Table 3.4 gives a comparison of adjacent solute selectivities at different
Figure 3.2  Solute retention with respect to chlorobenzene (k'/k'_{Bz-Cl}) vs [Brij 35] for different benzene derivatives.

Figure legend denotes solute identity.
Table 3.4 *Comparison of adjacent solute selectivity for Brij 35/SDS and SDS micellar systems*\(^a\)

<table>
<thead>
<tr>
<th>micellar medium</th>
<th>1/2</th>
<th>2/3</th>
<th>3/5</th>
<th>5/6</th>
<th>6/7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brij 35/SDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM</td>
<td>1.293</td>
<td>1.122</td>
<td>1.218</td>
<td>1.995</td>
<td>1.658</td>
</tr>
<tr>
<td>40 mM</td>
<td>1.238</td>
<td>1.127</td>
<td>1.209</td>
<td>1.957</td>
<td>1.653</td>
</tr>
<tr>
<td>50 mM</td>
<td>1.209</td>
<td>1.125</td>
<td>1.209</td>
<td>1.951</td>
<td>1.633</td>
</tr>
<tr>
<td>60 mM</td>
<td>1.165</td>
<td>1.128</td>
<td>1.194</td>
<td>1.941</td>
<td>1.617</td>
</tr>
<tr>
<td>mean</td>
<td>1.226</td>
<td>1.126</td>
<td>1.208</td>
<td>1.961</td>
<td>1.640</td>
</tr>
<tr>
<td>% RSD</td>
<td>4.38</td>
<td>0.235</td>
<td>0.824</td>
<td>1.20</td>
<td>1.15</td>
</tr>
<tr>
<td><strong>SDS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mM</td>
<td>1.996</td>
<td>1.274</td>
<td>1.257</td>
<td>1.717</td>
<td>1.295</td>
</tr>
<tr>
<td>60 mM</td>
<td>2.001</td>
<td>1.271</td>
<td>1.253</td>
<td>1.691</td>
<td>1.283</td>
</tr>
<tr>
<td>80 mM</td>
<td>2.025</td>
<td>1.262</td>
<td>1.248</td>
<td>1.709</td>
<td>1.282</td>
</tr>
<tr>
<td>100 mM</td>
<td>2.021</td>
<td>1.249</td>
<td>1.255</td>
<td>1.705</td>
<td>1.285</td>
</tr>
<tr>
<td>mean</td>
<td>2.011</td>
<td>1.264</td>
<td>1.253</td>
<td>1.706</td>
<td>1.286</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.715</td>
<td>0.888</td>
<td>0.308</td>
<td>0.638</td>
<td>0.464</td>
</tr>
</tbody>
</table>

\(^{a}\)Solute identity: 1 = phenol, 2 = nitrobenzene, 3 = benzene, 5 = anisole, 6 = toluene, and 6 = chlorobenzene.
surfactant concentrations in both micellar systems. Note that acetophenone was excluded from this investigation due to the extreme difference in its selectivity between the two systems. The relative standard deviation of solute selectivity for SDS and Brij 35/SDS was no more than 1.20%, indicating very little variation with respect to surfactant concentration for all solutes. However, differences were obvious between the two micellar systems. Lower selectivities were observed for the more hydrophilic solutes in Brij 35/SDS, whereas the selectivities for more hydrophobic solutes were higher for this system. In fact, some correlation in the selectivity differences between the two systems was evident.

Figure 3.3 illustrates these changes via a function of the selectivities, \( f(\alpha) \). This function is defined as

\[
f(\alpha) = \frac{\alpha_{\text{Brij 35}} - \alpha_{\text{SDS}}}{\alpha_{\text{SDS}}} \tag{3.9}
\]

where \( \alpha_{\text{Brij 35}} \) and \( \alpha_{\text{SDS}} \) are the solute selectivities in Brij 35/SDS and SDS, respectively. For hydrophilic solutes, the Brij 35/SDS system provided much less selectivity than SDS. This situation is not surprising since hydrophilic solutes should interact primarily with the POE layer rather than with the micellar core. Since the surface of the Brij 35/SDS micelles undergo substantial hydration (3.12), then the similar environments of the aqueous phase and the micellar surface should produce less discrimination between solutes and, thus, less selectivity. With this being the case, as solute hydrophobicity increases, then we would expect an increase in selectivity. As shown in Figure 3.3, the stated trend does exist.

If the inner micellar regions of each micellar system were similar, then we would expect the relative selectivity of the two systems, as defined by \( f(\alpha) \) (see eq
Figure 3.3  $f(\alpha)$ vs adjacent solute pairs. Solute identification follows numerical designation in Figure 3.1. The function $f(\alpha)$ is defined in Equation 3.9.
3.9), to approach zero. However, enhanced selectivity is observed in the Brij 35/SDS system for the latter two solute pairs. This certainly points to differences in microenvironments within the micelles of each system. It is well-known that nonionic/anionic surfactant mixtures form more compact and structurally stable micelles than pure anionic surfactants. This structural stability has been attributed to reduced electrostatic repulsion between anionic head groups and interaction between these head groups and oxonium ions formed at the oxygen atoms of the oxyethylene units (3.16). This property may contribute significantly to the differences in selectivity between the two systems since the compactness of nonionic/anionic micelles may inhibit hydration of regions where solubilization of moderately to highly hydrophobic solutes occurs; conversely, anionic micelles may be more prone to hydration in these regions due to their more diffuse structure.

3.3.4 Efficiency

High efficiencies are of extreme importance in any separation technique since narrower solute bands can improve resolution between closely eluting peaks (provided that $\alpha > 1$), aid in detection via an increase in band height (higher S/N ratio), and increase peak capacity. Differences in column efficiency between SDS and Brij 35/SDS have been previously documented in Chapter Two; however, operating conditions were substantially different meriting further study of trends in efficiencies. This particular study took advantage of equivalent column lengths for both systems; however, analyses were performed at different voltages producing nonequivalent operating currents. Previously, currents were lower for the Brij 35/SDS system (see Table 2.1). In this study, currents in the Brij 35/SDS systems were greater than those at the highest investigated concentration of pure SDS (100 mM).

Column efficiency, $N$, was measured using the equation
\[ N = 5.54 \left( \frac{t_R}{w_{0.5}} \right)^2 \]  

(3.10)

where \( t_R \) is the solute retention time and \( w_{0.5} \) is the peak width at half-height. Nitrobenzene was chosen as the model solute to show variation in efficiency. Table 3.5 compares efficiencies of each system at different surfactant concentrations. Eq 3.10 allows a good general comparison of the two micellar systems provided that all experimental conditions and the time which the solutes of interest remained in the capillary were constant. However, as previously mentioned, the applied voltage was different for these systems and in addition the column residence time of nitrobenzene in each system was significantly different (ranges of \( t_R \) were from 5.093 to 6.361 min for SDS system, 12.328 to 19.633 min for Brij 35/SDS over the surfactant concentration ranges). The higher residence times for the Brij 35/SDS system are attributed to lower electroosmotic velocities. These lower velocities may degrade column efficiency via a higher influence of longitudinal diffusion on observed plate heights (3.17). Other major contributors to reduction in efficiency such as sorption-desorption kinetics and polydispersity of the micelles were not considered since these factors primarily affect highly retained species \( (k' > 5) \), whereas the capacity factors of nitrobenzene in all surfactant systems never exceeded 3.5.

As shown in Table 3.5, efficiencies were slightly higher in the Brij 35/SDS systems. Since eq 3.10 is valid only for symmetrical peaks (3.18), the most suitable comparison can be made between higher surfactant concentrations of each system where peak asymmetries lie between 0.8 and 1.2, indicating reasonable symmetry. The differences in efficiencies are clearly not as great as observed in Chapter Two (see Tables 2.2 and 2.3); the efficiencies in the Brij 35/SDS system were slightly higher, although error in peak width measurements may make this difference negligible.
<table>
<thead>
<tr>
<th>micellar medium</th>
<th>efficiency</th>
<th>peak asymmetry</th>
<th>current (µA) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mM</td>
<td>64 400</td>
<td>0.465</td>
<td>16.3</td>
</tr>
<tr>
<td>60 mM</td>
<td>111 000</td>
<td>0.690</td>
<td>17.4</td>
</tr>
<tr>
<td>80 mM</td>
<td>108 000</td>
<td>0.906</td>
<td>19.2</td>
</tr>
<tr>
<td>100 mM</td>
<td>129 000</td>
<td>0.827</td>
<td>21.9</td>
</tr>
<tr>
<td><strong>Brij 35/SDS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM</td>
<td>72 900</td>
<td>1.171</td>
<td>27.3</td>
</tr>
<tr>
<td>40 mM</td>
<td>92 100</td>
<td>0.975</td>
<td>28.7</td>
</tr>
<tr>
<td>50 mM</td>
<td>139 000</td>
<td>1.134</td>
<td>27.7</td>
</tr>
<tr>
<td>60 mM</td>
<td>139 000</td>
<td>1.105</td>
<td>26.8</td>
</tr>
</tbody>
</table>

\(^a\)Applied voltages: SDS, 10 kV; Brij 35/SDS, 25 kV.
Very poor peak symmetry was observed at lower SDS concentrations. In the cases of 40 mM and 60 mM SDS, the nitrobenzene peak fronted significantly, giving asymmetry values < 0.7. This has led us to question whether sample overloading could be the predominant factor for these poor asymmetries. This was investigated by approximating the relative mole quantities of nitrobenzene and micelles present in the capillary. The concentration of nitrobenzene was ~1.5 mg/mL. With the following equation, one may elucidate the quantity of sample, Q, injected into the capillary

\[
Q = \frac{\rho g \pi r^4 \Delta h C t_i}{8 \eta L_{cap}}
\]  

where \(\rho\) and \(\eta\) are the density and viscosity of the solution within the capillary; \(r\) and \(L_{cap}\) the capillary radius and length, respectively; \(\Delta h\) the height differential between injection and run levels; \(C\) the analyte concentration; \(t_i\) the injection time; and \(g\) is the gravitational force. With aforementioned operating conditions, \(\Delta h\) of 9.8 cm, and assuming the density and viscosity of the filling solution were equivalent to water at 25°C, ~0.5 ng of nitrobenzene, or 4 pmol, were injected prior to each run. With a solute bandwidth of 6 sec (measured for nitrobenzene in 40 mM SDS), the number of SDS micelles at a 40 mM concentration within the corresponding volume (~21 \(\mu\)L for \(v_{eo} = 1.805 \text{ mm/sec and 50 \(\mu\text{m id}\)) was ~11 nmol. Clearly, a sufficient number of micelles should be present (~2750:1 micelle to solute mole ratio) to accommodate the quantity of analyte present within this region. Thus, it is doubtful that the poor peak symmetry observed at lower SDS concentrations could be attributed to an overloading effect.

We believe that the variation in asymmetry could be due to memory effects from the solvent used as the marker for electroosmotic velocity. Figure 3.4 illustrates...
the variation in asymmetry for phenol, nitrobenzene, and acetophenone over the investigated range of SDS concentrations. These compounds were chosen for comparison so that the variation in asymmetry could be viewed from a standpoint of solute elution relative to the $t_0$ marker. Nitrobenzene and acetophenone, with $k'$ ranges of 0.965-2.852 and 1.213-3.580 over the [SDS] range, respectively, followed the same trend in that asymmetries tended to stabilize above 60 mM SDS. However, the asymmetry of phenol, whose $k'$ range is 0.379-1.130, increased continuously over the entire [SDS] range. A clear illustration of this variation is shown in Figure 3.5. Obviously, elution of hydrophilic analytes near the solute marker can have a detrimental effect on peak asymmetry. This occurrence is most likely due to the discrete difference between the bulk properties of the micellar medium and sample mixture (prepared in 100% acetonitrile) which caused a perturbation in the retention characteristics of these analytes. Although this conclusion is speculative, sample preparation in a solvent which closely matches the composition of the micellar medium may alleviate this problem. Finally, phenol and acetophenone asymmetries ultimately exceeded 1.2 at higher [SDS]; the higher asymmetries for acetophenone were probably due to its coelution with anisole, whereas capillary wall interactions may have caused the tailing observed for phenol.

3.3.5 Comparison of electroosmotic and micellar electrophoretic velocities and their effect on $t_{me}/t_0$

Comparison of SDS and Brij 35/SDS systems with regard to relative velocities of the micelles and bulk solution presents some significant differences which merit discussion. Table 3.6 shows the electroosmotic and micellar electrophoretic velocities...
Figure 3.4  Comparison of peak asymmetry (b/a) variation vs [SDS] for three selected solutes: phenol ( ■ ), nitrobenzene ( ■ ), and acetophenone ( □ ).
Figure 3.5  Peak profile of phenol at different SDS concentrations. Run conditions are given in Figure 3.1.
Table 3.6 Comparison of electroosmotic and micellar electrophoretic velocities

<table>
<thead>
<tr>
<th>[SDS], M</th>
<th>$v_{eo}$</th>
<th>$v_{ep}$</th>
<th>$\frac{t_{me}}{t_o}$</th>
<th>[Brij 35], M$^b$</th>
<th>$v_{eo}$</th>
<th>$v_{ep}$</th>
<th>$\frac{t_{me}}{t_o}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>1.805</td>
<td>0.917</td>
<td>2.033</td>
<td>0.030</td>
<td>0.858</td>
<td>0.490</td>
<td>2.331</td>
</tr>
<tr>
<td>0.060</td>
<td>1.799</td>
<td>0.920</td>
<td>2.045</td>
<td>0.040</td>
<td>0.758</td>
<td>0.381</td>
<td>2.012</td>
</tr>
<tr>
<td>0.080</td>
<td>1.806</td>
<td>0.938</td>
<td>2.079</td>
<td>0.050</td>
<td>0.618</td>
<td>0.297</td>
<td>1.927</td>
</tr>
<tr>
<td>0.100</td>
<td>1.736</td>
<td>0.941</td>
<td>2.183</td>
<td>0.060</td>
<td>0.531</td>
<td>0.240</td>
<td>1.825</td>
</tr>
</tbody>
</table>

$^a$Velocities are in units of mm/sec.

$^b$Brij 35/SDS mixed surfactant system where [Brij 35] is varied.
for each system at different surfactant concentrations. The SDS system yields fairly constant velocities for the bulk solution over the [SDS] range; the micellar velocities increase slightly as [SDS] increases. Both observations are ascribed to the temperature rise in the capillary due to Joule heating and its effect on the viscosity of the solution. This has been discussed in detail elsewhere (3.1).

We would expect that viscosity effects should be more obvious for the Brij 35/SDS system, since the constant [SDS] concentration employed in this study should produce comparable Joule heating over the entire [Brij 35] range. As expected, both electroosmotic and electrophoretic velocities decrease with increasing [Brij 35]. However, the degree to which these velocities change is different, as shown in Figure 3.6, where reciprocal velocities are plotted versus [Brij 35]. Two factors may cause the observed decrease in $v_{eo}$: i) an increase in viscosity, and ii) adsorption of Brij 35 to the capillary surface. The latter possibility has been observed by Towns and Regnier (3.19), where they witnessed a decrease in $v_{eo}$ with increasing [Brij 35] at concentrations above and below the CMC. However, from our observations, we believe that adsorption processes are not significant and reach equilibrium conditions rather rapidly as evidenced by good run-to-run repeatability at individual Brij 35 concentrations. The decrease in $v_{ep}$ is attributed not only to the increase in viscosity but also to the decrease in the micelles' surface charge density as [Brij 35] increases.

As discussed in Chapter Two, we previously observed more constant $t_{mc}/t_{lo}$ ratios with the Brij 35/SDS systems. When referring to Table 3.6, it is obvious that $t_{mc}/t_{lo}$ changes significantly in both surfactant systems. The primary difference between the two studies was applied voltage. Previously, the applied voltage for the Brij 35/SDS system was lower than for pure SDS (18 kV for Brij 35/SDS, 25 kV for pure SDS compared to 25 kV for Brij 35/SDS, 10 kV for pure SDS in this study). Operating currents were also lower for Brij 35/SDS in the previous study (~17 µA
Figure 3.6 Reciprocal velocity vs [Brij 35] for the electroosmotic velocity (●) and Brij 35/SDS micelle electrophoretic velocity (■). Run conditions are given in Figure 3.1.
compared to $-27.5 \, \mu A$). This suggests that experimental conditions play an important role in variation of $t_{mc}/t_0$. However, the trend in $t_{mc}/t_0$ with respect to change in surfactant concentration is the same as previously observed, that is, $t_{mc}/t_0$ increases with increasing [Brij 35], whereas in the pure SDS system $t_{mc}/t_0$ decreases with increasing [SDS]. Explanations of these trends were given in Chapter Two.

The most interesting aspect of Figure 3.6 is the intersection of the lines at $-12$ mM Brij 35. If velocities below the lowest [Brij 35] employed in this study do indeed follow the same linearity, then we expect that the electroosmotic and micellar electrophoretic velocities to be equal at the intersection. Thus, the elution range (designated by $t_{mc}/t_0$) would be infinite. Figure 3.7 shows a chromatogram of alkylphenones using [Brij 35] of 11.8 mM, the concentration where convergence occurred in Figure 3.6. The $t_{mc}$ marker, C$_{24}$ in this particular case, did not elute in the length of time allowed for this analysis, making calculation of $k'$ on the basis of prescribed MECC equations impossible. This particular analysis gives a $t_{mc}/t_0$ ratio > 25, significantly higher than any reported ratio to date.

To gain further insight on the magnitude of $t_{mc}/t_0$, capacity factors were calculated using the relationship between $k'$ and retention time in conventional LC.

$$k' = \frac{t_R - t_0}{t_0}$$

(3.12)

Since a homologous series was used as the test sample, a plot of log $k'$ versus carbon number should be linear for an infinite elution range. Any deviation from linearity will be most prominent for later eluting compounds since the MECC equation contains an additional term in the denominator which becomes more significant as solute retention increases.
Figure 3.7  Electrokinetic chromatogram of alkylphenone homologues. Peak identity: 1) acetophenone, 2) propiophenone, 3) butyrophenone, 4) valerophenone, and 5) hexanophenone. Capillary: 50 μm i.d., 170 μm o.d., 47.5 cm (40.0 cm to detector). Detection wavelength: 254 nm. Micelle medium: 11.8 mM Brij 35/20 mM SDS in 10 mM phosphate buffer (pH 6.8); applied potential, 25 kV; current, 35.2 μA.
\[ k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \]  

(3.13)

Figure 3.8 shows log k' versus carbon number plots for data from Figure 3.7 and data from 30 mM Brij 35 (t_{mc}/t_0 = 2.32) plotted on the same basis as the currently investigated data. Note the degree of curvature for the separation in 30 mM Brij 35. If t_{mc} had any influence on the retention characteristics of the later eluting compounds when t_{mc}/t_0 > 25, then deviation from linearity would exist. Yet, for t_{mc}/t_0 > 25, no such deviation was observed (r^2=0.999). Therefore, we can conclude that near infinite elution ranges are possible using Brij 35/SDS surfactant systems in MECC.

3.4 CONCLUSIONS

Nonionic/anionic mixed micellar systems in MECC hold particular promise as alternatives to pure surfactant systems. In general, analyte retention was greater in the Brij 35/SDS system. Also, it was shown that the micellar POE layer plays an integral role in the retention of some compounds. Solute interactions with POE moieties were not limited to those groups which were present on the micellar surface; one solute capable of hydrogen bonding, i.e., phenol, seemed to interact with free nonionic surfactant monomers in solution also.

The determination of partition coefficients in mixed micellar systems was accomplished by developing a term for partial molar volume which was dependent on the relative mole quantities of the two surfactant systems. We observed slightly greater partition coefficients in the pure SDS system. Also, a significant advantage to using the Brij 35/SDS system was observed when comparing \( P_{wms} \) from our work to those in MLC. The \( P_{wms} \) from the Brij 35/SDS system correlated extremely well with those observed in MLC for pure Brij 35, whereas \( P_{wms} \) observed in the pure SDS system were systematically higher than in MLC. This may indicate that the
Figure 3.8  

$\log k'$ vs carbon number (Nc) for alkylphenones in Brij 35/SDS. Data were extracted from Figure 3.7 (●) and an alkylphenone separation under the conditions described in Figure 3.1 for Brij 35/SDS (○). Figure legend gives t_{mc}/t_{o} values for each data set.
solubilization properties of pure nonionic micellar systems could be characterized simply from data extracted from mixed micellar studies in MECC. This capability is probably feasible only if the nonionic surfactant is used at higher concentrations than the anionic surfactant, i.e., the anionic surfactant is used at low concentrations to provide electrophoretic mobility yet only marginally affect the micellar composition.

Selectivity variation was viewed from two standpoints: i) the effect of functionality on selectivity, and ii) the variation of adjacent solute selectivity with increased retention. In general, functional group selectivities of later eluting sample components were affected marginally by changing from pure SDS to the Brij 35/SDS systems. The selectivity of acetophenone, however, decreased dramatically by changing to Brij 35/SDS surfactant systems. Also, the selectivity of phenol increased with increasing [Brij 35], a trend not observed for any other solute in either surfactant system. Comparison of its retention to other solutes relative to chlorobenzene seems to indicate, as discussed above, that phenol interacts with Brij 35 molecules in both the micellar and monomer states.

Comparison of adjacent solute selectivities of the pure SDS and Brij 35/SDS micellar systems provided some interesting results regarding structural differences between the two types of micelles. First of all, selectivities in each system varied little with surfactant concentration. However, poorer selectivity was observed in Brij 35/SDS for early eluting compounds. Moreover, selectivity increased with solute hydrophobicity to values greater than observed in pure SDS. The poor selectivity for hydrophilic solutes points to the influence of the POE layer on retention and the effect of hydration of this micellar environment on selectivity. Higher selectivities for the more hydrophobic compounds in Brij 35/SDS can be attributed to the greater stability and compactness of these micelles in comparison to those of pure SDS.
When comparing separation efficiencies of the two micellar systems, very little advantage was gained by using the Brij 35/SDS systems. This finding is contrary to our previous work with PTH-amino acids in Chapter Two. However, operating currents for Brij 35/SDS were higher than those in pure SDS due to the difference in applied voltage between the two systems. This may have degraded Brij 35/SDS efficiencies significantly via Joule heating. Poor peak symmetry was observed in pure SDS, especially for early eluting peaks. This problem may have been caused by memory effects from the t₀ marker (acetonitrile). The same trend did not occur in Brij 35/SDS.

Finally, electroosmotic and micellar electrophoretic velocities were compared for the two systems. As expected, both velocities in pure SDS were fairly constant, whereas both velocities decreased with increasing [Brij 35] in Brij 35/SDS. Surprisingly, from a plot of reciprocal velocity versus [Brij 35], we observed convergence of these two velocities at a [Brij 35] well above its CMC. This convergence may indicate that an infinite elution range is possible using this micellar system. This hypothesis was tested by performing an analysis at the [Brij 35] with alkylphenones corresponding to the point of convergence. A \( t_{me}/t_0 > 25 \) was observed and was probably much greater as indicated by the linearity of \( \log k' \) versus carbon number where the \( k' \)s were calculated by the equation used in conventional LC.

This final observation is tremendously important to the development of MECC as an alternative to conventional LC in that the limited elution range is one of the most significant limitations of MECC. Infinite \( t_{me}/t_0 \) ratios could revolutionize MECC, making it the separation technique of choice for solute systems typically analyzed by conventional LC since the high efficiencies in MECC already make this technique extremely attractive. In closing, by optimizing surfactant concentration to provide an
infinite elution range, extremely short capillaries could be utilized to produce rapid, high efficiency separations.
CHAPTER THREE REFERENCES


CHAPTER 4

EFFECT OF ORGANIC SOLVENT ON THE RETENTION AND SELECTIVITY OF n-ALKYLPHENONE HOMOLOGUES IN SDS-MEDIATED MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY
4.1 INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) is a rapidly growing technique for the high resolution separation of electrically neutral compounds. Since its inception, MECC has been applied to chemical systems such as PTH-amino acids (4.1), illicit drugs (4.2), phenols (4.3, 4.4), vitamin B₆ metabolites (4.5), and nucleic acid constituents (4.6, 4.7). Several applications have also been published involving the use of additives to enhance the solubility of solutes, efficiency, and/or selectivity. For extremely hydrophobic compounds, MECC lacks the ability to separate adequately these compounds due to the high affinity of the solutes for the micelles. This results in the co-elution of these compounds with the micelles ($t_R = t_{mc}$). Therefore, some modification of the mobile phase is necessary to reduce the partitioning of extremely hydrophobic solutes into the micelles. The addition of cyclodextrins (4.8) has shown promise as a possible mobile phase modifier to aid in the separation of hydrophobic compounds in MECC; however, the bulk of research has focused upon the use of organic solvents as modifiers. The organic solvents typically employed are those such as methanol or acetonitrile which are commonly utilized in conventional reversed phase liquid chromatography (RPLC), or solvents such as 1-propanol which are effective in increasing efficiency in predominantly aqueous RPLC systems into which secondary chemical equilibria have been introduced, e.g., acid-base (4.9) and micellar equilibria (4.10, 4.11). Gorsc et al. (4.12) investigated the effects of methanol and acetonitrile as modifiers in MECC and found that both systems were satisfactory in increasing the solubilization of hydrophobic compounds and in extending the elution range. They also observed differences in polar group selectivity with different organic modifiers, which may be extremely useful in situations where poor resolution of compounds is a problem. Similar results were obtained for 2-propanol by Balchunas and Sepaniak (4.13).
To our knowledge, no work has been published concerning the effects of organic modifier on methylene selectivity and other retention characteristics of a homologous series. Several studies have been performed in micellar liquid chromatography (MLC), which also uses micelles although they serve as a mobile rather than a stationary phase. Khaledi et al. (4.14-4.16) have compared differences in hydrophobic selectivity of micellar and hydroorganic mobile phases along with hybrid systems consisting of micelles in hydroorganic solvents. They have concluded that solute type is integral to homologous series selectivity due to the location of solute retention in the microenvironments of the micelles. With the addition of organic modifiers to the micellar mobile phase, separation selectivity in MLC was frequently enhanced as the elution strength was increased. This paper will report our findings in MECC on the effect of organic modifiers on hydrophobic selectivity and other retention characteristics using sodium dodecyl sulfate (SDS) as the micelle-forming surfactant system.

4.2 EXPERIMENTAL

4.2.1 Apparatus

A Quanta 4000 Capillary Electrophoresis System was provided by Millipore Corporation, Waters Chromatography Division (Milford, MA, USA). This instrument was equipped with hydrostatic injection used for 1 sec intervals and a fixed-wavelength UV absorbance detector operated at 254 nm. Untreated fused-silica capillary tubing with dimensions of 50 mm i.d. and 170 mm o.d. was purchased from Alltech Associates, Inc. (Deerfield, IL, USA). The total capillary length was 82.5 cm with injector-to-detector lengths of 75 cm. Activation of the capillaries was performed using a modification of a procedure described previously (4.17). The capillary was initially rinsed with 1 M KOH for 15 min followed by subsequent rinses of 0.1 M
KOH and deionized water for 15 min each. The capillary was finally rinsed for 20 min with the operating buffer. Purges with the operating buffer were performed after each run for 5 min using a vacuum of ~14 inches Hg at the detector reservoir. All separations were performed with a positive voltage of 22 kV applied to the injection end of the capillary. Data were acquired on an IBM Personal Computer AT (Boca Raton, FL, USA) using a PE-Nelson Omega-2 chromatography application package obtained from Perkin-Elmer Corporation (Milford, CT, USA).

4.2.2 Materials and Methods

The n-alkylphenone homologous series was purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and consisted of C₈ (acetophenone), C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₆, and C₁₈ (dodecanophenone) homologs. Electrophoresis-grade SDS was purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA) and distilled water was deionized and redistilled with a Corning Mega-Pure™ Water Purification System (Corning, Inc., Corning, NY, USA). Methanol (MeOH) and acetonitrile (MeCN) were obtained from Mallinkrodt, Inc. (Paris, KY, USA) while 1-propanol (1-PrOH) was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

Stock phosphate buffer was prepared with NaH₂PO₄ • H₂O and NaOH to give a 100 mM concentration of pH 6.8. This buffer was diluted to a 10 mM concentration and used for preparation of surfactant solutions. Weighed amounts of SDS were dissolved in ~ 25 mL of 10 mM phosphate buffer followed by the addition of 15 mL of specified organic solvent (methanol, acetonitrile, or 1-propanol) and further dilution with phosphate buffer to yield a solution of 100 mL total volume. SDS concentrations of 25, 40, 55, and 70 mM were employed for each organic modifier. Stock n-alkylphenone standard solution was prepared in acetonitrile at solute concentrations of
~ 5 mg/mL. Samples for analyses were prepared by dilution of the stock solution with acetonitrile to solute concentrations of ~ 0.6 mg/mL.

4.3 RESULTS AND DISCUSSION

The retention behavior of homologues in MLC has been studied extensively (4.14-4.16) and was found to be fundamentally different from that observed in conventional RPLC with hydro-organic mobile phases. Explanations of this difference have centered on the locations of the solute within the micelle. Although useful information can be derived from MLC studies such as these, the inherent complexity of the separation system (4.18) makes a definitive interpretation of the results quite challenging. Figure 4.1A illustrates a currently accepted model of MLC retention. Solutes may partition among three pairs of coexisting phases within an HPLC column: (a) micellar phase/aqueous phase, (b) micellar phase/stationary phase, and (c) stationary phase/aqueous phase. Although this "pseudophase" model has been shown as experimentally sound, a number of assumptions were made in the development of theoretical expressions for retention (4.18). Deviations from expected retention behavior will result if any of these assumptions prove invalid.

Solute retention in MECC follows a much simpler model as illustrated in Figure 4.1B. Solutes partition between only two phases, micellar and aqueous. The lack of a third phase similar in nature to the micelle (as is the case in MLC) is the reason for both the intuitive and mathematical (vide infra) simplicity. Given the one-to-one correspondence of the micellar (pseudo)stationary phase and buffer mobile phase in MECC to the stationary phase and hydroorganic mobile phase in RPLC, it is not surprising that these systems have similar retention characteristics (cf. the three phase MLC system). Moreover, differences in retention characteristics between MECC and RPLC that are observed may provide valuable information on the effects
Figure 4.1  Mechanistic comparison of MLC to MECC: (A) the MLC three-phase (pseudophase) model, (B) the MECC retention model. Lowercase letters designate the various solute partitioning processes occurring in each system: (a) micellar phase/aqueous phase, (b) micellar phase/stationary phase, and (c) stationary phase/aqueous phase interactions.
of specific micellar microenvironments. Although MECC is plagued with several inherent problems not typically considered or avoidable in MLC, i.e., the effects of voltage, temperature, ionic strength, pH, etc. on various micellar parameters (CMC, aggregation number, micellar size and shape, etc.), it may nevertheless be preferable for the investigation solute/micelle interactions because of its greater mechanistic simplicity.

4.3.1 Methylene selectivity

4.3.1.1 General trends

The variation in hydrophobic selectivity is dependent on the change in retention with respect to a change in number of carbons in otherwise similar compounds. This variation is described by

\[
\log k' = (\log \alpha)N_c + (\log \beta)
\]

(4.1)

where \(k'\) is a solute's capacity factor, \(N_c\) is the carbon number of the solute, \(\beta\) is the phase ratio (\(= V_{mc}/V_{aq}\), where \(V_{mc}\) and \(V_{aq}\) are volumes of the micellar and aqueous phases, respectively), and \(\alpha\) is the methylene selectivity. Changes in methylene selectivity for different organic solvent modifications of the SDS system should be dependent on three factors: i) the change in the solvation properties of the bulk solvent (aqueous phase), ii) the possible inclusion of organic modifier in the micelles, and iii) the loci of solute solubilization within the micelles.

The last two factors are interrelated in that the presence of organic modifier in the micellar region where solute solubilization occurs would lead to reduced methylene selectivity due to the greater similarity of the aqueous and organic-modified micellar phases. On the other hand, in situations where the solute and organic solvent partition
into different regions of the micelle, selectivity is less likely to be affected. These possibilities are illustrated in Figure 4.2 in terms of the relative polarity of the phases. In a purely aqueous SDS system (4.2A), the differences in polarity are largest, thus providing the greatest selectivity according to solubility parameter theory (4.19). In Figure 4.2B, the added organic modifier is assumed to lower the polarity of the aqueous phase but not interact with the micelles (at least not in the vicinity of the solute); the difference in polarity between aqueous and micellar phases is thus reduced only moderately, and the resulting decrease in selectivity is also moderate. In Figure 4.2C, the added modifier is assumed both to lower the the polarity of the aqueous phase and to increase the polarity of the micelles in a region where the solute will partition; in this case the polarity difference in between aqueous and micellar phases is markedly reduced, and a significant decrease in selectivity is expected.

Table 4.1 shows values of methylene selectivity obtained with four SDS-based surfactant systems. Of the three SDS systems which contained MeOH, MeCN, or 1-PrOH, the one with MeOH most resembled the purely aqueous system in its selectivity. The fact that MeOH only lowered the selectivity slightly is not surprising since (i) MeOH has been shown to interact minimally with SDS (4.20) due to its general hydrophilic nature and strong H-bond interactions with water; and (ii) of the three organic solvents, MeOH is the most similar to water and its presence can be expected to induce the smallest change in polarity and hydrogen bonding characteristics of the bulk aqueous phase.

In contrast to MeOH, the addition of MeCN or 1-PrOH gave more significant decreases in methylene selectivity. For both the 1-PrOH/SDS and MeCN/SDS systems, the lower selectivity suggests not only the effect of these organic solvents on the aqueous phase but also their accumulation within and effect on the micelles. Such accumulation has been observed elsewhere for 1-PrOH (4.21), although we were
Figure 4.2 Qualitative depiction of the effect of organic solvent on polarity variation in micellar and aqueous phases of MECC. From left to right, 1) polarity difference between the micellar and aqueous phases with no organic modifier, 2) change in polarity difference when organic solvent is excluded from the micellar phase (Type I modifier), and 3) change in polarity difference when organic solvent is solvated in both phases (Type II modifier).
Table 4.1 Comparison of log ($\alpha_{CH_2}$) and log $\beta$ values.

| mobile phase | log (|$\alpha_{CH_2}$|) | log $\beta$ | log $\beta$ (calc) | $r^2$ (n) |
|--------------|----------------|-------------|-----------------|-----------|
| **0% organic modifier** | | | | |
| 25 mM SDS | 0.367 (±0.00) | -3.08 (±0.0) | -2.38 | 0.999 (7) |
| 40 mM SDS | 0.374 (±0.01) | -2.89 (±0.1) | -2.10 | 0.997 (5) |
| 55 mM SDS | 0.378 (±0.01) | -2.77 (±0.1) | -1.93 | 0.997 (5) |
| 70 mM SDS | 0.382 (±0.01) | -2.69 (±0.1) | -1.81 | 0.996 (5) |
| **15% v/v MeOH** | | | | |
| 25 mM SDS | 0.349 (±0.01) | -3.32 (±0.1) | - | 0.997 (7) |
| 40 mM SDS | 0.353 (±0.01) | -3.10 (±0.1) | - | 0.997 (7) |
| 55 mM SDS | 0.343 (±0.01) | -2.82 (±0.1) | - | 0.996 (6) |
| 70 mM SDS | 0.356 (±0.01) | -2.82 (±0.1) | - | 0.993 (6) |
| **15% v/v MeCN** | | | | |
| 25 mM SDS | 0.310 (±0.01) | -3.07 (±0.1) | - | 0.998 (7) |
| 40 mM SDS | 0.331 (±0.01) | -2.96 (±0.1) | - | 0.997 (7) |
| 55 mM SDS | 0.337 (±0.01) | -2.86 (±0.1) | - | 0.996 (7) |
| 70 mM SDS | 0.341 (±0.01) | -2.76 (±0.1) | - | 0.997 (7) |
| **15% v/v 1-PrOH** | | | | |
| 25 mM SDS | 0.314 (±0.005) | -2.97 (±0.1) | - | 0.998 (8) |
| 40 mM SDS | 0.311 (±0.005) | -2.73 (±0.1) | - | 0.999 (8) |
| 55 mM SDS | 0.311 (±0.005) | -2.59 (±0.1) | - | 0.999 (8) |
| 70 mM SDS | 0.325 (±0.005) | -2.53 (±0.1) | - | 0.998 (5) |
unable to find similar corroborative reports for MeCN. We nevertheless postulate the penetration of MeCN into the micelles (although not necessarily to the same region and/or degree as 1-PrOH), for two reasons: first, if MeCN did not penetrate the micelles we would expect its effect on selectivity to be closer to that observed with MeOH, and second, MeCN associates very little with H$_2$O ((4.22), ~5% of MeCN+H$_2$O at an apparent MeCN volume fraction of 0.5), presumably due to its inability to hydrogen bond with H$_2$O. Given these minimal interactions with water, even weak interactions with the micelle would not be precluded. Probably the most favorable location for MeCN in this micellar medium is an environment of moderate polarity, i.e., the palisade layer of the micelles (4.23).

4.3.1.2 Methylene selectivity between individual solute pairs

The previous discussion is very general in that the selectivities as measured by the slopes of log $k'$ vs carbon number are compared with no consideration to variations between individual solute pairs. Differences in solute pair selectivities are possible since all solutes do not necessarily reside in the same region of the micelle (4.14). In order to study the effects upon individual pairs, the selectivities of each pair were calculated on the basis of their individual capacity factors

$$\alpha = \frac{k'_{N_{c}+1}}{k'_{N_{c}}} \quad (4.2)$$

where $N_{c}$ represents the total number of carbons for the smaller homologue. Ratios of selectivity in the solvent modified systems to that in SDS/H$_2$O ($\alpha_{\text{modifier}}/\alpha_{\text{H}_2\text{O}}$) were employed to provide a common scale for comparison of solvent effects. Figure 4.3 shows the variation of these ratios over the experimental range of SDS concentration. In addition to scaling, ratios were used in an attempt to eliminate any selectivity variations that may exist at different surfactant concentrations. However, organic
Figure 4.3 \( \alpha_{\text{modifier}}/\alpha_{\text{H}_2\text{O}} \) vs [SDS] for n-alkylphenone solute pairs in each organic modifier system. The solvent and solute pair identities are given within the figure. For specific mobile phase and run conditions see Experimental Section.
solvents may interact with the micelles in such a way as to alter micellar structure. Therefore, any selectivity variations in these plots will reflect not only the effect of organic solvent on selectivity but also modifications to micellar structure caused by solvent/micelle interactions.

In general, the methylene selectivity in the MeOH system was slightly less than observed in the SDS/H₂O system for all solute pairs. This moderate change in selectivity suggests that the addition of MeOH does not appreciably modify the solvation properties of the aqueous and micellar phases with respect to one another. Since MeOH strongly associates with H₂O (4.22, 4.24), minimal solvent/micelle interaction was expected. However, since hydration of the micelle surface is prevalent, MeOH/micellar surface interactions may be favorable. Selectivity variation of more hydrophilic solutes, i.e., C₉/C₈ or C₁₀/C₉, should reflect surface modification by MeOH since these solutes are not retained significantly in SDS systems modified with organic solvent and, thus, should interact primarily with the micellar surface or penetrate slightly into the micelles. As Figure 4.3 illustrates, the selectivities of the aforementioned solute pairs do not differ significantly with the selectivities of the more hydrophobic solute pairs for SDS concentrations greater than 40 mM. However, some differences become evident in 25 mM SDS. As the solutes become more hydrophobic, the selectivities approach those of a purely aqueous SDS system. It is possible that at lower SDS concentrations the micellar structure may be more diffuse, permitting deeper penetration of highly water-miscible organic modifiers such as MeOH into the micelles. However, the depth of solvent penetration still seems to be limited since as solutes become more hydrophobic and penetrate deeper into the micelles, the selectivities approach that of SDS/H₂O. This suggests that more hydrophobic solutes are solvated in micellar microenvironments void of organic solvent.
The 1-PrOH system follows the same trend as MeOH in 25 mM SDS but MeCN does not. It follows that hydrogen bonding must play an integral role in the extent of solvent penetration into the micelles. The ability of an organic solvent to hydrogen bond with H₂O may set a "limit" to the depth that the solvent can partition into the micelles. On the other hand, MeCN, which remains essentially dissociated from H₂O, could partition freely, being constrained only by its solubility within the micellar regions. The lack of selectivity variation from the C₉ to C₁₂ homologs at all SDS concentrations suggests a fairly uniform MeCN concentration throughout the micelles or at least in those micellar regions where these solutes solubilize.

A comparison of the effect of each organic modifier upon the selectivity of the C₉/C₈ solute pair reveals some interesting results regarding retention of polar compounds. The most notable observance is the selectivity offset apparent in the MeCN system in comparison to other solute pairs. A similar but less pronounced offset is also present in the 1-PrOH system. The selectivity of this solute pair in the MeOH and MeCN systems are nearly equivalent. The similarity of selectivities in the MeOH and MeCN systems leads us to believe that these two solutes are retained at or near the micellar surface. If this is occurring, then a modification of the micellar surface by an organic solvent should decrease the selectivity between these solutes since the micellar surface environment would more closely resemble the aqueous phase. A selectivity decrease (~3%) is observed in the 1-PrOH system for this solute pair in comparison to selectivities in the MeOH and MeCN systems. Therefore, 1-PrOH possibly takes an active role in micelle formation; that is, 1-PrOH and SDS may form a mixed micellar system where the 1-PrOH hydroxyl groups make up a portion of the micellar surface and the propyl groups contribute hydrophobic character to the inner micellar regions.
An interesting trend was evident in the 1-PrOH system for the C_{12}/C_{11} solute pair. A ~10% decrease in selectivity was observed from 25 to 75 mM SDS with a general trend of lower selectivity than other solute pairs above 40 mM SDS. This selectivity reduction is rather puzzling since we would expect that as the solutes partition deeper into the micelles, the methylene selectivities between solutes should approach those of the purely aqueous SDS system via the greater polarity difference between the aqueous phase and micellar core. The depth which 1-PrOH partitions into the micelles should be limited due to H-bonding with H_{2}O (vide supra); therefore we would not expect significant concentrations of 1-PrOH in the inner micellar regions. Note that a decrease in selectivity was also present in the MeCN system, although the reduction was less pronounced. MeOH did not follow this trend.

The cause of this decrease is uncertain but may be due to slight modifications to the micellar structure caused by the presence of these organic solvents in the micelles. These structural modifications should be more evident for hydrophobic compounds since, by spending more time within the micelles, their retention is more influenced by changes to the micellar structure. The decrease in selectivity of the C_{12}/C_{11} solute pair with increasing SDS concentration suggests that the core is becoming less hydrophobic than in the SDS/H_{2}O system. This occurrence seems highly unlikely unless the presence of 1-PrOH within the micelles (and in the aqueous phase) dramatically influences the microenvironment where solute solubilization occurs. In other words, C_{11} and C_{12} may be retained much closer to the micellar surface in the 1-PrOH system than in SDS/H_{2}O. This explanation is speculative, however, so that studies involving more hydrophobic n-alkyphenone homologues are necessary to provide a more secure rationalization of this phenomenon.
4.3.2 Partition coefficients and CMC

4.3.2.1 Comparison of partition coefficients

Terabe et al. (4.25) have described the relationship between retention and a solute’s partition coefficient by

\[ k' = P_{wm}V([SDS]-CMC) \]  \hspace{1cm} (4.4)

where \( P_{wm} \) is the solute’s water/micelle partition coefficient of the solute and \( V \) is the partial molar volume of the surfactant monomer. Table 4.2 shows the slope and intercept values for n-alkylphenones in the investigated SDS systems. In order to determine the partition coefficients of the solutes in the organic solvent modified systems, the partial molar volume of SDS in each system must be known. Unfortunately, such data are unavailable in the literature at the specified organic solvent concentrations. However, approximate values can be derived using the approach described below.

From the plot of \( k' \) versus [SDS], the slopes represent the product of the solute’s partition coefficient and the partial molar volume of the surfactant. Since the partial molar volume is essentially constant over the SDS concentration range with its value for the SDS/H\(_2\)O system taken as 0.246 L/mol at 25°C (4.23), we can use this value as a reference for the determination of other partial molar volumes. A plot of the ratio of slopes (from \( k' \) versus [SDS]) in the organic modified systems to the SDS/H\(_2\)O system for each n-alkylphenone homolog versus carbon number yields a linear relationship. As the solute carbon number decreases, the time which the solute spends in the micelle decreases. Accordingly, as the carbon number approaches zero, the solutes are essentially excluded from the micelles. This means that the ratio of partition coefficients between an organic solvent modified system and pure SDS
Table 4.2  Partition coefficients and CMC values for members of n-alkylphenone homologous series.

<table>
<thead>
<tr>
<th>mobile phase</th>
<th>slope</th>
<th>Pwm</th>
<th>CMC, M (x 10^3)</th>
<th>r² (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS/H₂O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>39.1 (±0.3)</td>
<td>160</td>
<td>5.41 (±0.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₉</td>
<td>83.1 (±0.6)</td>
<td>338</td>
<td>5.63 (±0.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₀</td>
<td>190 (±2)</td>
<td>774</td>
<td>6.00 (±0.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₁</td>
<td>488 (±5)</td>
<td>1980</td>
<td>7.40 (±0.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₂</td>
<td>1430 (±4)</td>
<td>5800</td>
<td>10.4 (±0.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>15% v/v MeOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>20.0 (±0.6)</td>
<td>92.5</td>
<td>8.22 (±1.5)</td>
<td>0.998</td>
</tr>
<tr>
<td>C₉</td>
<td>38.5 (±0.9)</td>
<td>178</td>
<td>8.26 (±1.2)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₀</td>
<td>79.3 (±1)</td>
<td>367</td>
<td>8.44 (±1.0)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₁</td>
<td>182 (±4)</td>
<td>832</td>
<td>9.19 (±1.2)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₂</td>
<td>462 (±20)</td>
<td>2130</td>
<td>10.6 (±3)</td>
<td>0.996</td>
</tr>
<tr>
<td>C₁₃</td>
<td>1250 (±100)</td>
<td>5780</td>
<td>13.2 (±5)</td>
<td>0.984</td>
</tr>
<tr>
<td>15% v/v MeCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>17.2 (±0.3)</td>
<td>84.2</td>
<td>8.49 (±0.9)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₉</td>
<td>32.7 (±0.6)</td>
<td>160</td>
<td>8.59 (±1.0)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₀</td>
<td>66.5 (±1)</td>
<td>326</td>
<td>9.92 (±1.1)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₁</td>
<td>146 (±4)</td>
<td>715</td>
<td>11.1 (±1)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₂</td>
<td>346 (±10)</td>
<td>1690</td>
<td>12.5 (±2)</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₃</td>
<td>845 (±20)</td>
<td>4140</td>
<td>13.9 (±2)</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₄</td>
<td>2110 (±50)</td>
<td>10300</td>
<td>15.7 (±1)</td>
<td>0.999</td>
</tr>
<tr>
<td>15% v/v i-PrOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>15.3 (±0.4)</td>
<td>79.7</td>
<td>1.40 (±1.3)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₉</td>
<td>28.8 (±0.2)</td>
<td>150</td>
<td>0.81 (±0.4)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₀</td>
<td>56.8 (±0.2)</td>
<td>296</td>
<td>1.27 (±0.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₁</td>
<td>122 (±2)</td>
<td>639</td>
<td>2.83 (±0.9)</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₂</td>
<td>278 (±10)</td>
<td>1450</td>
<td>4.02 (±1.8)</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₃</td>
<td>616 (±30)</td>
<td>3200</td>
<td>4.38 (±2.3)</td>
<td>0.996</td>
</tr>
<tr>
<td>C₁₄</td>
<td>1340 (±100)</td>
<td>6950</td>
<td>5.19 (±4.0)</td>
<td>0.988</td>
</tr>
</tbody>
</table>
approaches unity. Therefore, the intercept of slope ratio versus $N_c$ should reflect a ratio of partial molar volumes ($V_{org}/V_{SDS}$ where $V_{org}$ is the partial molar volume of SDS in an organic solvent modified system). By using the aforementioned value for the partial molar volume of SDS in the purely aqueous system, partial molar volumes of SDS in the organic solvent systems can be determined. Values calculated from the intercepts were 0.218, 0.205, and 0.193 L/mol for the MeOH, MeCN, and 1-PrOH system, respectively.

Using these values, partition coefficients were determined from the $k'$ versus [SDS] slopes as shown in Table 4.2. There is a significant difference between the modified system $P_{wm}$ values and those found in H$_2$O. In comparing $P_{wm}$ values of the organic solvent modified system, we find slight but obvious differences from one solvent to another. We believe that the differences in $P_{wm}$ values can be attributed to the solvation properties of the organic modifiers in the bulk aqueous phase. This hypothesis is supported by extremely good correlation between $P_{wm}$ values and Hildebrand solubility parameter values (4.26) for the three investigated organic solvents (see Figure 4.4).

The effect of organic modifiers can be further illustrated by considering the free energy of solute transfer from the bulk solvent to the micelles. The relationship between partition coefficients and carbon number is defined by

$$\log P_{wm} = \text{(slope)} \times N_c + \text{intercept} \quad (4.5)$$

where $N_c$ is the carbon number of the solute (4.14). The slope is a measure of the free energy of transfer of a methylene group from the aqueous to micellar phase. The intercept reflects interaction of the residual phenone group with the micelles. Plots of $\log P_{wm}$ versus $N_c$ give slopes for the solvent modified systems which vary only
Figure 4.4 Correlation of partition coefficients ($P_{wm}$) of $n$-alkylphenones with Hildebrand solubility values ($\delta$) of each organic modifier. See figure legend for solute identities. All plots were correlated to $r^2 \geq 0.999$ except $C_{10}$ which gave $r^2 = 0.994$. 
slightly, as shown in Table 4.3. However, the slopes between the organic modifier systems and SDS/H₂O differed substantially. The same trend was also evident for intercept values (see Table 4.3).

In reference to the slope data, methylene affinity for the micellar phase was greater when no modifier was added. This is logical since polarity and solvation properties of the two phases (aqueous and micellar) differ most in the absence of organic solvent. With regard to the organic solvent systems, the micellar affinity of the methylene groups slightly decreased in the order 1-PrOH < MeCN < MeOH, which is consistent to differences in partition coefficients between solvent modified systems (see Table 4.2). These data do not necessarily give support to micellar modification with the organic solvents; rather, they show the general effect of organic solvent, whether it be in the aqueous or micellar phase, on solute retention.

On the other hand, intercepts of log P_{wm} versus N_c dictate the degree to which solutes interact with the micellar surface. We expect that the solute phenone groups should interact significantly with the micellar surface through various dipolar and electrostatic mechanisms. To inhibit interaction of the phenone group with the micellar surface, the modifier must essentially weaken the electrostatic "barrier" between the aqueous and micellar phases. This would be accomplished most easily through solvent/micelle coaggregation, which would decrease the surface charge of the micelles (4.27). Alternatively, accumulation of solvent at the micellar surface should decrease the dielectric constant of the solution surrounding the micelles, resulting in greater repulsion of the ionic head groups (4.28-4.31). This greater repulsion destabilizes the micelles, resulting in dissociation of a certain number of surfactant ions in order to reduce the repulsions. Concurrently, the charge density around the micelle decreases giving solutes freer access to the micellar interior. The data show that the phenone/micellar surface interactions are most prominent in the SDS/H₂O
**Table 4.3** Comparison of slope and intercept values for log $P_{wm}$ vs $N_c$ plots.

<table>
<thead>
<tr>
<th>mobile phase</th>
<th>slope</th>
<th>intercept</th>
<th>$r^2$ (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS/H$_2$O</td>
<td>0.389</td>
<td>-0.952</td>
<td>0.995</td>
</tr>
<tr>
<td>15% v/v MeOH</td>
<td>0.340</td>
<td>-0.790</td>
<td>0.995</td>
</tr>
<tr>
<td>15% v/v MeCN</td>
<td>0.326</td>
<td>-0.711</td>
<td>0.997</td>
</tr>
<tr>
<td>15% v/v 1-PrOH</td>
<td>0.315</td>
<td>-0.646</td>
<td>0.997</td>
</tr>
</tbody>
</table>
system. The order in which the organic solvents decrease this interaction is 1-PrOH < MeCN < MeOH. This order corroborates the relative degree of solvent/micelle interaction which we expect, i.e., 1-PrOH > MeCN > MeOH.

### 4.3.2.2 Trends in variation of CMC

From eq 4.4, the quotient of intercept and slope should produce a value corresponding to the critical micelle concentration (CMC). However, previous work (4.25) has demonstrated the unreliability of this method. Therefore, rather than attempt to measure CMC accurately, we have chosen to compare changes in CMC for each solvent system relative to SDS/H$_2$O.

As shown in Table 4.2, 1-PrOH significantly lowers the CMC and the other two solvents increase the CMC. The lower CMCs in the presence of 1-PrOH suggest coaggregation of SDS and this alcohol. It is well-known that coaggregation of alkanols with surfactants depresses the CMC (4.32-4.33). This phenomenon results from replacement of surfactant ions with alcohol molecules. With electrostatic repulsion of surfactant head groups being weaker, the association of surfactant ions together with alcohol molecules occurs at lower surfactant concentrations than in the absence of alcohol.

Several investigations have been performed regarding the penetration of 1-PrOH into SDS micelles (4.32-4.36). In each case, 1-PrOH was not solubilized in the hydrophobic core; yet, they did not rule out surface interactions. Birdi et al. (4.36) suggest that 1-PrOH does not interact with the micelles, claiming that trends in variation of CMC and number average micellar weights of 1-PrOH modified systems parallel studies where MeOH and ethanol were used as organic additives. These results may have been complicated, however, by the presence of added salt in each of the solvent modified systems. Studies involving the effect of SDS micelles upon
partial molar volumes of various alkanols indicates interaction between the alcoholic head groups and the micellized surfactant for all alkanols except MeOH (4.21). From analyses of CMC data, we believe that 1-PrOH interacts solely at the micellar surface in an alignment indicative of comicellization. A significant decrease in micellar electrophoretic velocity compared to the other solvent modified systems should therefore be evident due to the reduction of charge density at the micellar surface. This aspect will be discussed in the following section.

As for the other solvent systems, MeOH is extremely miscible in water resulting in weak micellar interactions through partitioning processes. The increase in CMC is probably due to increased solvation of surfactant monomers or a decrease in dielectric constant of the bulk solvent which causes an increased repulsion of the ionic heads of the surfactant molecules (4.37). For MeCN, we believe that micellar penetration of this solvent is suggested by selectivity data. However, the increase in CMC rivals the effects of MeOH. In light of this, the interaction of MeCN with the micellar phase may best be described as a more interactive partitioning behavior than observed for MeOH. This explanation supports both the effect of MeCN on CMC, which parallels the mechanism described for MeOH, and the presence of MeCN within the micelles.

4.3.3 Electroosmotic and micellar electrophoretic velocities

As was previously discussed, the presence of mixed micellar formation with a compound such as 1-PrOH would ultimately decrease the charge density of the micellar surface due to the presence of surface hydroxyl groups (4.27). The surface charge reduction should be reflected by a decrease in the electrophoretic mobility of the micelles. Table 4.4 gives the micellar electrophoretic velocities for each solvent system over the investigated SDS concentration range. In each solvent system, the
Table 4.4 Comparison of electroosmotic and micellar electrophoretic velocities.

<table>
<thead>
<tr>
<th>[SDS], M</th>
<th>$v_{eo}$ (mm/sec)</th>
<th>$v_{ep}$ (mm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>organic modifier</td>
<td>organic modifier</td>
</tr>
<tr>
<td>none</td>
<td>1.857</td>
<td>1.235</td>
</tr>
<tr>
<td>MeOH</td>
<td>1.455</td>
<td>0.913</td>
</tr>
<tr>
<td>MeCN</td>
<td>1.781</td>
<td>1.217</td>
</tr>
<tr>
<td>PrOH</td>
<td>1.036</td>
<td>0.805</td>
</tr>
<tr>
<td>0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.040</td>
<td>1.820</td>
<td>1.219</td>
</tr>
<tr>
<td></td>
<td>1.450</td>
<td>0.920</td>
</tr>
<tr>
<td></td>
<td>1.729</td>
<td>1.196</td>
</tr>
<tr>
<td></td>
<td>0.975</td>
<td>0.793</td>
</tr>
<tr>
<td>0.055</td>
<td>1.788</td>
<td>1.217</td>
</tr>
<tr>
<td></td>
<td>1.375</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>1.660</td>
<td>1.193</td>
</tr>
<tr>
<td></td>
<td>0.962</td>
<td>0.806</td>
</tr>
<tr>
<td>0.070</td>
<td>1.776</td>
<td>1.228</td>
</tr>
<tr>
<td></td>
<td>1.347</td>
<td>0.918</td>
</tr>
<tr>
<td></td>
<td>1.671</td>
<td>1.201</td>
</tr>
<tr>
<td></td>
<td>0.980</td>
<td>0.845</td>
</tr>
</tbody>
</table>
electrophoretic velocity did not change significantly with SDS concentration. Slight variations in electroosmotic velocities were evident and may be attributed to changes in viscosity with variation of SDS concentration (4.25). Micellar electrophoretic velocities in the H$_2$O and MeCN systems were approximately the same, indicating that no significant change in micellar surface charge density. As expected, the micellar velocities in the 1-PrOH system were slower than the velocities found in H$_2$O, suggesting possible coaggregation of SDS and 1-PrOH. Surprisingly, MeOH also significantly lowered the electrophoretic velocity but not as effectively as 1-PrOH.

The comparison of micellar velocities should be useful in showing the effect of organic modifiers on the micelles. However, factors other than those concerned with the micellar surface can affect these velocities, such as viscosity and permittivity. From the literature, changes in permittivity were not significant due to the aqueous nature of the bulk solvent. In the case of each organic modifier system, dielectric constants of the bulk solvent ranged from $>$ 70 to the dielectric constant of H$_2$O (4.38, 4.39) and the presence of SDS at such low concentrations should not change permittivity appreciably (4.25). On the other hand, the differences in viscosities of the bulk solvent systems were fairly large, ranging from $\sim$ 1 g/mL (H$_2$O) to $\sim$ 1.7 g/mL (1-PrOH) (4.40).

If we were able to negate the effects of viscosity and permittivity upon the electrophoretic velocity, determination of the micelle's electrokinetic ($\zeta$-) potential would be possible. Under certain conditions (4.41), the $\zeta$-potential can be used to determine the micellar surface charge density directly which, if lower than the micellar $\zeta$-potential in the SDS/H$_2$O system, would indicate surface modification. However, the effects of temperature and SDS concentration upon viscosity and permittivity enable us only to reach order of magnitude values of surface charge. With the slight
surface modifications that are expected, attempts to correlate these calculated values with modifications to the micellar surface would be futile.

4.4 CONCLUSIONS

Organic solvent modifiers in MECC have been previously found useful for separation of solute systems containing moderately to highly hydrophobic compounds. However, a fundamental study of these systems was necessary to gain a better understanding of their role in the separation process. The use of n-alkylphenone homologs permits investigation of several parameters related to the separation mechanism, i.e., selectivity, partition coefficients, and free energies of transfer. Also, we have shown that this solute system may be useful in the study of variation in micellar structure due to the presence of organic solvents.

In general, we observed reduced methylene selectivities for SDS systems modified with organic solvent. Of the three investigated solvents (MeOH, MeCN, and 1-PrOH), selectivities in the MeOH system most resembled those in SDS/H2O, presumably due to its strong H-bonding with H2O and resultant exclusion from the micelles. Methylene selectivity substantially decreased in the MeCN and 1-PrOH systems. Both solvents were presumed to accumulate within the micelles on the basis of these findings, yet by different mechanisms. Comparison of CMC data between organic solvent modified systems and SDS/H2O suggests that 1-PrOH coaggregates with SDS to form mixed micelles. On the other hand, MeCN apparently accumulates in the micelles via active partitioning into micellar regions of favorable polarity and/or solvation.

From this study, the organic solvents can be classified into three categories with respect to their effect on the micelles: i) noninteractive (MeOH), ii) interactive through partitioning processes (MeCN), and iii) interactive through coaggregation (1-
PrOH). Any water miscible organic solvent may fall into one of these categories depending on its relative interaction between the bulk aqueous phase and the micellar phase.

In closing, we hope this study provides a clearer picture of how organic solvents can affect retention, selectivity, and other parameters in SDS-mediated MECC. Also, our findings may be extremely useful as a basis for comparing effects of organic solvents on other surfactant systems, i.e., nonionic/anionic surfactant mixtures. This topic is addressed in the following Chapter.
CHAPTER FOUR REFERENCES


CHAPTER 5

EFFECT OF ORGANIC SOLVENT ON THE RETENTION AND
SELECTIVITY OF n-ALKYLPHENONE HOMOLOGUES IN BRIJ
35/SDS-MEDIATED MICELLAR ELECTROKINETIC CAPILLARY
CHROMATOGRAPHY
5.1 INTRODUCTION

Micellar electrokinetic capillary chromatography (MECC) is a rapidly growing technique utilizing micelles in capillary zone electrophoresis (CZE) for separation of neutral compounds. Since its introduction (5.1, 5.2), several modifications to the buffer medium have been implemented to improve the applicability of this technique. Several micellar systems have been introduced to exploit certain properties of the analytes of interest. Burton et al. (5.3) investigated some common anionic and cationic surfactant systems. Bile salt micelles have received attention for the separation of enantiomeric mixtures (5.4, 5.5). Otsuka et al. (5.6) investigated the effect of SDS and dodecyltrimethylammonium bromide (DTAB) on the retention of phenylthiohydantoin (PTH)-derivatized amino acids and found significant differences in retention characteristics between the two systems.

Limited use of nonionic/anionic micellar mixtures is evident in MECC. Otsuka and Terabe (5.7) illustrated the usefulness of digitonin/SDS surfactant systems for optical resolution of PTH-amino acid mixtures. Rasmussen et al. (5.8) first introduced Brij® 35, polyoxyethylene(23)dodecanol, as a possible micellar phase in MECC. When mixed micelles were formed from Brij 35 and SDS, significant modification of net micellar velocity and, moreover, separation selectivity was observed.

Organic modifiers can serve to increase the solubility of extremely lipophilic compounds in the aqueous phase and moderately affect the elution range. Gorse et al. (5.9) studied the effect of 1-20% v/v of methanol and acetonitrile on analyte retention and elution range. They observed significant extension of elution range with each modifier and decreased retention of hydrophobic solutes. Addition of these solvents also affected selectivity and efficiency. Methanol in the buffer medium has also aided in the separation of isotopically substituted compounds (5.10). Finally, various
studies have employed 1- or 2-propanol to increase solute solubilization, enhance solute-micelle mass transfer, and decrease solute-capillary wall interactions (5.11, 5.12).

In this study, the properties of Brij 35/SDS micellar systems were investigated under the influence of dilute solutions of three organic solvents, methanol (MeOH), acetonitrile (MeCN), and 1-propanol (1-PrOH). Specifically, differences in retention, selectivity, and partitioning characteristics of a homologous series will carry the bulk of discussion. The influence of the polyoxyethylene (POE) surface of the micelles on micellar solvation will also be discussed.

5.2 EXPERIMENTAL

5.2.1 Apparatus

A Quanta 4000 Capillary Electrophoresis System was provided by Millipore Corporation, Waters Chromatography Division (Milford, MA, USA). This instrument was equipped with hydrostatic injection used for 1 sec intervals and a fixed-wavelength UV absorbance detector operated at 254 nm. Untreated fused-silica capillary tubing with dimensions of 50 μm i.d. and 170 μm o.d. was purchased from Alltech Associates, Inc. (Deerfield, IL, USA). The total capillary length was 47.5 cm with injector-to-detector lengths of 40 cm. Activation of the capillaries was performed using a modification of a procedure described previously (5.13). The capillary was initially rinsed with 1 M KOH for 15 min followed by subsequent rinses of 0.1 M KOH and deionized water for 15 min each. The capillary was then rinsed for 20 min with the operating buffer. Purges with the operating buffer were performed after each run for 5 min using a vacuum of ~14 inches Hg at the detector reservoir. All separations were performed using a 25 kV applied voltage. Data were acquired on an
Apple MacintoshPlus computer (Cupertino, CA, USA) equipped with a Rainin Dynamax® Method Manager data acquisition package (Woburn, MA, USA).

5.2.2 Materials and Methods

The n-alkylphenone homologous series was purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and consisted of C₈ (acetophenone), C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₆, and C₁₈ (dodecanophenone) homologs. Brij® 35 (polyoxyethylene(23)dodecanol) was obtained from Aldrich and electrophoresis-grade SDS was purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA). Distilled water was deionized and redistilled with a Corning Mega-Pure™ Water Purification System (Corning, Inc., Corning, NY, USA). Methanol (MeOH) and acetonitrile (MeCN) were obtained from Mallinkrodt, Inc. (Paris, KY, USA) while 1-propanol (1-PrOH) was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA).

Stock phosphate buffer was prepared with NaH₂PO₄ • H₂O and NaOH to give a 50 mM concentration of pH 6.8. This buffer was diluted to a 10 mM concentration and used for preparation of surfactant solutions. Stock solutions of Brij 35 and SDS were prepared at concentrations of 0.1 M each in the diluted phosphate buffer. Fifteen mL of specified organic solvent (methanol, acetonitrile, or 1-propanol) was added to volumetric amounts of Brij 35 and SDS stock solutions and further diluted with phosphate buffer to yield a solution of 100 mL total volume. Brij 35 concentrations of 30, 40, 50, and 60 mM and 20 mM SDS were employed with each organic modifier. Stock n-alkylphenone standard solution was prepared in acetonitrile at solute concentrations of ~5 mg/mL. Samples for analyses were prepared by dilution of the stock solution with acetonitrile to solute concentrations of ~0.6 mg/mL.
5.3 RESULTS AND DISCUSSION

Nonionic/anionic surfactant mixtures have been utilized recently in MECC to provide different separation selectivity compared to when using an anionic surfactant system exclusively (5.8). Unique hydrophilic solute retention via interaction with the POE micellar surface layer may be extremely useful in the development of optimization schemes similar to those currently employed in reversed-phase HPLC, i.e., modification of selectivity by varying relative percentages of organic solvents in the mobile phase. By addition of organic solvents to nonionic/anionic surfactant mixtures, further modification of separation selectivity may be possible; these solvents should also increase hydrophobic analyte solvation, facilitating their separation within a limited time frame. The following sections will discuss the effects of three organic solvents; methanol, acetonitrile, and 1-propanol; on various parameters involved in the separation of an alkylphenone homologous series.

5.3.1 Variation in retention

Solute retention in a nonionic/anionic surfactant system may occur by two mechanisms: i) solute interaction with the moderately polar POE surface layer of the micelles and ii) solute interaction with the nonpolar micellar core. Therefore, the buffer medium in which separation occurs may be visualized as three distinct phases: a highly polar aqueous phase, a moderately polar POE phase, and a nonpolar micelle core phase. Separation of the aqueous phase and micellar POE surface into discrete phases may be idealized; however, recent studies indicate that desolvation of monomeric POE chains occurs upon micellization (5.14) which would exclude the aqueous and POE phases from one another to some degree. Organic solvent in the micellar system may affect the model in a number of ways: i) the solvent may be located exclusively in the aqueous phase in which case the model retains its defined
phases, ii) the solvent may be solvated in the aqueous and POE phases at nearly equivalent concentrations thereby reducing or eliminating distinction between these two phases, or iii) the solvent may saturate the micelle, making division of all layers less discrete.

In order to ascertain the effects of organic solvents on retention, homologue retention in organic solvent modified systems was compared to pure Brij 35/SDS. The fractional change in retention between micellar systems was determined by the ratio $k'_{\text{mod}}/k'_{\text{pure}}$ where, at a specified Brij 35 concentration, $k'_{\text{pure}}$ is the solute capacity factor in the pure Brij 35/SDS system and $k'_{\text{mod}}$ is its capacity factor in the organic solvent modified system. A plot of this function against carbon number, as shown in Figure 5.1, illustrates each organic solvent's effect on the retention of the various homologues. As was expected, all organic solvents affected homologue retention similarly; hydrophobic homologues experienced greater retention changes via their increased affinity for the aqueous phase, whereas retention of hydrophilic solutes did not decrease as dramatically owing to their preferential solvation in the aqueous phase prior to addition of organic solvent.

Changes in net retention between homologues with increasing carbon number at different Brij 35 concentrations were minimal (25-35% decrease in retention from $C_8$ to $C_{12}$); however, in some cases, Brij 35 concentration notably affected the magnitude of retention variation for the set of homologues in comparison to pure Brij 35/SDS. The most significant anomaly occurred in the MeOH modified system at 30 mM Brij 35. To elaborate, for Brij 35 concentrations above 30 mM, the retention of $C_8$ was 30% less than in pure Brij 35/SDS. At 30 mM Brij 35, however, the retention of $C_8$ decreased only 15%. This trend was evident for all alkylphenone homologues, creating an offset of 30 mM Brij 35 data from the other plots.
Figure 5.1  $k'$ ratio of organic solvent modified to pure Brij 35/SDS vs carbon number for n-alkylphenones at different Brij 35 concentrations. Headings above each plot denote the organic solvent added. See figure legend for specific Brij 35 concentrations.
The solvation properties of the organic solvents and micelle structural stability may explain this phenomenon. It is well-known that nonionic/anionic micelles are more compact than micelles formed from the individual surfactants due to the interaction of anionic head groups and oxonium ions. These oxonium ions form between hydrogen or sodium ions and negatively polarized ether oxygen atoms of the oxyethylene groups (5.15). In addition, the greater micellar compactness tends to increase the solubilization of analytes as demonstrated by Nishikido (5.15). At 30 mM Brij 35, the surfactant mole fraction of SDS ($n_{SDS}/n_{total}$, where $n_{total}$ is the sum of Brij 35 and SDS mole quantities), which was present at a 20 mM concentration in all Brij 35/SDS systems studied, is at its highest value. Interaction between anionic head groups and oxonium ions should be at a maximum producing the most structurally stable micellar system in this study.

This high structural stability may explain the offset previously discussed (vide supra). In Chapter Four, we found that MeOH was least interactive with an SDS micellar phase due to its strong H-bond interactions with water and general hydrophilic nature. It follows that MeOH may interact poorly with this surfactant system as well; however, the surface of Brij 35/SDS micelles consists of a polyoxyethylene (POE) "shell" which is prone to hydration (5.16). With this in mind, MeOH should interact readily with these groups much in the same manner as water. However, with decreasing Brij 35 mole fraction, the Brij 35/SDS micelles should become more compact due to greater SDS ionic head group/POE oxonium ion interactions. This greater interaction reduces the extension of the POE groups into solution and should also reduce the degree of POE hydration (or solvation). In other words, the increased compactness of the micelles may effectively exclude MeOH from the POE environment making the aqueous and POE phases more dissimilar (in 30 mM Brij 35). Therefore, in the MeOH system, retention of the n-alkylphenone
homologues in 30 mM Brij 35 should more resemble their retention in pure Brij 35/SDS than at other Brij 35 concentrations.

The degree to which Brij 35/SDS coaggregation occurs can be viewed through changes in micellar electrophoretic velocity with [Brij 35]. The electroosmotic velocity, \( v_{eo} \), is given by

\[
v_{eo} = \frac{\varepsilon \zeta}{\eta} E
\]  

(5.1)

where \( \varepsilon \) and \( \eta \) are the dielectric constant and viscosity of the solution, respectively; \( \zeta \) is the zeta-potential at the solid-liquid interface; and \( E \) is the electrical field strength, equivalent to applied voltage per unit length. The electrophoretic velocity of a micelle is described by eq 5.2

\[
v_{ep} = \frac{2\varepsilon \zeta}{3\eta} f(\kappa a) E
\]  

(5.2)

where \( f(\kappa a) \) depends on the micellar shape, having a value of 1.5 for a sphere of \( \kappa a = \infty \). The ratio of \( v_{ep} \) to \( v_{eo} \) can be used to view the relative change in micellar electrophoretic velocity through negation of viscosity and permittivity fluctuations which may accompany changes in Brij 35 concentration. Figure 5.2 shows a plot of \( v_{ep}/v_{eo} \) versus [Brij 35]. Note that as [Brij 35] increases, the micellar velocity in relation to the electroosmotic velocity decreases. Thus, SDS monomers within the Brij 35 micelles become more disperse with increasing Brij 35 concentration and, consequently, the SDS anionic head groups cannot interact as efficiently with the increased number of oxonium ions present at higher [Brij 35].
Figure 5.2  Ratio of net micellar (\(v_{\text{net}}\)) to electroosmotic (\(v_{\text{eo}}\)) velocities vs [Brij 35] for the Brij 35/SDS mixed surfactant system. Velocities were measured by migration times of acetonitrile (\(v_{\text{eo}}\)) and decanophenone (\(v_{\text{net}}\)).
The lack of the offset at 30 mM Brij 35 in MeCN or 1-PrOH is unknown but probably involves their different solvating properties of Brij 35 POE groups. The data in Figure 5.1 clearly suggests similarity in interactions of MeCN and PrOH with the micelles. It is likely that increased solvation of the POE groups would explain the similarity of 30 mM Brij 35 with the other surfactant concentrations in each solvent but this explanation is speculative. Further studies involving variation of organic solvent content and Brij 35/SDS mole fractions may reveal more on the micelle/solvent interactions.

Figure 5.3 allows comparison of retention characteristics in Brij 35/SDS/solvent systems with those in SDS/organic solvent media. The magnitude of retention change in SDS/organic solvents was much greater than in the Brij 35 systems (50 to 80% retention decrease over the homologue range). Also, the magnitude was fairly consistent for all organic solvent modified systems, that is, the influence of the organic solvents on the retention change compared to pure Brij 35/SDS was approximately the same. From these observations, it is clear that the general retention characteristics of Brij 35/SDS micelles are affected less than SDS micelles in organic solvents. This further supports the greater compactness of Brij 35/SDS micelles. A slight anomaly was observed for 25 mM SDS in 1-PrOH which may indicate SDS/PrOH coaggregation (5.17), but the small magnitude of this deviation precludes this assumption.

5.3.2 Variation of selectivity

5.3.2.1 General methylene selectivity

The retention change from modification of carbon number in otherwise similar compounds permits determination of hydrophobic, or methylene, selectivity. The relationship of carbon number and retention is described by
Figure 5.3  \( k' \) ratio of organic solvent modified to pure SDS vs carbon number for \( n \)-alkylphenones at different SDS concentrations. Headings above each plot denote the organic solvent added. See figure legend for specific SDS concentrations.
\[ \log k' = (\log \alpha)N_c + (\log \beta) \]  

(5.3)

where \( k' \) is a solute's capacity factor, \( N_c \) is the carbon number of the solute, \( \beta \) is the phase ratio (\( = V_{mc}/V_{aq} \), where \( V_{mc} \) and \( V_{aq} \) are volumes of the micellar and aqueous phases, respectively), and \( \alpha \) is the methylene selectivity. In Chapter Four, selectivity studies on the effect of organic solvent on SDS micelles indicated possible inclusion of organic solvent into the micellar structure. Since we have concluded that Brij 35/SDS micelles are probably more structurally stable and compact than SDS micelles, we expect greater methylene selectivities in the Brij 35/SDS systems due to solvent exclusion from inner micellar microenvironments and, thus, a greater polarity difference between the aqueous and micellar phases.

Table 5.1 gives values for \( \log (\alpha_{CH_2}) \) at different surfactant concentrations for each organic solvent modified system. The methylene selectivities in Brij 35/SDS/organic solvent systems were generally greater than in SDS/organic solvent. Also, variation in \( \log (\alpha_{CH_2}) \) between Brij 35 concentrations in each organic solvent was systematically less, excluding MeOH, than in the SDS systems (1.23, 2.64, 1.68, and 0.64% RSD for Brij 35/SDS and 1.70, 1.60, 4.18, and 2.11% RSD for SDS in H_2O, MeOH, MeCN, and 1-PrOH, respectively). As was noted previously, however, the retention characteristics of 30 mM Brij 35/SDS in MeOH differ from those in other MeOH-modified Brij 35 systems; exclusion of this data gives deviations similar to those in higher SDS and Brij 35 concentrations (1.38% RSD and 1.60% RSD for Brij 35/SDS and SDS, respectively). Homologue retention through inner micellar, rather than POE, interaction probably contributes to the higher selectivities in the Brij 35 systems. From these selectivity data, we propose that these organic solvents do not interact as strenuously with Brij 35/SDS micelles compared to pure
<table>
<thead>
<tr>
<th>mobile phase</th>
<th>log (α_{CH2})</th>
<th>log β</th>
<th>log β (calc)</th>
<th>r² (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% organic modifier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM Brij 35</td>
<td>0.395 (±0.01)</td>
<td>-3.17 (±0.1)</td>
<td>-1.47</td>
<td>0.998</td>
</tr>
<tr>
<td>40 mM Brij 35</td>
<td>0.401 (±0.01)</td>
<td>-3.13 (±0.1)</td>
<td>-1.34</td>
<td>0.998</td>
</tr>
<tr>
<td>50 mM Brij 35</td>
<td>0.399 (±0.01)</td>
<td>-3.05 (±0.1)</td>
<td>-1.23</td>
<td>0.998</td>
</tr>
<tr>
<td>60 mM Brij 35</td>
<td>0.390 (±0.01)</td>
<td>-2.91 (±0.1)</td>
<td>-1.15</td>
<td>0.998</td>
</tr>
<tr>
<td>15% v/v MeOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM Brij 35</td>
<td>0.360 (±0.01)</td>
<td>-2.98 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
<tr>
<td>40 mM Brij 35</td>
<td>0.347 (±0.01)</td>
<td>-2.85 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
<tr>
<td>50 mM Brij 35</td>
<td>0.345 (±0.01)</td>
<td>-2.76 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
<tr>
<td>60 mM Brij 35</td>
<td>0.338 (±0.01)</td>
<td>-2.64 (±0.1)</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>15% v/v MeCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM Brij 35</td>
<td>0.355 (±0.01)</td>
<td>-3.01 (±0.1)</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>40 mM Brij 35</td>
<td>0.351 (±0.01)</td>
<td>-2.90 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
<tr>
<td>50 mM Brij 35</td>
<td>0.342 (±0.01)</td>
<td>-2.77 (±0.1)</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>60 mM Brij 35</td>
<td>0.345 (±0.01)</td>
<td>-2.72 (±0.1)</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>15% v/v 1-PrOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mM Brij 35</td>
<td>0.326 (±0.01)</td>
<td>-2.76 (±0.1)</td>
<td>-</td>
<td>0.997</td>
</tr>
<tr>
<td>40 mM Brij 35</td>
<td>0.325 (±0.01)</td>
<td>-2.67 (±0.1)</td>
<td>-</td>
<td>0.997</td>
</tr>
<tr>
<td>50 mM Brij 35</td>
<td>0.322 (±0.01)</td>
<td>-2.58 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
<tr>
<td>60 mM Brij 35</td>
<td>0.322 (±0.01)</td>
<td>-2.51 (±0.1)</td>
<td>-</td>
<td>0.998</td>
</tr>
</tbody>
</table>

*Table 5.1 Comparison of log (α_{CH2}) and log β values.*
SDS micelles. However, in order to understand better organic solvent interaction with these micellar systems, examination of individual solute pair selectivities may be more appropriate.

5.3.2.2 Methylene selectivity between individual solute pairs

Since the slopes compared in the previous section give only a generalized view of methylene selectivity in the organic solvent modified systems, we have chosen to investigate methylene selectivity changes between individual solute pairs at different Brij 35 concentrations. Methylene selectivity between homologue pairs was determined by

$$\alpha = \frac{k_{N_c+1}}{k_{N_c}}$$ (5.4)

where \(N_c\) represents the total number of carbons for individual homologues. Plots of fractional change in selectivity vs Brij 35 concentration are shown in Figure 5.4 for each organic solvent system. The ordinate represents a ratio of selectivities between the organic solvent modified systems and Brij 35/SDS/H_2O \((\alpha_{\text{modifier}}/\alpha_{\text{H}_2\text{O}})\). This particular relationship was used in an attempt to eliminate selectivity variations from changes in Brij 35 concentration. However, each organic solvent may interact in some manner with the micelles to cause structural variations. Therefore, the selectivity variations shown in these plots will reflect the effects of organic modifier and micellar modifications.

Variation in selectivity ratios at different Brij 35 concentrations for each organic modifier was obvious. However, close examination reveals that these variations were nearly identical for each solute pair. For instance in the MeCN system, a gradual decrease in selectivity change followed by a rapid increase in 60 mM
Figure 5.4  $\alpha_{\text{modifier}}/\alpha_{\text{H}2\text{O}}$ vs [Brij 35] for n-alkylphenone solute pairs in each organic modifier system. The solvent and solute pair identities are given within the figure. For specific mobile phase and run conditions see Experimental Section.
Brij 35 is evident for each homologue pair. It seems that higher retention of individual homologues magnifies these variations. The consistency of these variations suggests possible error in the measurement of homologue capacity factors at different Brij 35 concentrations. The capacity factor in MECC is measured by

\[ k' = \frac{t_R - t_0}{t_0(1 - (t_R/t_{mc}))} \]

where \( t_R, t_0, \) and \( t_{mc} \) are the elution times of the analyte, an unretracted solute, and an infinitely retained solute, respectively. Error in \( k' \) can occur from faulty measurement of any of these values. Of the three variables, \( t_{mc} \) is most suspect since later eluting peaks generally reflect error in this value more significantly and, as stated above, the error seems to become greater as solute retention increases. An iterative method of determining \( t_{mc} \) using a homologous series has been described (5.10) which is preferable to using a marker since i) reliable measurement of a single eluting species is difficult for fairly broad peaks and ii) the presence of organic modifier increases solubility of hydrophobic compounds making the determined values for \( t_{mc} \) questionable. We attempted to apply this method to our systems in an effort to reduce \( \alpha \) ratio variation between Brij 35 concentrations. In comparison to Figure 5.4, the changes in \( \alpha \) ratio by this iterative scheme were minimal. Therefore, we have no basis to assume that the variation in \( \alpha \) ratio was due to \( t_{mc} \) measurement errors.

In general, MeOH and MeCN affected selectivity similarly, that is, the two plots for these solvents in Figure 5.4 are extremely similar. As is expected, 1-PrOH decreased \( \alpha \) much more than the other two solvents due to the lesser polarity of 1-PrOH and, hence, greater resemblance to the micellar interior.

The most striking feature of these plots is the change in \( \alpha \) ratio of the C9/C8 homologue pair compared to other solute pairs. In MeCN, the \( \alpha \) ratio decreased with
increasing carbon number. However, in MeOH and 1-PrOH, the C9/C8 ratio was significantly less, lying between C11/C10 and C12/C11 solute pairs. This occurrence probably stems from interactions between these solutes and the POE micelle layer. Lesser retained compounds such as C8 and C9 should interact significantly with the POE layer. The capability of MeOH and 1-PrOH to hydrogen bond with oxygen atoms of the oxyethylene groups yields a surface concentration of organic solvent. The presence of these solvents in the micellar region primarily responsible for solute retention should produce micellar microenvironments with polarity similar to the aqueous phase. Since selectivity is a measure of solute discrimination between two phases, a reduction in separation selectivity should predominate for those solutes that interact with these micellar regions.

5.3.3 Partition coefficients and CMC

5.3.3.1 Effect of organic solvent on partition coefficients

Terabe et al. (5.2) described the following relationship between solute retention and surfactant concentration.

\[ k' = P_{wm}V([\text{SURF}] - \text{CMC}) \]  

Each term has been defined in preceding text. With knowledge of the surfactant monomer’s partial molar volume (V), the slope provides the water-micelle partition coefficient of the solute. The addition of organic solvent should increase the solute’s affinity for the aqueous phase resulting in lower P_{wm} values. Table 5.2 illustrates this effect. In each organic solvent, the partial molar volume of Brij 35 was taken as equivalent to its value in H2O (1.105 L-mol⁻¹). However, variation of this value is inevitable in the presence of SDS and organic solvents. Also, lack of accurate
### Table 5.2 Partition coefficients for members of n-alkylphenone homologous series.

<table>
<thead>
<tr>
<th>mobile phase</th>
<th>slope</th>
<th>Pw,m</th>
<th>intercept</th>
<th>-(int/slope x 100)</th>
<th>r² (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 35/H₂O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>22.2 (±0.4)</td>
<td>20.1</td>
<td>0.362 (±0.02)</td>
<td>1.63</td>
<td>0.999</td>
</tr>
<tr>
<td>C₉</td>
<td>51.8 (±0.5)</td>
<td>46.9</td>
<td>0.876 (±0.02)</td>
<td>1.69</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₀</td>
<td>117 (±2)</td>
<td>106</td>
<td>2.18 (±0.08)</td>
<td>1.86</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₁</td>
<td>307 (±20)</td>
<td>278</td>
<td>5.28 (±0.6)</td>
<td>1.72</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₂</td>
<td>966 (±110)</td>
<td>875</td>
<td>11.6 (±4)</td>
<td>1.20</td>
<td>0.988</td>
</tr>
<tr>
<td>15% w/v MeOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>16.1 (±0.7)</td>
<td>14.5</td>
<td>0.235 (±0.03)</td>
<td>1.47</td>
<td>0.998</td>
</tr>
<tr>
<td>C₉</td>
<td>32.7 (±1)</td>
<td>29.6</td>
<td>0.538 (±0.07)</td>
<td>1.65</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₀</td>
<td>64.4 (±2)</td>
<td>58.3</td>
<td>1.28 (±0.1)</td>
<td>1.99</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₁</td>
<td>139 (±1)</td>
<td>122</td>
<td>3.52 (±0.1)</td>
<td>2.65</td>
<td>1.000</td>
</tr>
<tr>
<td>C₁₂</td>
<td>276 (±20)</td>
<td>250</td>
<td>11.0 (±1)</td>
<td>3.96</td>
<td>0.994</td>
</tr>
<tr>
<td>15% w/v MeCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>14.0 (±0.3)</td>
<td>12.6</td>
<td>0.292 (±0.01)</td>
<td>2.08</td>
<td>1.000</td>
</tr>
<tr>
<td>C₉</td>
<td>29.3 (±0.8)</td>
<td>26.55</td>
<td>0.670 (±0.04)</td>
<td>2.28</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₀</td>
<td>59.4 (±2)</td>
<td>53.7</td>
<td>1.52 (±0.1)</td>
<td>2.57</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₁</td>
<td>127 (±5)</td>
<td>115</td>
<td>3.88 (±0.2)</td>
<td>3.05</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₂</td>
<td>278 (±3)</td>
<td>251</td>
<td>11.0 (±0.1)</td>
<td>3.92</td>
<td>1.000</td>
</tr>
<tr>
<td>15% w/v 1-PrOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₈</td>
<td>15.8 (±0.3)</td>
<td>14.3</td>
<td>0.270 (±0.01)</td>
<td>1.71</td>
<td>0.999</td>
</tr>
<tr>
<td>C₉</td>
<td>31.5 (±0.7)</td>
<td>28.5</td>
<td>0.520 (±0.03)</td>
<td>1.65</td>
<td>0.999</td>
</tr>
<tr>
<td>C₁₀</td>
<td>62.5 (±2)</td>
<td>56.6</td>
<td>1.05 (±0.1)</td>
<td>1.68</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₁</td>
<td>133 (±5)</td>
<td>120</td>
<td>2.40 (±0.2)</td>
<td>1.81</td>
<td>0.998</td>
</tr>
<tr>
<td>C₁₂</td>
<td>285 (±10)</td>
<td>258</td>
<td>6.71 (±0.5)</td>
<td>2.36</td>
<td>0.997</td>
</tr>
</tbody>
</table>
temperature measurements within the capillary prohibits comparison of \( P_{wm} \) values with the literature to determine what effect the solvent system has upon the partial molar volume of the Brij 35/SDS system. Thus, \( P_{wm} \) values allow only relative comparisons of solute-micelle binding activity between homologues; comparisons between organic solvent systems are approximate at best.

Unlike SDS/organic solvent systems, organic solvents introduced only slight differences in \( P_{wm} \) values for the Brij 35/organic solvent systems. This indicates that identity of organic solvent has little effect on solute partitioning between the two phases. Since homologue retention should occur initially by interaction with or penetration through the POE layer, then the degree to which the POE layer is solvated probably governs the partitioning process. If the POE layer is sufficiently solvated, there is no true "barrier" to distinguish micellar phase from aqueous phase (unlike SDS micellar systems where an analyte must traverse an extremely ionic environment prior to retention). Lack of this barrier should ease solute mass transfer between micellar and aqueous phases.

To investigate homologue interaction in the modified micellar systems further, the free energy of solute transfer from the bulk solution to micellar phase was deduced. The following equation defines the relationship between partition coefficients and carbon number (5.18).

\[
\log P_{wm} = (\text{slope})N_c + \text{intercept} \tag{5.6}
\]

The slope is a measure of free energy of transfer of a methylene group from the aqueous to micellar phase. The intercept reflects interaction of the residual phenone group with the micelles. Table 5.3 shows slope and intercept values for each Brij 35/SDS system. Methylene affinities in the Brij 35 systems closely resembled
Table 5.3 *Comparison of slope and intercept values for log $P_{wm}$ vs $N_c$ plots.*

<table>
<thead>
<tr>
<th>mobile phase</th>
<th>slope</th>
<th>intercept</th>
<th>$r^2$ (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS/H$_2$O</td>
<td>0.377</td>
<td>-1.724</td>
<td>0.999</td>
</tr>
<tr>
<td>15% v/v MeOH</td>
<td>0.309</td>
<td>-1.309</td>
<td>1.000</td>
</tr>
<tr>
<td>15% v/v MeCN</td>
<td>0.323</td>
<td>-1.485</td>
<td>1.000</td>
</tr>
<tr>
<td>15% v/v 1-PrOH</td>
<td>0.314</td>
<td>-1.368</td>
<td>0.999</td>
</tr>
</tbody>
</table>
corresponding values in SDS suggesting that similar microenvironments exist within
the micellar cores of both systems.

Differences in intercept values between Brij 35 systems reveal greater phenone
group interaction with Brij 35/SDS micelles in the presence of organic solvent. In
Chapter Four, this observation was attributed to greater interaction of the electron-rich
phenone groups with the dense positively charged microenvironment surrounding the
anionic head groups. The same explanation probably holds for these systems. In
comparing the SDS and Brij 35 systems, the intercept values in the Brij 35 systems
were much lower. This reflects significantly less phenone interaction with the
micelles. Following the explanation suggested earlier in this paragraph, since surface
charge density surrounding Brij 35/SDS micelles is less than for pure SDS micelles, it
follows that the interaction of the phenone groups with the micellar surface would
decrease. Note also that in the SDS/organic solvent systems (Table 4.3) intercept
values closely followed the degree of solvent interaction with the micelles where the
magnitude of phenone interaction was in the order of 1-PrOH > MeCN > MeOH. The
Brij 35 systems follow the same trend in that phenone interaction with the micellar
phase increases in the presence of organic solvents. However, the trend in relative
magnitude between organic solvents is different being in the order of MeOH > 1-
PrOH > MeCN. This suggests substantial solvation of the POE layer by MeOH in
comparison to the other solvents.

5.3.3.2 Trends in variation of CMC

From eq 5.6, the quotient of intercept to slope theoretically determines the
CMC for a particular micellar system. Table 5.2 gives intercept values for each Brij
35 micellar system. Note that the values are positive; thus, these values cannot be
used to determine accurately the CMC. However, these data can give some useful
information while keeping in mind that, from Table 5.2, the quotient of intercept to slope is negative. Excluding C_{12} data which in most cases carried the bulk of error, the presence of organic modifier in the Brij 35/SDS micellar system does not appreciably affect CMC; nevertheless, the Brij 35/MeCN system yielded slightly more negative intercept/slope values. These findings were in contrast to SDS/organic solvent data where the relative CMCs were substantially higher in the presence of MeOH and MeCN and lower in 1-PrOH. This lack of CMC variation adds additional support to our previous findings on Brij 35/SDS micelle stability. An attempt was made to determine the CMCs by including the SDS concentration. In some cases negative intercepts were evident giving CMCs of the same order of magnitude as the literature value for pure Brij 35 (0.001 M). However, the variation between these calculated CMCs made their accurate determination impossible.

5.4 CONCLUSIONS

The application of nonionic surfactants such as Brij 35 to MECC holds promise as an alternative to purely anionic or cationic surfactants. Their primary advantages are unique separation selectivity and lack of electrical charge (which facilitates control of Joule heating within the separation capillary). Addition of organic solvents to MECC surfactant systems can be advantageous in permitting separation of hydrophobic compounds and increasing the solubility of otherwise insoluble analytes.

The addition of organic solvents to Brij 35/SDS micellar systems produces significantly different effects on homologue retention than observed in SDS/organic solvent systems. Brij 35/SDS systems resist change in retention much more effectively than pure SDS in the presence of organic solvent. In parallel, the methylene selectivity of Brij 35/SDS/organic solvent systems is higher than in SDS/organic solvent systems. Variation of selectivity ratios of organic solvent
modified to pure Brij 35/SDS micellar systems indicate that MeOH and MeCN were less effective in changing selectivity than 1-PrOH.

Minimal differences in partition coefficients were observed indicating that the identity of organic solvent has very little effect on solute partitioning between the two phases. Also, plots of $P_{wm}$ versus carbon number show little difference in free energies of methylene transfer between Brij 35/SDS and pure SDS systems. However, the Brij 35/SDS micellar systems exhibited a substantial decrease in interaction with the residual phenone groups. These results suggest that the preferential solvation of the POE layer provides more efficient penetration of analytes into the micelles, unlike pure SDS systems where an electrostatic "barrier" exists between phases.
CHAPTER FIVE REFERENCES


APPENDIX

SEQUENTIAL MULTIMODAL ELUTION FOR PSEUDO-MULTIDIMENSIONAL LIQUID CHROMATOGRAPHY
ON A SINGLE COLUMN
March 9, 1992

Barbara Polanski
Publications Division
American Chemical Society
1155 16th St. Northwest
Washington, DC 20036

Dear Ms. Polanski,

I am writing to you in reference to the article entitled “Sequential Multimodal Elution for Pseudomultidimensional Liquid Chromatography on a Single Column”, published in Analytical Chemistry, Volume 63, pp. 33-44, 1991, of which I am an author. I would like to include this manuscript as part of my Ph.D. dissertation. The completed dissertation will be submitted to University Microfilms, Incorporated. Please forward permission for the reprint of this manuscript.

Thank you for your prompt reply on this matter by mail or by FAX.

Sincerely,

Edward L. Little
Principal author

Prof. Joe P. Foley
Co-author

Edward L. Little
Principal author
A.1 INTRODUCTION

Liquid chromatography has emerged as the separation method of choice for numerous complex solute mixtures. The introduction of high-performance liquid chromatography (HPLC) has further increased its usefulness due to greater column efficiency and overall reproducibility. Reversed-phase high-performance liquid chromatography (RPLC) has received more attention than any other separation mode due to its broad applicability. Other advantages of RPLC include greater column stability than in conventional liquid-liquid chromatography (LLC); the variety, economy, and low toxicity of common RPLC solvents; and the ease of gradient elution.

As samples become more complex, the ability of a particular separation method to resolve all components decreases. Several factors may lead to insufficient resolution for complex samples, including inadequate column efficiency and/or gradient optimization. Even in cases where these factors are optimized, however, the separation may still be unsuitable due to limitations of selectivity and/or peak capacity. With regard to the latter, a statistical study of component overlap has shown that "a chromatogram must be approximately 95% vacant to provide a 90% probability that a given compound of interest will appear as an isolated peak." (A.1) In instances where this condition is not met, the additional resolution of components within a complex sample would typically require supplemental separation steps, thereby reducing the speed and convenience of using HPLC. Due to this problem, separation methods with greater selectivity and peak capacity but otherwise similar attributes are desirable.

Multidimensional separations have become popular due to the dramatic improvement in resolution they usually provide. Although the improved resolution is usually attributed to the much greater peak capacity, it is also a result of selectivity-related improvements (vide infra). Numerous examples of multidimensional
separations have been reported, particularly those utilizing thin-layer chromatography (TLC), paper chromatography, or electrophoretic methods. Isoelectric focusing/gel electrophoresis perhaps best exemplifies the advantages of multidimensional separations by providing peak capacities in the thousands (A.2). More recently, multidimensional separations have been applied to column chromatography, i.e., GC/GC (A.3, A.4), HPLC/HPLC (A.5-A.7), and HPLC/GC (A.8-A.10), with a reasonable amount of success. However, there has been some degree of difficulty in interfacing column chromatographic dimensions (A.11) and the additional instrumentation or instrumental modifications can be costly. These problems may discourage one from considering coupled-column multidimensional separations as the technique of choice for complex samples.

In light of the above shortcomings, we have investigated the feasibility of performing multidimensional-like separations on a single column, made possible by sequential multimodal elution using secondary chemical equilibria and organic solvents. Our efforts have resulted in a reversed-phase gradient technique capable of separating compounds by chemical class as well as resolving sample components within each class. This technique significantly increases the attainable peak capacity and between-class selectivity for a given column.

A.2 THEORY
A.2.1 Basics of Multidimensional Separations

The basic criteria described by Giddings for true multidimensional separations are as follows: i) (all) components of a sample are subjected to two or more independent separation modes; and ii) the resolution of components from one displacement is not reduced by subsequent separations (A.12, A.13). These criteria are satisfied by a number of techniques including those mentioned in the introduction.
To summarize briefly in terms of TLC, suppose the components of a sample, introduced in a corner, undergo a displacement along one axis of the plate. The degree of migration of each component is independent of all others, yet there may be insufficient resolution due to inadequate selectivity or peak capacity. A subsequent separation along the other axis using a solvent of different strength and/or selectivity increases the overall area of separation with a concurrent increase in the resolution of sample components.

Giddings has explained the overall increase in resolving power of multidimensional separations in terms of increased peak capacity (A.14). By constructing a gridwork of areas corresponding to resolution units within the plane of separation, the total (or maximum) peak capacity can be given by the summation of the number of area increments or, in simpler terms, the product of individual peak capacities, $\phi_y$ and $\phi_z$.

$$\phi_{2-D} = \phi_y \phi_z$$  \hspace{1cm} (A.1)

Although the general superiority of multidimensional separations over one-dimensional separations can be attributed to the dramatically increased peak capacity, it can also be viewed from the perspective of increased selectivity. More precisely, it is the increased probability of obtaining sufficient selectivity to achieve a separation. Since in the ideal case of a two-dimensional separation the two separation modes are independent, the probability that one mode or the other will provide sufficient selectivity to separate a given pair of compounds is the sum of the individual probabilities. Because the sum of two (positive) probabilities, however small, is always greater than either individual probability, the odds of achieving sufficient selectivity with multidimensional approaches are increased. In summary, it is both the
increased peak capacity and selectivity of multidimensional separations that results in increased resolution, and makes multidimensional approaches so beneficial for complex samples.

A.2.2 Sequential Multimodal Elution (SME)

The basis of our elution scheme is the sequential use of one or more selectively strong mobile phase(s) followed by a universally strong mobile phase, as illustrated in Figure A.1A for the case where only one selective mobile phase is employed. Shown for comparison in Figure A.1B is the conventional RPLC elution scheme.

In the multimodal elution scheme of Figure A.1A, a specific class or classes of compounds are first eluted and resolved by a selective mobile phase; the remaining classes of compounds are then eluted and resolved by the universal mobile phase. Selective mobile phase(s) are designed so that any compounds to be separated in later elution modes are highly retained (see eq A.15 and related text), negligibly broadened, and thus unaffected during the selective mobile phase elution. The sequential use of these elution modes is the key to increasing peak capacity, selectivity, resolution and decreasing the randomness (disorder) of the separation. The simultaneous use of these elution modes is inadequate, as will be illustrated later. Note that more than one selective elution mode may be used consecutively prior to the general (solvent) elution mode. The only requirement is that the analytes to be eluted in a given elution mode be relatively unaffected during any prior elution mode. Thus trimodal and perhaps even tetramodal mobile phase elution schemes may also be feasible.

The sequential application of two or more independent elution modes is, to our knowledge, a novel approach for liquid chromatographic separations. Glajch and Kirkland have provided (A.15) a systematic classification of mobile phase programs
Figure A.1 Illustration of a typical sequential multimodal elution separation (A) and a conventional reversed-phase separation (B). Mobile phases are described in the text. The dotted line in A represents the time at which the second elution mode begins.
based on changes (or lack thereof) in mobile phase strength and/or selectivity: simple isocratic (SI), isocratic multisolvant programming (IMP), isoselective multisolvant gradient elution (IMGE), and selective multisolvant gradient elution (SMGE—note that their use of "selective" differs from ours). Upon inspection, it can be shown that SME does not fall within any of the four mobile phase programs mentioned above. First, SME couples two or more independent elution modes (isocratic or gradient), whereas SI, IMP, IMGE, and SMGE employ only one. Furthermore, despite their differences the latter share a common characteristic that distinguishes them from SME. Regardless of how the solvent strength and/or selectivity changes with time during SI, IMP, IMGE, and SMGE, the changes are felt more or less equally by all solutes. In contrast, during the selective elution mode(s) of SME, the mobile phase strength changes for only a portion (class) of the sample while remaining of fixed strength (i.e., very weak) for the remainder. This is why it is possible to separate compounds by class using SME (vide infra). Finally, it is important to realize that SME is not merely a form of multisegmented mobile phase programming. Although the latter can be employed as necessary in IMP, IMGE, and SMGE, or within one or more elution modes of SME, it is not the same as SME.

Does the sequential multimodal elution (SME) scheme of Figure A.1A result in a multidimensional separation as defined earlier? As we show later in this paper, the separation of sample components achieved during a given elution mode need not be significantly affected by earlier or later elution modes. Thus SME fulfills the second criterion of multidimensionality discussed above. In addition, sample components eluted during the second and later separation modes are subjected to more than one independent displacement. For these components the first criterion of multidimensionality is also fulfilled. On the other hand, sample components eluted during the first separative mode are not subjected to more than one independent
displacement. Thus by design a portion of the sample does not fulfill the first criterion and therefore, from a rigorous viewpoint, SME does not represent a true multidimensional approach. As we shall show, however, the sequential application of two or more elution modes on a single column does result in significantly increased peak capacity and selectivity, two characteristic features of multidimensional separations. In view of these improvements over one-dimensional separations, and the fulfillment of most of the criteria for multidimensionality, we believe it is appropriate to refer to SME-based separations based as "pseudo-multidimensional" or "multidimensional-like" to reflect the common features of increased peak capacity and selectivity that they share with true multidimensional separations.

In general, there are four selective mobile phase systems (predominantly aqueous) that can be utilized in SME in the reversed phase mode (SME-RPLC): i) pH buffers, ii) ion-pairing agents, iii) chelating agents, and iv) metal ions. These selective mobile phases can be used to elute and separate ionizable compounds (acids and bases), permanent ions, metal cations, and/or analyte ligands in the presence of neutral compounds or other species unaffected by these mobile phase systems. Obviously, all of these mobile phases are based on the exploitation of secondary chemical equilibria (SCE) in which the selected analytes can participate (A.16). Analytes not participating in SCE can be eluted last by either of two possible universal mobile phase systems: organic solvents or micellar solutions.

The primary requirement for the sequential use of selective and universal mobile phases is their compatibility (miscibility, etc.). In most cases, the concentration of SCE reagent required for the selective elution mode(s) is relatively low so that precipitation of the reagent does not occur under low organic conditions. In the present research, our efforts were centered around the (bimodal) combination of pH gradients and organic solvents due to the ease of mobile phase preparation and its
suitability to a vast number of chemical systems. An ion-pairing agent/micellar solution scheme is also an interesting bimodal combination since, in principle, only water and a surfactant, serving first as the ion-pair reagent and then as the monomer component of the micelle, would be required for this approach.

As mentioned earlier, more than one selective elution mode may be used prior to the general (solvent or micellar) elution mode, provided that the analytes eluted in a given separation mode are relatively unaffected during any prior elution mode. Thus trimodal and tetramodal mobile phase systems may also be possible. One promising trimodal system would be the combination of an ion-pairing agent, pH buffer, and organic solvent or micellar solution for the separation of permanent ions, ionizable compounds, and neutral compounds, respectively. Although the gradients of trimodal and tetramodal systems would be more difficult to generate sequentially (probably necessitating the use of a quaternary LC pumping system), the scope of separation could feasibly encompass virtually all possible chemical classes of a diverse, complex sample.

A.2.3 Peak Capacity

If SME-LC were a true multidimensional technique, the total peak capacity would be the product of the peak capacities of each separation mode (provided the modes were orthogonal). Our experimental results indicate that although significant increases in peak capacity are achieved with SME-LC, the peak capacity is less than with an optimized multidimensional system; it is therefore appropriate to consider peak capacity from a theoretical standpoint. Mathematical treatments of peak capacity have been reported by Giddings (A.14) and Westerlund (A.17), and numerous multidimensional column chromatographic applications can be found in the literature (A.18). For an individual elution mode, peak capacity can be expressed as
\[ \phi_i = 1 + \frac{\sqrt{N}}{m} \ln (1 + k'_z) \] (A.2)

where \( N \) is the number of theoretical plates, \( k'_z \) is the capacity factor of the last eluting peak, and \( m \) represents a factor pertaining to the peak width for minimum separation (\( m \)\( \sigma \)), where \( m \) is usually equal to four. Since each sequential elution mode results in the elution and separation of only a subset (class) of the analytes, peak capacities are not multiplicative as in true two-dimensional separations, but instead are (assumed to be) additive in nature:

\[ \phi_{\text{total}} = \phi_1 + \phi_2 + \ldots + \phi_n \] (A.3)

In order for eq A.3 to be applicable in all cases, it is imperative that each gradient within the sequential framework be highly selective for a particular class of compounds. Also, those components that are not being selectively eluted during a given gradient should be highly, if not infinitely retained at the head of the column (see eq A.15 and related text). With this assumption, combining eqs A.2 and A.3 along with the identity \( 1 + k'_z = \frac{V^*_r}{V^*_o} \), where \( V^*_r \) is the retention volume of the last eluting peak and \( V^*_o \) is the column void volume, gives an equation to describe the total peak capacity for the sequential separation, where \( n \) is the number of sequential elution modes.

\[ \phi_{\text{total}} = n + \frac{1}{m} \left\{ \sqrt{N_1} \ln \left( \frac{V^*_1}{V^*_{o,1}} \right) + \sqrt{N_2} \ln \left( \frac{V^*_2}{V^*_{o,2}} \right) + \ldots + \sqrt{N_n} \ln \left( \frac{V^*_n}{V^*_{o,n}} \right) \right\} \] (A.4)
Equation A.4 is a very general expression for the total peak capacity, allowing for differences, however minor, in void volume, efficiency (plate count), and retention volume of the last peak among the various elution modes employed sequentially on a given column. Minor differences in \( V_0 \) might be expected due to differences in the stationary phase wetting capabilities of the mobile phases used for the specific elution modes; if necessary, these differences could be virtually eliminated by adding to the mobile phase of each elution mode a small amount of solvent that preferentially solvates the stationary phase relative to the other mobile phase components (e.g., 3-6% \( n \)-propanol for typical reversed phase situations). Somewhat greater differences in \( N \) might be expected since it is affected by both stationary phase mass transfer effects due to the wetting differences discussed above as well as differences in mobile phase mass transfer also arising from compositional differences. In practice, we believe that the potentially less efficient elution mode(s) of a given column can be made nearly equal in efficiency to the more (most) efficient elution mode via judicious control over various mobile phase properties, as we have demonstrated for predominantly aqueous buffers in comparison with conventional hydroorganic mobile phases in RPLC (A.19).

In its present form, the peak capacity expression (eq A.4) is somewhat difficult to interpret. Recognizing or assuming that differences in \( V_0 \) and \( N \) are negligible, eq A.4 can be simplified to

\[
\Phi_{\text{total}} = n + \frac{\sqrt{N}}{m \ln V_0} \left( \ln \left( V_{r_1}^* \right) + \ln \left( V_{r_2}^* \right) + \ldots + \ln \left( V_{r_n}^* \right) \right)
\]

\[
= n + \frac{\sqrt{N}}{m \ln V_0} \sum_{i=1}^{n} \ln V_{r_i}^*
\]

(A.4a)
In addition, if the retention volume of the last peak is the same among the various elution modes, i.e., \( V_{r1}^* = V_{r2}^* = \ldots = V_{rn}^* \), then \( \sum \ln V_{rj}^* = n \ln V_r^* \), and eq A.4a can be further simplified to

\[
\Phi_{\text{total}} = n + \frac{n \sqrt{N}}{m} \ln \left( \frac{V_r^*}{V_0} \right) \tag{A.5}
\]

At this point, the number of sequential elution modes, \( n \), can be factored from each term and the retention volume ratio can be redefined in terms of \( k' \) to yield

\[
\Phi_{\text{total}} = n \left( 1 + \frac{\sqrt{N}}{m} \ln \left( 1 + k'_z \right) \right) \tag{A.6}
\]

Eq A.6 is directly related to eq A.2 and thus shows that the total peak capacity in SME-LC is given approximately by

\[
\Phi_{\text{total}} = n \Phi_i \tag{A.7}
\]

where \( \Phi_i \) is the peak capacity of an individual elution mode (eq A.2) and \( n \) is the number of elution modes sequentially applied.

Although the peak capacity described by eq A.7 is much less than what would be expected for multidimensional separations in which the separation modes are orthogonal, it is still a great improvement over one-dimensional separations (eq A.2). In practice, the observed peak capacity may differ from that predicted by eq A.7. In cases where solute band broadening may be less than expected (\( \sigma_{\text{obs}} < \sigma_{\text{pred}} \)) due to the focusing effects of a gradient, the observed peak capacity may be higher and vice-versa. As a general rule, however, the peak capacity of the elution modes should not
differ significantly from each other unless one or more of the mobile phases has a detrimental effect on column efficiency.

A.2.4 Information Content

As we will presently show, SME-LC provides more information than conventional unimodal elution chromatography. To describe this mathematically, the inverse relationship between informing power and entropy can be exploited. In other words, to show that SME-LC provides an increase in information content, we must show that SME-LC results in a lower entropy for a given separation.

The entropy of a system can be simply described by

\[ S = k \ln W \]  \hspace{1cm} (A.8)

where \( k \) is Boltzmann's constant and \( W \) is interpreted as a probability (A.20). This equation can be applied to chromatographic separations by letting \( W \) represent the number of possible elution orders for a given chromatographic technique.

Figure A.2A illustrates the possible elution orders for a sample containing eight components. For the unimodal elution case, the number of possible elution orders is \( W = 8! \) or 40,320. For the sequential bimodal elution case, if there are two different chemical classes containing 4 components each, as shown in Figure A.2B, then the number of elution orders for SME-LC will be the product of the number of elution orders within each separative mode, \( W = (4!)(4!) \) or 576. This can be generalized as

\[ W = \binom{n_1}{r} \binom{n_2}{r} \cdots \binom{n_r}{r} = \prod_{i=1}^{r} \binom{n_i}{r} \]  \hspace{1cm} (A.9)
Figure A.2  Examples of possible elution orders for a one-dimensional separation (A) and a sequential multimodal elution separation (B). Dotted line as in Figure A.1.
where \( n_j \) is the number of components eluted during the \( i \)th elution mode, \( r \) is the total number of elution modes, and the \( \frac{n_j}{r} \) ratios are understood to be rounded to the nearest whole number. Assuming that the number of components eluted by each mode is the same (i.e., \( n_1 = n_2 = \ldots = n_r \)), eq A.9 simplifies to

\[
W = \left( \frac{n_i}{r} \right)^r
\]  

(A.9a)

From eqs A.8 and A.9a it is evident that the entropy of the separation is much lower for SME-LC than for a one-dimensional separation. Thus, more information is obtained from SME-LC because the elution orders are more predictable.

The difference in entropy between SME-LC and one-dimensional chromatographic techniques can be described by

\[
\Delta S = S_{SME} - S_{1-D} = k \ln \frac{W_{SME}}{W_{1-D}}
\]  

(A.10)

where \( S_{SME} \) and \( S_{1-D} \) are the individual entropies and \( W_{SME} \) and \( W_{1-D} \) are the number of possible elution orders for a SME-LC and a one-dimensional separation, respectively. Substitution of eq A.9a into eq A.10 yields after rearrangement gives

\[
\frac{\Delta S}{k} = \ln \left( \frac{n!}{r!} \right)^r - \ln n! = r \ln \left( \frac{n!}{r!} \right) - \ln n!
\]  

(A.11)

Using Stirling's approximation (\( \ln x! \approx x \ln x - x \)), eq A.11 can be transformed to

\[
\frac{\Delta S}{k} = r \ln \frac{n}{r} - \ln \left( \frac{n!}{r!} \right) - (n \ln n - n) = -n \ln r
\]  

(A.12)
A plot of $\Delta S/k$ versus $n$ (calculated via eq A.11) is shown in Fig. A.3 for several multimodal possibilities. Note that the relative disorder of separation (relative number of possible elution orders) decreases as the number of elution modes, $r$, increases. This is predicted by eq A.11 but is more obvious in eq A.12. Since informing power and entropy are inversely proportional, this is equivalent to stating that, for a given number of sample components, as the number of separative modes increases, the information content increases. This result is logical since fewer components will be eluted per separation mode and, from a statistical viewpoint, the overall elution order will be more predictable. Figure A.3 and eq A.12 also show that the reduction in disorder achieved by using SME-LC increases as the number of sample components increases, a result also consistent with intuitive notions.

In cases where an unequal number of components elute in different modes, the entropy increases steadily with increasing disparity in the relative proportion of components as indicated in Figure A.3 for the bimodal sequential elution mode ($r=2$: 1:1, 1:2, and 1:3). If all of the sample components should elute within a single mode during sequential multimodal elution, then SME-RPLC would be only slightly advantageous in terms of information content over conventional RPLC ($r = 1$). One can then assume that either (i) only one class of compounds is present; or (ii) the mobile phase being used in the given elution mode is inappropriate for the sample. If assumption (i) is true, then the results provided by SME-LC enables one to eliminate the possibility of one or more specific classes of compounds being present in the sample. For example, if no components elute during a well-designed pH gradient, then none of the components are weak acids or bases over the range of the pH gradient. On the other hand, SME-LC would be somewhat disadvantageous in terms of analysis time if the sample components all eluted within a single separation mode since all of the SME-LC modes except one would be wasted. As for assumption (ii),
Figure A.3  Comparison of the disorder of separation (randomness of elution order) for a one-dimensional elution technique ($r = 1$) and sequential multimodal chromatography ($r = 2-4$). $n$ is the number of sample components, and $r$ is the number of sequential elution modes. Numbers in parentheses for $r = 2$ indicate relative proportion of components that elute in the first and second separation modes. See eqs A.9-A.12 and related text.
the potential selection of an inappropriate elution mode is usually of little concern since typically one already suspects the presence of different chemical classes within the sample and will accordingly choose reasonable mobile phase conditions for the selective elution mode(s).

A.2.5 Selectivity

As we will show, SME-LC can provide significantly better selectivity than conventional HPLC. In simple isocratic elution, selectivity (relative retention) is defined as the ratio of the retention factors or adjusted retention times of a pair of solutes:

\[
\alpha = \frac{k_2'}{k_1'} = \frac{t_{R_2}'}{t_{R_1}'} = \frac{t_{R_2} - t_o}{t_{R_1} - t_o} \tag{A.13}
\]

where \( t_o \) is the retention time of an unretained peak. With gradient elution, only the adjusted retention time definition is practical since the retention factors (k's) are usually changing.

To compare SME-LC with conventional (unimodal) separations, it is useful to differentiate between two types of selectivity: within-class selectivity, \( \alpha_{wc} \), and between-class selectivity, \( \alpha_{bc} \). For \( \alpha_{wc} \), both \( t_{R_1}' \) and \( t_{R_2}' \) in eq A.13 refer to compounds of the same class, whereas for \( \alpha_{bc} \), \( t_{R_1}' \) and \( t_{R_2}' \) in eq A.13 correspond to compounds from different classes. Unfortunately, the present definition of the latter (\( \alpha_{bc} \)) would, after exhaustive application, result in far too many combinations to be useful. An appropriate and useful simplification is to use average values for either \( t_{R_1}' \), \( t_{R_2}' \), or both in eq A.13. When both averages are used the result is

\[
\bar{\alpha}_{bc} = \frac{t_{R_2}'(avg)}{t_{R_1}'(avg)}, \tag{A.14}
\]
where $t_{R1(\text{avg})}$ and $t_{R2(\text{avg})}$ refer to the average retention time of the first and second class of compounds, respectively.

Although we will postpone our detailed discussion of within-class and between-class selectivity until later (for specific separations), it is easy to appreciate qualitatively the dramatic increase in the average between-class selectivity, $\alpha_{bc}$, provided by sequential multimodal elution (SME). By simple inspection of Fig. A.1, $\alpha_{bc} \approx 3$ for SME compared to $\alpha_{bc} = 1$ for a conventional unimodal separation. It should be noted that, in theory, if there is sufficient control of solute retention and band-broadening in the later elution mode, $\alpha_{bc}$ could be made extremely large by purposely delaying the onset of the later elution mode. In general, this would be undesirable since it would result in a very large gap (delay) between elution modes and hence an excessive analysis time.

Before concluding our this discussion on selectivity, we offer two precautions: First, in comparing the selectivity provided during gradient runs among various elution strategies, it is best if the initial mobile phase strength and gradient steepness for the modes to be compared are similar (A.21). Otherwise, the retention times of one or both runs may need to be adjusted to reflect these differences. Second, when evaluating the within-class selectivity in SME-LC, one should use the retention time of the analytes relative to the start of the separation mode in which they eluted. That is, the time prior to the beginning of the pertinent elution mode should be subtracted from the analytes’ retention time since the duration of the prior elution mode(s) are ideally independent of the pertinent separation mode.

In summary, SME-LC is more informative than conventional HPLC (a one-dimensional, unimodal technique) due to the greater predictability of elution order and the ability to eliminate specific chemical classes from the list of possible classes within
the sample. As shown by eq A.7, it can provide a peak capacity significantly higher than conventional HPLC. It also provides a dramatic between-class selectivity and, as we will later show, may also provide improved within-class selectivity in the selective elution modes over that furnished by conventional HPLC.

A.2.6 Retention Requirements

As mentioned earlier, it is important that compounds to be separated in later elution modes be highly retained during earlier separation mode(s). For the bimodal case, it is possible to derive an expression for the minimum retention factor (during the first elution mode) of an analyte to be eluted in the second mode, $k'_{\text{min, mode 2}}$, using only simple, well-known identities such as $d = v \cdot t$ (distance = velocity x time) and $u_s = u_{\text{mp}}/(1 + k')$, where $u_s$ and $u_{\text{mp}}$ are the linear velocity of the solute and mobile phase, respectively. The result is

$$k'_{\text{min, mode 2}} = \left(\frac{1}{f_1} - 1\right)\frac{V_1}{V_0}.$$  \hspace{1cm} (A.15)

where $f_1$ is the distance migrated down the column by the solute relative to column length ($x/L$) during the first elution mode, $V_1$ is the total volume of the first elution mode ($= V^*_{\text{f1}}$ of eq A.4), and $V_0$ is the column void volume. Assuming the first elution mode is complete after 15 column volumes ($V_1 / V_0 = 15$) and that analytes to be eluted during the second mode are to migrate less than 20% during the first mode ($f_1 = 0.2$), eq A.16 indicates that the minimum retention factor for these analytes in the first mode is 60. Although the constraint of 20% migration might be viewed as too liberal, our experience has shown that up to 30% migration can be tolerated without significant adverse effects on the second elution mode. Even with a more conservative approach, however, in which $V_1 / V_0 = 30$ and only 5% migration is allowed ($f_m = \ldots$)
0.05), the minimum retention factor is still only 570. Although 570 may seem to be a prohibitively high degree of retention, this condition can easily be met in RPLC by using only a small amount of organic solvent (e.g., < 10%) in the first elution mode. In fact, even larger retention factors are routinely observed in RPLC under these conditions.

Eq A.15 can also be used to estimate the necessary retention for late-eluting solutes in the trimodal and tetramodal cases. For solutes that elute during the second mode, eq A.15 can be used directly without any assumptions. For solutes that elute during later modes, \( V_1 \) in eq A.15 should be replaced by the total volume of the early elution modes. It is also necessary to assume that solute retention is exactly the same during all of the early elution modes; some changes in retention could conceivably occur due to secondary effects such as changes in ionic strength, etc.

A.3 EXPERIMENTAL

A.3.1 LC System

A ternary liquid chromatograph (Rainin Instruments, Woburn, MA) was used, along with a Model 7125 injection valve (Rheodyne, Cotati, CA) with a 20 \( \mu \)L loop, a Model V4 UV-visible absorbance detector (Isco, Lincoln, Nebraska), and a Model LC-4B thin-layer electrochemical detector (Bioanalytical Systems, Lafayette, IN). Silica-based reversed phase columns (C1, C8, and C18; 150 x 4.6 mm; \( d_p = 5 \mu m \); Rainin Instruments, Woburn, MA) were used for analyses of samples containing benzoic acid derivatives or peptides. A 150 x 4.1 mm PRP-1 column (Hamilton Co., Reno, NV) was used for samples containing phenols and polycyclic aromatic hydrocarbons (PAHs). All columns were washed periodically with 100% organic solvent to remove any buildup of highly retained compounds that might have been present in the samples. The columns were thermostated at 25.0 ± 0.1°C using a glass
water jacket and a Model RMS-6 circulating bath (Brinkmann Instruments, Westbury, NY). All mobile phase solutions were filtered through 0.45 μm Nylon-66 filters (Rainin Instruments, Woburn, MA) before use. Subsequent degassing of the mobile phase was accomplished by placing the solutions in a heated ultrasonic bath and then applying a partial vacuum for a few minutes.

A.3.2 Reagents/Standards

Mobile phase components methanol, acetonitrile, and water were HPLC grade, as was the sodium carbonate, sodium acetate, and sodium perchlorate. Sodium bicarbonate, formic acid, sodium formate, and acetic acid were ACS reagent grade. All chromatographic solutes (peptides, benzoic acids, neutral compounds, etc.) were reagent grade or better and used without further purification. They were dissolved either in the aqueous buffer or in a minimal amount of methanol or acetonitrile and then diluted with the aqueous buffer. The creosote sludge sample was acquired from LSU's Institute of Environmental Studies. The presence of phenols and polyaromatic hydrocarbons (PAHs) in the creosote sludge was confirmed by GC/MS prior to the SME-LC analysis.

A.3.3 Mobile Phase Preparation

A.3.3.1 Peptide/Neutral Samples

The mobile phase system consisted of 100 mM formic acid/sodium formate buffer and methanol. Sodium perchlorate was added to the acidic component of the buffer in order to ensure a constant ionic strength (100 mM) throughout the pH gradient. Detection of peptides and neutral compounds was accomplished at 254 nm. The flow rate was 1.0 mL/min. Measurement of pH gradients was accomplished either manually (fraction collection, Vfrac = 100 μL) or with a Model FTPH-2 Micro
Flow Through pH system (Lazar Research, Los Angeles, CA). In both cases, a Model 920 research grade pH meter (Orion, Cambridge, MA) was calibrated with pH 4 and 7 buffers so that the pH could be accurately determined at any given time during the gradient.

A.3.3.2 Benzoic Acid/Neutral Samples

The mobile phase system consisted of 43.5 mM acetic acid/sodium acetate buffer with either methanol or acetonitrile as the organic solvent. The pH was measured as described for the peptide samples. Three different hydrocarbonaceous silica columns were used for this sample mixture: C$_1$, C$_8$, and C$_{18}$. Methanol was used as the organic solvent for separations employing the C$_1$ and C$_8$ columns, whereas acetonitrile was used with the C$_{18}$ column to avoid exceeding an arbitrary pressure limit for the column. The ionic strength of the mobile phase was not adjusted for these separations. Benzoic acid derivatives were detected at 280 nm followed by detection of the neutral compounds at 260 nm at 0.1 AUFS. The flow rate was 1.5 mL/min.

A.3.3.3 Creosote Sample

The selective mobile phase consisted of an aqueous sodium carbonate/sodium bicarbonate buffer and 30% acetonitrile. The sodium bicarbonate solution also contained 50 mM sodium perchlorate so that the ionic strength would remain constant as the pH was varied (ca. 35 mM after the buffer was mixed with acetonitrile). Continuous degassing (e.g., by sparging with helium) was avoided in order to minimize loss of the carbonate buffer. The pH of the aqueous buffer was measured after calibrating the pH meter with pH 7 and 10 standard buffers. Separation of phenolic substituents was performed at a constant pH of 9.11 to alleviate baseline drift.
during electrochemical detection. A solvent gradient from 30% to 100% acetonitrile was then used to elute and separate the polycyclic aromatic hydrocarbons.

A.3.4 Generation of Linear pH Gradient

The conjugate acid and base of a particular buffer system were segregated so that the pH of the mobile phase could be changed by varying the proportions of the two solutions. Due to the logarithmic relationship between acid/base ratios and pH, linear pH gradient programming was accomplished by the use of "sigmoidal" composition gradients as illustrated in Figure A.4. The required changes in the relative proportions of the buffer were deduced from pH calculations facilitated by an in-house MS-BASIC computer program. Calculations were performed in 1% increments of conjugate base, taking into account ionic strength and the constant percentage of organic solvent present in the mobile phase (during the pH gradient of a given run).

A.3.5 Bimodal Gradient Programming

Bimodal gradients were performed by linking selective and universal gradients. In all cases, a step gradient from the final conditions of the (selective) pH gradient to the initial conditions of the (universal) solvent gradient was necessary. Slight baseline disturbances were typically observed as a result of the dramatic change in solvent composition between gradients, but these disturbances did not interfere with any separation. For the peptide separations, chromatograms were cosmetically altered by subtracting a blank SME run from the actual SME separation.
Figure A.4  Examples of mobile-phase composition gradients necessary for linear pH gradients. The ordinate represents the percentage of conjugate base. Gradient identification: curve A, $\Delta p\text{H/min} = 1$; curve B, $\Delta p\text{H/min} = 0.5$; curve C, $\Delta p\text{H/min} = 0.25$. Curves represent a pH gradient from 3.50 to 6.00 and were calculated for a 96 mM formic acid/sodium formate buffer with a constant ionic strength and 5% organic solvent.
A.4 RESULTS AND DISCUSSION

A.4.1 Chromatographic Expectations and Experimental Verification

Hypothetical SME-LC and one-dimensional separations of a complex sample are shown in Figure A.1. A one-dimensional separation (Fig. A.1B) is typically unable to provide separations by class, and due to insufficient peak capacity, might not be able to resolve all components of a complex sample. SME-LC (Fig. A.1A), with its increased peak capacity and selectivity, can separate and resolve two (or more) particular classes of compounds via selective and nonselective mobile phases. The region between chemical classes in Fig. A.1A can be minimized in most cases by starting the second elution mode prior to the completion of the first mode, but this may impair the resolution of late eluting components of the first separation mode. In general, this spacing should be considered an advantage rather than a disadvantage since a clearer definition of different chemical classes is facilitated with such gaps.

An experimental demonstration of SME-LC is illustrated in Figure A.5 using a reversed-phase C\textsubscript{1} column. The “complex sample” in this case consists of benzoic acid derivatives and neutral aromatics. The benzoic acids were eluted by a pH gradient, whereas the neutral compounds loaded onto the top of the column and were not eluted until a methanol gradient was applied. The separation of different chemical classes (acids and neutrals) is clearly demonstrated. The time required for analysis could easily be shortened by starting the solvent gradient earlier or by increasing its initial strength. The sharpness of the neutral bands indicates that, at least for this bimodal pH/solvent elution scheme, excessive broadening of the later-eluting neutral compounds during the first elution mode (pH gradient) does not occur.

Finally, to illustrate that SME-LC is possible with a broad range of reversed-phase columns and not just a C\textsubscript{1} column, the sample of Figure A.5 was separated on
Figure A.5 Sequential multimodal reversed-phase separation of a sample of acids and neutrals on a C$_1$ column with UV detection at 280 nm (acids) and 260 nm (neutrals). Analyte identification: 2-CBA, 2-chlorobenzoic acid; 3-NBA, 3-nitrobenzoic acid; BA, benzoic acid; 3-CBA, 3-chlorobenzoic acid; 3-BBA, 3-bromobenzoic acid; NB, nitrobenzene; ACP, acetophenone; BB, bromobenzene; 4-dCB, 1,4-dichlorobenzene; 2,2'-dBBP, 2,2'-dibromobiphenyl. Mobile-phase components: reservoir A = 43.5 mM acetic acid, B = 43.5 mM sodium acetate, and C = methanol. The pH gradient, initiated at the time of sample injection, was linear from pH 3.55 to 5.5 at 0.8 pH units/min and held constant at the final pH until all benzoic acid derivatives had eluted. The methanol gradient, shown in the figure, was then applied.
C<sub>8</sub> and C<sub>18</sub> columns in Figure A.6 with only slight modifications to the bimodal elution conditions.

One of the primary concerns in using SME-LC is solvent and reagent purity. The extraneous peaks present in the pH and solvent gradients of Figure A.5 (and A.6) are due, respectively, to relatively polar and nonpolar impurities in the water or buffer reagents. The level of nonpolar impurities present in the water is probably somewhat exaggerated since they accumulate continuously on the column during the first separation mode. Nevertheless, if one takes precautions to use high purity mobile phases, such impurity peaks can be minimized. Note also that the multimodal separation terminates naturally with a 100% methanol wash, thus eluting any highly retained compounds from the column at that time and avoiding their elution in subsequent runs.

To examine the necessity of using a sequential bimodal approach, the separation of Fig. A.5 was attempted with a simultaneous bimodal gradient and also a unimodal solvent gradient for comparison. As shown in Figure A.7, neither the use of (A) a simultaneous bimodal gradient (pH and organic solvent) nor the use of (B) a unimodal organic solvent gradient results in satisfactory resolution. This was anticipated since the peak capacity is less for these separation modes than for the SME-LC separations of Figures A.5 and A.6. SME-LC clearly represents an effective approach for increasing the peak capacity of a given column.

In addition to the obvious increase in peak capacity for the SME-LC separation of Figs. A.5 and A.6, an increase in both within-class and between-class selectivity (eqs A.13 and A.14 and related text) was also observed. In the present example, the weak acids comprise the first class of compounds and the neutral solutes comprise the second class. Table A.1 shows the between-class selectivity, α<sub>bc</sub>, for each of the acidic and neutral solutes achieved by the unimodal (Figure A.7B) and
Sequential multimodal elution for RPLC separations using a C$_8$ (A) and a C$_{18}$ (B) column. Detection and sample components as in Figure A.5, except that nitrobenzene was omitted.
Figure A.7  Alternative reversed-phase separations (cf. Figure A.5) using (A) simultaneous bimodal elution (pH/methanol gradients) and (B) unimodal elution (methanol gradient at 5%/min). Detection at 260 nm. Sample components as in Figure A.6. pH and methanol gradients of A as in Figure A.5, except that both were started at the time of injection.
Table A.1  Comparison of between-class selectivity, $\alpha_{bc}$, for unimodal (conventional) elution and sequential multimodal elution in RPLC. a

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>$\alpha_{bc}$ (unimodal)</th>
<th>$\alpha_{bc}$ (bimodal)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEAK ACIDS</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2-chlorobenzoic acid</td>
<td>1.95</td>
<td>10.16</td>
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<td>3-nitrobenzoic acid</td>
<td>1.74</td>
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<td>1.74</td>
<td>3.84</td>
<td>120</td>
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<td>3-chlorobenzoic acid</td>
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</tr>
<tr>
<td>3-bromobenzoic acid</td>
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<tr>
<td>NEUTRALS</td>
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<td></td>
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<tr>
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<td>58</td>
</tr>
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<td>bromobenzene</td>
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<td>78</td>
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<tr>
<td>4-dichlorobenzene</td>
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<td>64</td>
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<td>2,2'-dibromobiphenyl</td>
<td>2.43</td>
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</tr>
<tr>
<td>AVERAGE b</td>
<td>1.85</td>
<td>4.26</td>
<td>131</td>
</tr>
</tbody>
</table>

a Selectivity reported as relative retention, defined as $t_{R_{ACP}}/t_{R_{acid}}$ for acidic solutes and as $t_{R_{neutral}}/t_{R_{3CBA}}$ for neutral solutes, where ACP = acetophenone and 3CBA = 3-chlorobenzoic acid. Data correspond to those of Figs. A.5 (bimodal) and A.7B (unimodal). Since nitrobenzene is not present in the sample of Fig. A.7, it is necessarily excluded from these calculations.

b Calculated according to eq A.14.
bimodal (Figure A.5) separations, as well as the average value, $\bar{\alpha}_{bc}$, defined according to eq A.14. For both the individual solutes and the average value, $\alpha_{bc}$ is dramatically greater for the bimodal separation. This increase in $\alpha_{bc}$ is directly attributable to the class separation achieved by stepwise modification of mobile phase selectivity using SME-LC.

In light of the differences in the between-class selectivity provided by conventional (unimodal) RPLC and SME-LC, it is appropriate to compare the within-class selectivity, $\alpha_{wc}$, for these approaches. As shown in Table A.2, $\alpha_{wc,\text{bimodal}}$ is significantly larger than $\alpha_{wc,\text{unimodal}}$ for the acidic compounds. Perhaps the most dramatic change occurs with 3-nitrobenzoic acid and benzoic acid. Whereas they coelute in ca. 6 min in conventional unimodal RPLC (Figure A.7B), they are baseline resolved in less than 4 min by SME-LC (Figure A.5). The differences in $\alpha_{wc}$ were anticipated since the retention mechanism for the ionizable solutes was changed from one that depended primarily on distribution coefficients to one in which the ionization constants played a greater role (RPLC vs SCE-LC, ref A.16). Moreover, these differences in $\alpha_{wc}$ are consistent with the results of Buck and Tomellini (A.22), who performed a systematic study of the unique selectivity provided by pH gradients in RPLC.

In contrast to the different retention mechanisms experienced by the acids in the unimodal and bimodal elution, the neutral compounds experience essentially the same mechanism in both cases (a methanol gradient), since they are relatively unaffected by the pH gradient of the bimodal elution scheme. It is not surprising, therefore, that the within-class selectivity of the neutral compounds is essentially the same; the minor differences apparent in Table A.2 are due to the somewhat different gradient conditions employed and, possibly for acetophenone, non-negligible migration during the pH gradient.
Table A.2 Comparison of Within-Class Selectivity, $\alpha_{wc}$, for Unimodal (Conventional) Elution and Sequential Multimodal Elution in RPLC.$^a$

<table>
<thead>
<tr>
<th></th>
<th>$\alpha_{wc}$ (unimodal)</th>
<th>$\alpha_{wc}$ (bimodal)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WEAK ACIDS$^b$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-chlorobenzoic acid</td>
<td>1.00</td>
<td>1.00</td>
<td>NA</td>
</tr>
<tr>
<td>3-nitrobenzoic acid</td>
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</tr>
<tr>
<td>benzoic acid</td>
<td>1.12</td>
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<td>136</td>
</tr>
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<td>3-chlorobenzoic acid</td>
<td>1.24</td>
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<td>3-bromobenzoic acid</td>
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<td>164</td>
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<tr>
<td><strong>NEUTRALS$^c$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetophenone</td>
<td>1.55</td>
<td>2.10</td>
<td>36</td>
</tr>
<tr>
<td>bromobenzene</td>
<td>1.49</td>
<td>1.34</td>
<td>-10</td>
</tr>
<tr>
<td>4-dichlorobenzene</td>
<td>1.32</td>
<td>1.21</td>
<td>-8</td>
</tr>
<tr>
<td>2,2'-dibromobiphenyl</td>
<td>1.00</td>
<td>1.00</td>
<td>NA</td>
</tr>
</tbody>
</table>

$^a$ Data correspond to those of Figs. A.5 (bimodal) and A.7B (unimodal).

$^b$ Selectivity reported as relative retention ($t_{R_{acid}}/t_{R_{ref}}$), with 2-chlorobenzoic acid as the reference compound.

$^c$ Selectivity reported as relative retention ($t_{R_{ref}}/t_{R_{neutral}}$), with 2,2'-dibromobiphenyl as the reference compound. Since nitrobenzene is not present in the sample of Fig. A.7, it is necessarily excluded from these calculations.
In summary, the anticipated increases in both peak capacity and selectivity provided by SME-LC appear to have been realized. Although our results are limited to the samples of the present study, we believe that SME-LC could provide improved results for a variety of samples.

**A.4.2 Feasibility of Biological Sample Analyses**

Figure A.8 illustrates the capability of SME-LC to separate and resolve two- and three-residue peptides from neutral aromatics (or other potential neutral interferents). Although these peptides contain both carboxyl- and amino-terminal groups, separation is based primarily upon the deprotonation of the carboxyl-terminal groups since, under the present conditions, all of the amino-terminal groups remain fully protonated over the pH gradient employed. The effect of this constant amino-terminal group protonation at low pH is simply a more rapid elution of the peptides than one would find for analogous compounds without the amino group. The hydrophobicity of the side chains, however, is more than adequate for reasonable retention of the peptides. Somewhat longer peptides and even small proteins could possibly be separated by similar SME-LC elution schemes; in these instances, the reduced risk of denaturation due to the aqueous nature of the selective mobile phase(s) could be an important advantage.

**A.4.3 Control of Retention**

One prerequisite for the separation of different classes of compounds via SME-LC is sufficient control of retention of solutes during their elution in the desired elution mode and an almost infinite retention (eq A.15) of later-eluting compounds during earlier elution modes. Figure A.9 illustrates the control of retention possible for the neutral compounds of the peptide sample of Figure A.8. The second elution mode
Figure A.8  Sequential multimodal elution RPLC separation of a complex sample containing di- and tripeptides and neutral compounds. Detection conditions: \( \lambda = 254 \text{ nm} \), sensitivity = 0.2 AUFS. Mobile-phase program: reservoir A = 100 mM formic acid + NaClO₄, B = 100 mM sodium formate, and C = methanol. Solutions A and B contained 6% 1-propanol to improve column efficiency. pH gradient profile: initial pH held at 3.20 for 0.5 min, followed by linear pH gradient of 1.1 pH units/min to pH 4.30 and held for 1.5 min. MeOH gradient profile: a step gradient from 0 to 65% MeOH was performed after 3 min and then maintained at 65% for 1 min, followed by a linear gradient at 10%/min to 95% MeOH and then held at 95% for 5 additional min.
Figure A.9  Example of control of solute retention in sequential multimodal elution RPLC. The elution of a second class of compounds during a second separation mode can be delayed (or accelerated) simply by delaying (or accelerating) the start of the second elution mode. Delay of second elution mode (methanol gradient): (A) no delay; (B) 1 min; and (C) 2 min. Sample and other conditions as in Figure A.8.
(solvent gradient) was started at the usual time in the top chromatogram but was delayed by one and two minutes in the middle and bottom chromatograms, respectively. As shown in Figures A.9B and A.9C, the retention times of the neutral compounds were increased almost exactly by these delays. Except for this predictable increase in the retention times, the separation of the neutral compounds was otherwise relatively unaffected, i.e., efficiency, selectivity, and resolution were preserved. This indicates that no significant migration or band spreading of the neutrals occurs until after the universal elution mode (solvent gradient) is applied. Because a small amount of very strong reversed phase solvent (6% 1-propanol) was used in the selective elution mode to promote good column efficiency, it was originally anticipated that some migration and band-spreading of neutrals might occur. Our results show that these effects are minimal, however, at least on the time scale of the present separations.

One other point needs noting. The final two peaks (peptides) in the first half of Fig. A.9A have smaller bandwidths and are less retained than the same peaks of Figs. A.9B and A.9C. This apparent discrepancy is explained as follows: In Fig. A.9A the organic solvent gradient elution mode was purposely begun just before the pH gradient was finished and the last two peptides had been eluted, whereas in Fig. A.9B and A.9C the solvent gradient was not started until well after the pH gradient/peptide elution was completed. Thus for a small fraction of the time that the two peptides were on-column in Fig. A.9A, and they felt the additional influence of the early portion of the organic solvent gradient which caused them to elute more rapidly but with decreased selectivity. The overlap of the pH and organic solvent gradients in Fig. A.9A was done to minimize analysis time and is of little concern in the present example, but it does bring to attention a precaution that should generally be
observed, i.e., it is best to avoid overlap of the elution modes in SME-LC; such overlap may decrease both within-class and between-class selectivity.

A.4.4 Enhancement of Detection

Another advantage of SME-LC is the enhanced detection of different classes of compounds. In the case of the separation shown in Figure A.5, a wavelength of 280 nm was used to detect the benzoic acids. Following their elution, the wavelength was adjusted to 260 nm for better detection of the neutral aromatics. Conventional one-dimensional RPLC does not allow such an enhancement since there is no guarantee that specific chemical classes will elute in a specific region of the chromatogram. The separation-by-class feature of SME-LC can also be an advantage when more than one detector is necessary. An excellent example is shown in Figure A.10 for the separation of phenols from polycyclic aromatic hydrocarbons (PAHs) in creosote sludge. Detection of the low levels of phenols (ppm to ppb) was performed using electrochemical detection while UV detection was used for the PAHs. The presence of phenols and PAHs in the sludge was verified prior to HPLC analysis by GC/MS; their elution during the pH and solvent modes, respectively, was confirmed by comparison of the retention times of the unknown peaks of Fig. A.10 with those of representative standards of each class of compounds (e.g., phenol, p-cresol, p-xylenol; benzene; naphthalene). The sequential detection of the phenols and PAHs made possible by SME-LC is quite advantageous; a simultaneous detection scheme for these compounds in one-dimensional RPLC would be difficult if not impossible to devise since: (i) the gradient necessary to elute all components in a reasonable period of time would cause extreme baseline drift in the electrochemical detection; and (ii) the absorbance of the phenols could potentially interfere with the detection of the PAHs and vice-versa.
Figure A.10  Separation of phenolic priority pollutants and polyaromatic hydrocarbons (PAHs) in creosote sludge using sequential multimodal elution RPLC. Mobile-phase program: reservoir A = 50 mM sodium carbonate, B = 50 mM sodium bicarbonate + NaClO₄, and C = acetonitrile. The pH was held constant at a pH_{opt} = 9.11 with 30% acetonitrile to accommodate electrochemical detection of phenols (alleviate baseline drift). The ACN gradient profile, initiated at 13 min, was 30-95% ACN at a rate of 5%/min with an isocratic hold at 95% ACN for 2 min.
A.4.5 Repeatability

Tables A.3 and A.4 show the repeatability of retention times and peak areas, respectively, for various benzoic acids and neutral compounds that have been resolved by bimodal pH/organic solvent gradient elution similar to that of Figure A.5. The relative standard deviation was less than 0.3% and 2.6%, respectively, for all sample components and is certainly comparable to that which can be achieved by conventional RPLC. Both the qualitative and quantitative precision are excellent since some systematic and random error is certainly present due to the imprecision of manual injection, HPLC flow rate anomalies (A.23), and the complex gradient programming required to achieve a linear pH gradient, e.g., the use of a "sigmoidal" conjugate acid/conjugate base gradient as shown in Figure A.4 to provide a linear change in pH with time. Even better retention and area reproducibility may be possible for separations involving selective (SCE) elution modes other than pH gradients, since the logarithmic mobile phase programming described above for pH gradients may be unnecessary. Note that the amount of sample required (injected on-column) is the same for SME-LC as for conventional RPLC; all comparisons between SME-LC and RPLC in this report were made using identical sample volumes.

A.4.6 Additional Considerations

A couple of points about SME-LC need to be emphasized. First, with regard to the pH/solvent bimodal elution system, the retention mechanism of the initial elution mode is not the same as ion suppression. Elution of the ionogenic compounds is due to the gradual ionization of their functional groups and, therefore, their lower affinity for the nonpolar stationary phase. In order to have control over the elution of ionizables, initial conditions of the gradient program should take into account the ionization constants of these components. For instance, if one wishes to
Table A.3 Reproducibility of Retention Times Using Sequential Multimodal Elution in RPLC.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound Type</th>
<th>Compound</th>
<th>Trial #1</th>
<th>Trial #2</th>
<th>Trial #3</th>
<th>Mean tR</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weak Acids</strong></td>
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<tr>
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<td>BA</td>
<td>3-CBA</td>
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<tr>
<td>Trial #1</td>
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<tr>
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<tr>
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<tr>
<td>RSD</td>
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<td>0.27%</td>
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<td>0.25%</td>
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<tr>
<td><strong>Neutrals</strong></td>
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<tr>
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<td>10.968</td>
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<td></td>
</tr>
<tr>
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<td>9.847</td>
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<td>10.968</td>
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</tr>
<tr>
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<tr>
<td>RSD</td>
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<td>0.06%</td>
<td>0.05%</td>
<td>0.03%</td>
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</tbody>
</table>

\textsuperscript{a} Conditions as in Figure A.5, except that nitrobenzene was omitted from the sample.

\textsuperscript{b} Eluted during pH gradient as shown in Fig. A.5. Solute identification: 2-CBA, 2-chlorobenzoic acid; 3-NBA, 3-nitrobenzoic acid; BA, benzoic acid; 3-CBA, 3-chlorobenzoic acid; 3-BBA, 3-bromobenzoic acid;

\textsuperscript{c} Eluted during methanol gradient as shown in Fig. A.5. Solute identification: ACP, acetophenone; BB, bromobenzene; 4-DCB, 1,4-dichlorobenzene; 2,2'-DBBP, 2,2'-dibromobiphenyl.
Table A.4 Area Reproducibility Using Sequential Multimodal Elution.\textsuperscript{a}

<table>
<thead>
<tr>
<th>compound</th>
<th>2-CBA</th>
<th>3-NBA</th>
<th>BA</th>
<th>3-CBA</th>
<th>3-BBA</th>
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<td>289609</td>
<td>183707</td>
</tr>
<tr>
<td>RSD</td>
<td>1.24%</td>
<td>1.03%</td>
<td>1.66%</td>
<td>2.55%</td>
<td>1.86%</td>
</tr>
</tbody>
</table>

\textbf{WEAK ACIDS}

<table>
<thead>
<tr>
<th>compound</th>
<th>ACP</th>
<th>BB</th>
<th>4-DCB</th>
<th>2,2'-DBBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>trial #1</td>
<td>343545</td>
<td>169522</td>
<td>98445</td>
<td>156330</td>
</tr>
<tr>
<td># 2</td>
<td>341853</td>
<td>170884</td>
<td>96863</td>
<td>152864</td>
</tr>
<tr>
<td># 3</td>
<td>334647</td>
<td>167190</td>
<td>94312</td>
<td>156933</td>
</tr>
<tr>
<td>RSD</td>
<td>1.39%</td>
<td>1.10%</td>
<td>2.16%</td>
<td>1.41%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Areas are in units of $\mu$V-sec. Conditions as in Table A.3.
separate components on the basis of acidity and/or basicity, their ionization constants should fall well within the range of the pH program being performed. Those components whose \( \text{pK}_a \)'s or \( \text{pK}_b \)'s fall outside this range may be eluted too rapidly and be poorly separated, or too slowly and confused with neutral species during the universal solvent gradient.

Second, an ideal feature of the pH/solvent bimodal elution scheme would be the capability to separate by class any group of ionizable species from any group of neutral compounds, regardless of systematic disparities in other chemical and physical properties such as molecular weight. Whether or not this ideal can be approached is difficult to predict at this stage. So far, however, we have demonstrated that the pH/solvent elution scheme works well for different classes of compounds of comparable molecular weight.

A.4.7 Comparison with Stationary Phase-Generated Multimodal HPLC

Another interesting aspect of SME-LC is its relationship to separations performed with multimodal (mixed-mode) stationary phases. Many mixed-mode stationary phases have been studied recently (A.24-A.28), each having its own particular applications and advantages over other separation techniques. In the comparison that follows, we shall refer broadly to these mixed-mode separations as "stationary phase-generated multimodal LC (SPGM-LC)".

Although SME-LC and SPGM-LC are both multimodal and require mobile phases of comparable complexity, significant differences between these separation methods are apparent almost immediately upon inspection. First, whereas SME-LC is multimodal with respect to the mobile phase, SPGM-LC is multimodal with respect to the stationary phase. Moreover, SME-LC is a sequential multimodal separation scheme whereas SPGM-LC is a simultaneous multimodal separation scheme and is
the stationary phase analogue to the simultaneous multimodal mobile phase elution scheme of Fig. A.6A. This difference is important in terms of the total peak capacity, the between-class selectivity ($\alpha_{bc}$), and the randomness of the separation. Whereas SME-LC provides considerable improvement in all three parameters over conventional one-dimensional HPLC or simultaneous multimodal mobile phase schemes, SPGM-LC does not; it is the stationary phase analogue of the simultaneous multimodal elution discussed earlier (Fig. A.7A). Another difference between SME-LC and SPGM-LC is the type of column required. SME-LC merely requires a conventional RPLC column which is relatively inexpensive and can be found in almost any HPLC laboratory. SPGM-LC requires a specialized column that at present is more expensive, less versatile, and frequently less efficient. In summary, although mixed-mode stationary phase separations are certainly very useful in many situations, in many instances a properly designed SME-LC separation is a less expensive, superior alternative.

A.4.8 Final Comparisons

For samples consisting of more than one class of compounds, SME-LC is much better than conventional (unimodal) or simultaneous multimodal reversed-phase separations in terms of peak capacity, selectivity between classes, and resolution. Little or no sacrifice in analysis time, solute bandwidths, or qualitative and quantitative reproducibility is required. In some cases SME-LC is actually faster because of the compression of the elution modes made possible by the greater predictability of the separation. Limitations of SME-LC include (i) the additive instead of multiplicative nature of the peak capacities of the individual separation modes, resulting in a distinctly lower peak capacity than true two-dimensional techniques with orthogonal separation modes; (ii) the possibility that various classes of compounds may elute
during the "wrong" separation mode if the sequential mobile phase conditions are improperly selected; and (iii) the fact that SME-LC technique is not superior to (but somewhat more complex than) conventional one-dimensional RPLC if the sample contains only one class of compounds.

A.5 CONCLUSIONS

The analysis of complex samples often requires the use of multidimensional chromatographic techniques. The effectiveness of a technique, however, is not only based on the peak capacity and separating power which can be attained, but also on whether the technique is laborious and/or economically feasible. In this paper, we have presented a theory and some representative examples of a sequential multimodal elution technique that is applicable to the separation of simple to moderately complex samples. This technique has many features of multidimensional separations, and requires little or no hardware modification to conventional, one-dimensional HPLC equipment. The advantages and disadvantages of this technique over alternative separation modes such as conventional reversed phase chromatography have been noted throughout. In general, we feel that the benefits of sequential multimodal elution greatly outweigh any shortcomings.

Registry No. 2-chlorobenzoic acid, 118-91-2; 3-nitrobenzoic acid, 121-92-6; benzoic acid, 65-85-0; 3-chlorobenzoic acid, 535-80-8; 3-bromobenzoic acid, 585-76-2; nitrobenzene, 98-95-3; acetophenone, 98-86-2; bromobenzene, 108-86-1; 1,4-dichlorobenzene, 106-46-7; 2,2'-dibromo-1,1'-biphenyl, 13029-09-9; anisole, 100-66-3; toluene, 108-88-3; N-(N-L-phenylalanylglycyl) glycine, 23576-42-3; N-L-valyl-L-tyrosine, 3061-91-4; N-L-tyrosyl-L-valine, 17355-09-8; N-L-leucyl-L-tyrosine, 968-21-8; N-glycyl-L-tryptophan, 2390-74-1; N-L-valyl-L-phenylalanine, 3918-92-1.
APPENDIX REFERENCES


VITA

Edward "Skip" L. Little, Jr. was born in Monroe, Louisiana on November 19, 1962, the son of E. L. Little, Sr. and Patricia Modz. He attended Northeast Louisiana University in Monroe, Louisiana where he received Bachelor of Science and Master of Science degrees in Chemistry in May of 1985 and August of 1987, respectively. In August of 1987, he began graduate studies at Louisiana State University.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Edward Lester Little

Major Field: Chemistry

Title of Dissertation: Comparative Study of Anionic and Nonionic/Anionic Surfactant Systems in Micellar Electrokinetic Capillary Chromatography

Approved:

[Signatures]

Dean of the Graduate School

EXAMINING COMMITTEE:

Robert J. Gale

[Signatures]

Date of Examination:

March 6, 1992