New concepts that may extend the useful range of supercritical fluid chromatography

Giddings, Luther Dennis, Ph.D.

The Ohio State University, 1993
NEW CONCEPTS THAT MAY EXTEND THE USEFUL RANGE OF
SUPERCRITICAL FLUID CHROMATOGRAPHY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree
Doctor of Philosophy in the Graduate School of the Ohio State University

By

Luther Dennis Giddings

The Ohio State University

1993

Dissertation Committee:
Dr. Susan Olesik
Dr. Patrick Gallagher
Dr. Richard McCreery

Approved by

Dr. Susan V. Olesik
Department of Chemistry
to Kit
ACKNOWLEDGMENTS

There are many people who have helped me successfully complete my graduate studies. I would like to thank Dr. Susan Olesik for her guidance and patience. I am grateful for all I have learned during the last six years. I truly appreciate the help and long-suffering shown me by members of the Olesik group, past and present. Two group members merit especial recognition. I thank Tina Engel for the hours of help she patiently gave me on a variety of projects and for allowing me to benefit from her many, many years of chromatographic experience. I am also much indebted to Bill Larkins, my equally irreverent and iconoclastic co-partner in crime. We spent thousands of hours engaged in or discussing many things, even chemistry on occasions.

I wish to thank Carl Engelman and Dirk Friedrich for the tremendous service they provided me during my NMR experiments. I am also grateful to Drew Kuzmission, Erv O' Brian, and John Pudelski for teaching me how to use the NMR spectrometer without hurting it or myself. I appreciate Curt Miller's efforts in doing much of the preliminary work for the AOT studies. Likewise, I appreciate Mark Diener's synthesis of the polymer that served as the precursor in the PGC studies and timely comments from John Pudelski on the topic of PGC.

Although I place great value on my newly earned degree, I treasure far more the friendships I will take with me from Columbus. The countless friends I have made at
play and at work are to me an essential part of life and happiness. I wish I could acknowledge each individually, but only two - Chris and Mark McDermott - will be mentioned. I am especially grateful to my teammates on the Degenerate HOMOs, the W.H.L. Mules, BAMF, and the Gates of Hell for accepting and befriending me, for tolerating my limited skills and aging body, and for providing me with a new set of glory years to reflect on as middle age sets in.

My family has been of paramount importance during graduate school. The love and the support of my mother and father have been unlimited and unconditional. Without them I would have faded into academic and economic oblivion long ago. The kindness and generosity of my mother and father-in-law, Phyllis and John, have also been of inestimable value during my long educational exile from home. Most significant has been the support of my wife and children. I am grateful for the tolerance that Kate, Doug, and Dan have demonstrated during the absences, the neglect, and the general crankiness they have silently suffered for the last six years. Words cannot adequately express my love and gratitude to Kit, whose vision of me and my capabilities has always exceeded my own. Her gentle reminders that I owe life more than another desert rat are always sympathetic but timely and have softly eased me down life's path to greater responsibility and participation as a functional member of society. Her love and confidence have encouraged me to persevere through the many long dark periods when nothing was going right and when it seemed that school would never end. Thank you, thank you with all my heart.
VITA

October 16, 1956
Born Greely, Colorado

June, 1985
B. S., University of Utah, Salt Lake City, Utah, Chemistry

September, 1987 - March, 1993
Teaching Assistant and Research Assistant, Department of Chemistry, The Ohio State University, Columbus, Ohio

PUBLICATIONS


FIELDS OF STUDY

Major Field: Chemistry

Analytical chemistry including supercritical fluid chromatography and other separation techniques, under the supervision of Dr. Susan V. Olesik.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ................................................................. iii

VITA .............................................................................................. v

TABLE OF CONTENTS ................................................................. vi

LIST OF TABLES ............................................................................ ix

LIST OF FIGURES .......................................................................... xi

CHAPTER I
SUPERCRITICAL FLUID CHROMATOGRAPHY: A PRIMER ................. 1
   INTRODUCTION ........................................................................... 1
   Properties of Supercritical Fluids ............................................. 1
   Supercritical Fluid Chromatography ....................................... 4
   A Brief History of Supercritical Fluid Chromatography .......... 5

CURRENT PROBLEMS IN SUPERCRITICAL FLUID CHROMATOGRAPHY ................................................................. 6
   The Fundamentals of Supercritical Fluid Chromatography ....... 8
   Instrumentation Concerns ....................................................... 8
   Mobile Phase Problems ......................................................... 9
   Stationary Phase Developments .......................................... 13

GOALS OF RESEARCH ................................................................. 14
List of References ......................................................................... 15

CHAPTER II
RETENTION CHARACTERISTICS OF HIGH MOLECULAR WEIGHT COMPOUNDS IN CAPILLARY
SUPERCRITICAL FLUID CHROMATOGRAPHY ...................................... 17
   INTRODUCTION ........................................................................... 17
   EXPERIMENTAL SECTION ....................................................... 18
   Polymers and Samples ......................................................... 18
   Instrumentation: MMA/BA Studies ....................................... 21
   Instrumentation: Polydisulfide Studies .................................... 21

RESULTS AND DISCUSSION ....................................................... 22
LIST OF TABLES

Table 1. Critical parameters of some compounds of interest ................. 2
Table 2. A comparison of several physical properties of gases, liquids, and supercritical fluids ................................................................. 3
Table 3. Areas of SFC in need of additional research. ............................ 7
Table 4. Per cent polysiloxane stationary phase remaining after treatment with supercritical ammonia for 24 hours at 200 atm and 145°C .... 12
Table 5. Physical properties of polymers studied .................................... 20
Table 6. HETP (μm) per peak ................................................................. 30
Table 7. Capacity factors (k') and selectivities (α) for 20-meter DB-17, 20-meter DB-225, and 1-meter DB-17 columns ................................. 31
Table 8. A comparison of the number of theoretical plates and resolution observed for the 20-meter DB-17 and 1-meter DB-17 columns ...... 32
Table 9. Reported CMC values for AOT at ambient pressure ................. 53
Table 10. A comparison of CMC values from water, AOT C-1, and AOT C-1' protons at ambient pressure ......................................................... 63
Table 11. CMC values for AOT in CDCl₃ and C₆D₆ at ambient pressure and at 136 bar based on H₂O ¹H chemical shift values ..................... 70
Table 12. CMC values for AOT in C₆D₆ at 136 bar .................................. 73
Table 13. Capacity factors and other parameters of the analytes used in GC experiments .......................................................... 97
Table 14. Solutes that would not elute under GC conditions ................... 98
Table 15. Retention models for Restek column using gas chromatography ... 101
Table 16. Statistics for the models presented in Table 15 .................. 102
Table 17. Capacity factors and parameters of analytes used in SFC experiments. .......................................................... 110
Table 18. Solutes that would not elute under SFC conditions ............ 111
Table 19. Retention models for SFC .................................................. 113
Table 20. Statistics for the models presented in Table 19 .................. 114
Table 21. Capacity factors and pKₐ values for pyridine and cyano-substituted pyridines. ......................................................... 120
LIST OF FIGURES

Figure 1. Separation of Thiokol LP-3 on the 12.2-meter BP-10 column . . . . 23
Figure 2. Separation of Thiokol LP-3 on the 12.2-meter fused silica tube . . 24
Figure 3. Separation of MMA/BA on the 20-meter DB-17 column ............. 26
Figure 4. Separation of MMA/BA on the 1-meter DB-17 column ............... 28
Figure 5. Chromatographic resolution on the 1 meter DB-17 column and on the 20 meter DB-17 column ....................................................... 29
Figure 6. Separation of MMA/BA on the 20-meter DB-225 column ............. 35
Figure 7. The molecular structure of AOT ................................................... 43
Figure 8. High pressure sample apparatus .................................................. 46
Figure 9. 250 MHz spectrum of 200 mM AOT in C₆D₆ (w₀=6.5) at ambient pressure and 40 °C .............................................................. 48
Figure 10. Changes in H₂O ¹H peak position (ppm) in CDCl₃ (w₀=2.0, ambient pressure, 40°C) as a function of AOT concentration ......................... 50
Figure 11. Changes in H₂O ¹H peak position (ppm) in C₆D₆ (w₀=6.5, ambient pressure, 40°C) as a function of AOT concentration ......................... 51
Figure 12. Changes in peak width (PWHM, in Hz) in CDCl₃ (w₀=2.0, ambient pressure, 40°C) as a function of AOT concentration ......................... 55
Figure 13. AOT in C₆D₆: changes in H₂O ¹H peak position (ppm) and spectral appearance at different surfactant concentrations (w₀=6.5, ambient pressure, 40°C) ............................................. 57
Figure 14. Changes in peak width (PWHM, in Hz) in C₆D₆
(w₀=6.5, ambient pressure, 40°C) as a function of AOT concentration ........................................... 58

Figure 15. Chemical shift of the C-1 (in CDCl₃) AOT proton as a function of surfactant concentration .................................................. 59

Figure 16. Chemical shift of the C-1' (in CDCl₃) AOT protons as a function of surfactant concentration .................................................. 60

Figure 17. Chemical shifts of the C-1 (in C₆D₆) AOT protons as a function of surfactant concentration ........................................... 61

Figure 18. Chemical shifts of the C-1' (in C₆D₆) AOT protons as a function of surfactant concentration ........................................... 62

Figure 19. H₂O ¹H chemical shifts as a function of AOT concentration in C₆D₆ ................................................................. 67

Figure 20. C-1 AOT proton chemical shifts as a function of surfactant concentration in C₆D₆ at 136 bar ........................................... 68

Figure 21. C-1' AOT proton chemical shifts as a function of surfactant concentration in C₆D₆ at 136 bar ........................................... 69

Figure 22. H₂O ¹H chemical shifts as a function of AOT concentration in CDCl₃ ................................................................. 72

Figure 23. H₂O ¹H peak chemical shifts as a function of AOT concentration at 136 bar ................................................................. 76

Figure 24. A proposed structure for PGC .................................................. 89

Figure 25. A plot of ln k'observed versus ln k'predicted by GC model #1 ........................................... 103

Figure 26. A plot of ln k'observed versus ln k'predicted by SFC model #1 ........................................... 115

Figure 27. A plot of ln k' versus pKa for pyridine compounds ........................................... 124
CHAPTER I

SUPERCRITICAL FLUID CHROMATOGRAPHY: A PRIMER

INTRODUCTION

Properties of Supercritical Fluids

A supercritical fluid is a gas or liquid that has been heated in excess of its critical temperature, \( T_c \). At temperatures in excess of \( T_c \), the phase boundary between the gas and liquid phases disappears and a single phase, a supercritical fluid, exists. An increase in pressure cannot bring the return of the phase boundary and the liquid phase when temperatures exceed \( T_c \). The pressure necessary to liquefy a fluid at its critical temperature is its critical pressure \( P_c \). The density of a fluid at its critical temperature and pressure is its critical density \( \rho_c \). The critical parameters of some compounds of interest are listed in Table 1.

Supercritical fluids possess physical properties intermediate to those of gases and liquids. A comparison of several of these can be seen in Table 2. The density of a supercritical fluid is directly related to the system pressure. As a consequence, the density of a supercritical fluid can be manipulated through pressure adjustments to the system. The solubility parameter of a supercritical fluid is related to its critical pressure and its density through the equation\(^2\)
Table 1. Critical parameters of some compounds of interest.\(^3\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(T_c) (°C)</th>
<th>(P_c) (atm)</th>
<th>(\rho_c) (g/cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbon dioxide</td>
<td>31.04</td>
<td>72.85</td>
<td>0.468</td>
</tr>
<tr>
<td>ammonia</td>
<td>132.4</td>
<td>111.3</td>
<td>0.235</td>
</tr>
<tr>
<td>ethane</td>
<td>32.28</td>
<td>48.16</td>
<td>0.203</td>
</tr>
<tr>
<td>n-propane</td>
<td>96.67</td>
<td>41.94</td>
<td>0.217</td>
</tr>
<tr>
<td>n-butane</td>
<td>152.01</td>
<td>37.7</td>
<td>0.228</td>
</tr>
<tr>
<td>n-pentane</td>
<td>196.5</td>
<td>33.35</td>
<td>0.237</td>
</tr>
<tr>
<td>n-hexane</td>
<td>234.2</td>
<td>29.3</td>
<td>0.233</td>
</tr>
<tr>
<td>nitrous oxide</td>
<td>36.43</td>
<td>71.6</td>
<td>0.453</td>
</tr>
<tr>
<td>air</td>
<td>-140.6</td>
<td>37.2</td>
<td>0.313</td>
</tr>
<tr>
<td>water</td>
<td>374.2</td>
<td>218.3</td>
<td>0.325</td>
</tr>
<tr>
<td>methanol</td>
<td>239.43</td>
<td>79.9</td>
<td>0.272</td>
</tr>
<tr>
<td>ethanol</td>
<td>243.1</td>
<td>62.96</td>
<td>0.276</td>
</tr>
<tr>
<td>sulfur hexafluoride</td>
<td>45.55</td>
<td>37.11</td>
<td>0.734</td>
</tr>
<tr>
<td>hydrogen sulfide</td>
<td>100.4</td>
<td>88.9</td>
<td>0.31</td>
</tr>
<tr>
<td>chlorotrifluoromethane</td>
<td>28.9</td>
<td>38.7</td>
<td>0.579</td>
</tr>
<tr>
<td>dichlorodifluoromethane</td>
<td>111.80</td>
<td>40.71</td>
<td>0.558</td>
</tr>
<tr>
<td>trichlorofluoromethane</td>
<td>198.0</td>
<td>43.5</td>
<td>0.554</td>
</tr>
</tbody>
</table>
Table 2. A comparison of typical physical properties of gases, liquids, and supercritical fluids.4

<table>
<thead>
<tr>
<th>Property</th>
<th>Gas</th>
<th>Liquid</th>
<th>Supercritical Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (g/cm$^3$)</td>
<td>$10^{-3}$</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Diffusivity (cm$^2$/sec)</td>
<td>$10^{-1}$</td>
<td>$5 \times 10^{-6}$</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>Viscosity (g cm$^{-1}$ s$^{-1}$)</td>
<td>$10^{-4}$</td>
<td>$10^{-2}$</td>
<td>$10^{-4}$</td>
</tr>
</tbody>
</table>
\[ \delta = 1.25 P_c^{0.5} \left( \frac{\rho_r}{\rho_{r,\text{liq}}} \right) \]  

(1)

where \( P_c \) is the critical pressure of the fluid, \( \rho_r \) is the reduced density of the supercritical fluid and \( \rho_{r,\text{liq}} \) is the reduced density of the liquid.

**Supercritical Fluid Chromatography**

Supercritical fluid chromatography (SFC) is a separation technique that employs a supercritical fluid as a mobile phase. By exploiting the unique properties of supercritical fluids SFC offers several distinct advantages over gas chromatography (GC) and liquid chromatography (LC). Through the selection of a supercritical fluid with a sufficiently low critical temperature SFC can be used to analyze substances that are thermally labile. The enhanced solvent strength of supercritical fluids relative to GC mobile phases facilitates separation of analytes with high molecular weights and low volatilities that cannot be examined with GC. A supercritical mobile phase can solubilize analytes while in GC solute transfer to the mobile phase is dependent on the volatility of the solute. Supercritical fluids generally possess lower viscosities and higher diffusivities than solvents commonly used in LC. This makes it possible to accomplish many separations with SFC with greater chromatographic efficiency and with a shorter time of analysis than by using comparable LC techniques. SFC can use several of the "universal" detectors that are popular in GC but which cannot be used in LC, such as the flame
ionization detector (FID). It is also much easier to interface SFC systems than LC systems with information-intensive detectors, such as mass spectrometers.

**A Brief History of Supercritical Fluid Chromatography**

Although SFC has experienced the bulk of its development since 1981 its history is nearly two hundred years old. The discovery of the "critical state" occurred in 1822. Studies of the critical region and of enhanced solubility in supercritical fluid were made during the latter portion of the Nineteenth Century.

Nearly one hundred years passed before supercritical fluids were considered for use in chromatography. In 1961 porphyrins, which could not be separated by conventional GC, were successfully eluted from a column with a dense gas mobile phase. During the mid and late 1960s the theoretical effects of increasing the pressure of the mobile phase in GC were recognized and experimentally verified.2,7-9

Several factors slowed the development of SFC during the 1970s. The corrosive nature of some supercritical fluids on the seal and gasket materials of the day were made of resulted in problems with leaks.2 Detector difficulties also created problems for those interested in SFC.2,4 Perhaps most important, an explosion in the growth of liquid chromatography, especially high performance liquid chromatography (HPLC), obscured the benefits of SFC as the potential of HPLC began to be exploited.

Advances in HPLC instrumentation benefitted SFC. In the early 1980s technological improvements to LC equipment were utilized to overcome many of the numerous difficulties that had plagued earlier workers in the field, including the leakage
problem, a need for independent temperature and pressure control and programming, and control of mobile phase composition and flow rate. The first use in SFC of open tubular columns in 1981, also known as capillary columns, made it possible to begin to realize some of the efficiency and speed of analysis advantages of SFC over GC that had been described in theory twenty years earlier. The smaller volume of mobile phase that flowed through these columns also facilitated the adaptation of popular universal detectors, such as the FID, for use with SFC. These developments, coupled with the commercial availability of SFC instrumentation (which first occurred in 1986) and the exploding popularity of supercritical fluids as extraction media, have helped to carry SFC to its current position as a separation technique.

**CURRENT PROBLEMS IN SUPERCRITICAL FLUID CHROMATOGRAPHY**

Supercritical fluid chromatography is in many ways a relatively new technique, inasmuch as the bulk of its development has occurred during the last twelve years. It has not been spared the pains and the problems that attend the growth and popularization of a new technique. In 1987 a discussion of areas within SFC in need of additional research was published. These are listed in Table 3. While a great deal of progress has been made, there are still numerous fundamental and applied challenges facing those who study SFC.
Table 3. Areas of SFC in need of additional research.\textsuperscript{11}

<table>
<thead>
<tr>
<th>General</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Theory of supercritical solvents</td>
<td></td>
</tr>
<tr>
<td>Behavior of supercritical mixtures</td>
<td></td>
</tr>
<tr>
<td>New (more polar) mobile phases</td>
<td></td>
</tr>
<tr>
<td>Non-corroding materials</td>
<td></td>
</tr>
<tr>
<td>Injection of solids</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Packed-column SFC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufficiently inert stationary phases</td>
<td></td>
</tr>
<tr>
<td>Adequate flow or pressure control</td>
<td></td>
</tr>
<tr>
<td>Universal and sensitive detectors</td>
<td></td>
</tr>
<tr>
<td>Effects of column pressure drop</td>
<td></td>
</tr>
<tr>
<td>Effects of column and particle dimensions</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Capillary SFC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproducible restrictors</td>
<td></td>
</tr>
<tr>
<td>Splitless injectors</td>
<td></td>
</tr>
<tr>
<td>Stable and efficient stationary phases</td>
<td></td>
</tr>
<tr>
<td>Narrow-bore columns (d. $\leq$ 15 $\mu$m)</td>
<td></td>
</tr>
<tr>
<td>Selective detectors</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Applications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Establish unique applications (not covered by GC or LC)</td>
<td></td>
</tr>
</tbody>
</table>
**The Fundamentals of Supercritical Fluid Chromatography**

There are still many dark areas in the current understanding of supercritical fluids and SFC. In a recent review of literature that was published in 1990 and 1991, over 40 articles were cited that treated the areas of SFC theory and fundamental measurements.\(^{12}\) Predicting the appropriate mobile phase or mobile phase - modifier combination (modifiers will be discussed below) for as yet unstudied analytes is often vague, confusing, and difficult. The dependence of solute retention on various chromatographic conditions such as mobile phase composition and density, the nature of the stationary phase, and the physical and chemical attributes of the analyte molecules is often unclear. A basic understanding of the relationship between retention and solute solubility in the mobile phase is lacking in many instances. Even information of fundamental importance, such as the extent of solubility of solvents commonly used as modifiers and the conditions affecting their solubility (e.g., temperature and pressure), is often unavailable.

**Instrumentation Concerns**

In a review of SFC written in 1987, one of the limitations to SFC cited by the authors addressed difficulties associated with making reproducible sample injections on SFC columns.\(^{13}\) The pursuit of reproducible peaks is affected by injection problems and it is an ongoing problem.\(^{12,14}\) The introduction of the sample to the system is more difficult in SFC than in GC or in HPLC. The small columns most popular in SFC introduce very stringent requirements on acceptable injector dead volume. Capillary
columns are easily overloaded by either the sample or its solvent, which creates further problems in quantitative or trace analyses.

Pumping systems and fittings put an effective ceiling on the useful range of many SFC analyses. The most common commercially available precision syringe pumps in use in SFC and HPLC cannot exceed a maximum pressure of 7500 to 10,000 psi. The most common commercially available fittings for use in SFC and HPLC are not rated for use beyond 10,000 psi. Yet, it was clearly demonstrated in several of the early SFC studies that many analytes of interest do not become soluble in supercritical carbon dioxide or ammonia until pressures of 11,000 to 30,000 psi are reached.\textsuperscript{2,8,9}

The flow of mobile phase through SFC columns is generally regulated by mechanical restrictors. The most commonly used restrictors are not adjustable with respect to the amount of flow that they permit. Flow characteristics are often difficult to predict. Frequent plugging and the need for replacement is an ongoing headache, especially when consistent mobile phase flow is required in experiments. An additional problem is often encountered when high molecular weight analytes are studied. If too drastic a pressure drop occurs across a restrictor, the mobile phase density may decrease to the point where analytes are no longer soluble in the mobile phase and they precipitate onto the interior wall of the restrictor before they reach the detector.

**Mobile Phase Problems**

One of the most serious challenges to supercritical fluid chromatography (SFC) is that of analyzing large or polar molecules. The most commonly used
supercritical fluid mobile phases, carbon dioxide, ethane, and propane, lack the solvent strength necessary to solvate many analytes of interest due to their inherently low polarizability and dipolarity.15

Three approaches have been employed in attempts to circumvent this limitation to SFC. Modifiers, organic solvents added in small amounts, may be mixed with supercritical fluids to form binary fluids with enhanced solvent strength. Solubility can be increased by a factor of several hundred percent for some solute-fluid systems.16,17 Tri-n-butylphosphate, a strong Lewis base, has been used to enhance the solubility of hydroquinone in supercritical carbon dioxide by nearly three orders of magnitude.18 The beneficial properties of modifiers are ultimately limited since the addition of an excess of modifier may induce a variety of undesirable side effects. These may include significant changes in the critical properties of the binary fluid (e.g., an increase in the critical temperature of the fluid), an increase in fluid viscosity, a diminution of solute diffusivity, and interference with detectors commonly used in SFC.

Surfactants such as Aerosol-OT19 can act as more potent additives than organic modifiers, but the usefulness of these substances is restricted by similar limitations to those of organic modifiers. In addition, less effort has been devoted to the study of these systems. As a consequence supercritical fluid-surfactant systems are much less understood than supercritical fluid-modifier systems. Several problems arise from this. Inadequate theoretical underpinnings make it difficult to predict important aspects of the system, such as the phase behavior of the supercritical fluid-surfactant system and the solubility of analytes in the binary fluid. Only a relatively few surfactants are known to
form reverse micelles in supercritical fluids. This imposes restrictions on the number and variety of tools available for use when attempting difficult analyses. And, as will be pointed out in another chapter in this document, the behavior of surfactants in pressurized systems is poorly understood.

The use of supercritical fluids with greater polarity than those that are commonly used has also been attempted. The ability of supercritical ammonia to dissolve highly polar analytes which are insoluble in supercritical carbon dioxide such as amines, amino acids, small peptides, and mono and disaccharides has been reported.\textsuperscript{2,9,20} Factors besides solubility may also favor the use of supercritical ammonia or other supercritical mobile phases than supercritical carbon dioxide since it can react with primary amines and other basic compounds.\textsuperscript{20,21} However, supercritical ammonia remains of limited utility to practitioners of SFC. In one study the solubility of several stationary phases commonly used in SFC was reported.\textsuperscript{20} The instability of stationary phases exposed to an ammonia mobile phase can be seen in Table 4. The loss of stationary phase is a serious problem to those desirous to use polar supercritical fluid mobile phases since SFC separations rely on interactions between analytes and the stationary phase as well as on interactions between analytes and the mobile phase. It seems reasonable to assume that supercritical fluids more polar than ammonia, such as supercritical methanol and ethanol, may exacerbate the problem of stationary phase stability if they are used as mobile phases in supercritical fluid chromatography.
Table 4. Per cent polysiloxane stationary phase remaining after treatment with supercritical ammonia for 24 hours at 200 atm and 145°C.\textsuperscript{20}

<table>
<thead>
<tr>
<th>polysiloxane stationary phase</th>
<th>percent remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% n-nonyl</td>
<td>100</td>
</tr>
<tr>
<td>50% n-octyl</td>
<td>100</td>
</tr>
<tr>
<td>50% cyanopropyl</td>
<td>0</td>
</tr>
<tr>
<td>50% phenyl</td>
<td>40</td>
</tr>
<tr>
<td>30% biphenyl</td>
<td>30</td>
</tr>
<tr>
<td>SE-54 (5% phenyl)</td>
<td>0</td>
</tr>
</tbody>
</table>
Stationary Phase Developments

Most stationary phases currently used in SFC were originally developed for GC or for LC. Often the solubility of these stationary phases in the supercritical fluid mobile phase requires that the stationary phase be crosslinked or immobilized on either the support or on the interior wall of a capillary column. This is generally adequate to prevent most common mobile phases from stripping the stationary phase from the column, but there are exceptions. The combination of carbon dioxide and certain modifiers, such as a 4% formic acid/CO₂ mobile phase, will strip the stationary phase from a column.²² As was seen above and in Table 4, mobile phases more polar than carbon dioxide, such as ammonia, will also strip the stationary phase from columns. A study of the literature indicates that while a great deal of effort is being focussed on the production of stationary phases capable of novel separations, very little work is being done to provide SFC with stationary phases capable of withstanding the rigors of exposure to the more polar supercritical fluids.¹²

Most stationary phases commonly used in SFC are founded on silica supports. While silica appears to be inert to supercritical ammonia it's use in SFC is not devoid of problems. Siloxyl groups, which are generally present on silica surfaces, interact with polar analytes to the extent that it is widely thought that analyte-siloxyl interactions are principal contributors to observed retention in SFC. These interactions are sufficiently strong to alter the expected elution behavior of polar analytes, even to the extent that some substances are completely and irreversibly retained by the stationary phase. Efforts to chemically deactivate silica surfaces, e.g. through endcapping, have
only been partially successful. Other stationary phases that affect solute retention through surface adsorption, e.g., alumina, are similarly problematic. The development of a stationary phase that is not only inert to polar supercritical fluids but that is also unreactive with polar analytes could be extremely beneficial to practitioners of SFC.

**GOALS OF RESEARCH**

It has been demonstrated above that the relative newness of SFC as a technique has left its practitioners with numerous problems to face. Some of these problems are trivial and easily forgotten or overlooked. Others are of sufficient import that failure to resolve them in a satisfactory manner will result in the ultimate limitation of SFC as a viable technique for many types of analyses. It is the goal of the studies in this dissertation to make contributions to the solution of two of the problems described above. Chapter two describes work that provides some insight into the mechanism by which polymer samples are separated when a pressure gradient is used. Chapters three and four address the problem of analysis of polar and high molecular weight analytes, but from two different directions. Chapter three discusses fundamental research done on pressurized solvent-surfactant systems. The work discussed in chapter three is intended to serve as a bridge to the study of supercritical fluid-surfactant systems. In chapter four the results of the characterization of a novel column for SFC are presented. The capillary column that was examined was coated with a film of porous glassy carbon, a material that has recently shown a great deal of promise as a stationary phase in SFC because of its physical and chemical properties.
List of References

19. see Chapter 3 in this document, for example.


CHAPTER II

RETENTION CHARACTERISTICS OF HIGH MOLECULAR WEIGHT COMPOUNDS IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

INTRODUCTION

Supercritical fluid chromatography (SFC) provides a powerful technique for the separation of oligomers in polymer samples. A wide variety of natural and synthetic polymers have been separated. Many combinations of mobile phase, stationary phase, and operating conditions are available for use. Such diversity may make it possible to separate polymers that are presently problematic when using other techniques.

Interactions between the mobile phase and the oligomers are of primary importance to polymer separations in SFC. While the stationary phase affects retention, the mechanism by which it contributes is unclear. For packed-SFC columns the postulated separation process involves continuous reprecipitation and redissolution of the oligomers as they move down the column. As a result of the pressure drop across the column and the existence of threshold pressures or threshold densities for each oligomer, the higher molecular weight oligomers follow the smaller ones down the column. The validity of this retention mechanism has been substantiated by studies which have shown that the nature of the stationary phase has little impact on the selectivity of the oligomer...
Due to the nature of the retention mechanism in packed-column SFC of oligomers, gradient programming is required to achieve optimum separation of the oligomers. Pressure programming and the resultant density programming were the first gradient methods used to separate polymers and remain the most common gradient methods used for oligomer separations.

Capillary columns of the dimensions used in this study have negligible pressure drop across the column. The retention mechanism based on precipitation-redissolution as a function of pressure drop is therefore not applicable to capillary columns. This chapter illustrates the relative importance of oligomer solubility and oligomer-stationary phase interactions for two very different polymer systems, a polydisulfide and a methylmethacrylate/butylacrylate (MMA/BA) copolymer.

**EXPERIMENTAL SECTION**

**Polymers and Samples**

Thiokol LP-3 is a polydisulfide elastomer that is produced by Morton Thiokol, Inc. It is used as a sealant and as an additive for high molecular weight polymer synthesis. The polydisulfides are good sealants because they exhibit high resistance to organic solvents, i.e. the solubility of these elastomers in organic solvents is minimal. It has been demonstrated that the LP-3 polymer is soluble in supercritical CO₂. Although none of the possible crosslinking is shown, the approximate molecular structure of the polydisulfide repeating unit is as follows:
HS-(C₂H₄O-CH₂O-C₂H₄-SS)n-C₂H₄O-CH₂O-C₂H₄-SH

Thiokol LP-3 was obtained from Polysciences, Inc., Warrington, PA and was used without further purification.

The repeating unit of the methyl-methacrylate/n-butylacrylate (MMA/BA) copolymer has the following molecular structure:

\[
\begin{array}{c}
\text{CH}_3 \\
\text{CH} \\
\text{COOCH}_3 \\
\end{array} m
\quad \quad \quad \quad
\begin{array}{c}
\text{H} \\
\text{C} \\
\text{COOC}_4\text{H}_8 \\
\end{array} n
\]

where \(m\) usually ranges from 10 to 18 and \(n\) values vary between 0 and 7, with \(n=2\) being the most common.\(^7\) Oligomers ranging in molecular weight from 600 amu to 3500 amu have been identified using mass spectroscopy.\(^7\) There are at least 72 oligomers theoretically possible in the molecular weight range from 1000 amu to 2700 amu. These copolymers are used for the manufacture of many types of plastics. The specific MMA/BA copolymer studied was made by combining 80%/20% (w/w) of the monomers. The polymer was obtained from the Marshall Laboratories of E.I. duPont de Nemours & Co., Inc.

Table 5 shows other properties of the two oligomer systems used in this study. The analyzed samples were made by diluting bulk polymer to 10% (w/w) with reagent-grade carbon disulfide (J.T. Baker, Inc.). This concentration, although high, was
Table 5. Physical properties of polymers studied. $^a$ number average molecular weight, amu; $^b$ weight average molecular weight, amu; $^c$ polydispersity; $D = M_w/M_n$

<table>
<thead>
<tr>
<th></th>
<th>$M_n^a$</th>
<th>$M_w^{b}$</th>
<th>$D^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiokol LP-3</td>
<td>540</td>
<td>1500</td>
<td>2.8</td>
</tr>
<tr>
<td>MMA/BA</td>
<td>750</td>
<td>1300</td>
<td>1.70</td>
</tr>
</tbody>
</table>
necessary because many of the oligomers in the samples were not detectable at lower bulk sample concentrations.

**Instrumentation: MMA/BA Studies**

The chromatograph in the MMA/BA studies consisted of a Varian 3700 gas chromatographic oven and a Varian flame ionization detector. A 20-meter DB-17 (polyphenylmethylsiloxane) open tubular column with an internal diameter of 100 μm and a film thickness of 0.2 μm was used as purchased (J&W Scientific). A 20-meter DB-225 (polycyanopropylphenylmethylsiloxane) open tubular column with an internal diameter of 100 μm and a film thickness of 0.1 μm was also used as purchased (J&W Scientific). The mobile phase consisted of SFC-grade carbon dioxide (Matheson) modified with 0.5-1.3% (v/v) of 95-97% formic acid (Aldrich). An Isco 260D syringe pump was used to deliver the mobile phase. Sample introduction was accomplished with a Valco CI4W injector with a 60-nL sample loop. Upon injection the sample loop was left in line with the column for the duration of the run. This is to prevent discrimination against high molecular weight analytes. Cycling of the valve introduces a pressure drop in the injection loop, and the concomitant density reduction may render the mobile phase solvent strength inadequate for solvation of heavier components in a sample.

**Instrumentation: Polydisulfide Studies**

The chromatographic system and operating conditions used have been described in detail elsewhere.\(^6\) A Hewlett-Packard 5890A gas chromatograph and a Hewlett-Packard Model 19256A flame photometric detector (FPD) served as the system oven and
detector. A 12.2-meter BP-10 (polyphenylmethylsiloxane) open tubular column with a 220 \( \mu m \) internal diameter and a film thickness of 0.25 \( \mu m \) was used (Scientific Glass Engineering, Austin, TX). The stationary phase was cross-linked with azo-tert-butane before exposure to a supercritical fluid mobile phase. A 12.2-meter fused silica capillary (Polymicro Technologies, Inc., Phoenix, AZ) with an internal diameter of 250 \( \mu m \) was also employed as a column without any additional treatment. The mobile phase used in the polydisulfide studies consisted of supercritical grade carbon dioxide (Scott Specialty Gases). An Isco \( \mu \text{L}-\text{LC500} \) syringe pump delivered the mobile phase. Sample introduction was accomplished with a Valco CI4W injector with a 200-nL sample loop. As in the MMA/BA studies, upon injection the sample loop was left in line with the column for the duration of the analysis.

In all studies homemade integral restrictors constructed from 50 \( \mu m \) i.d. fused silica tubing (Polymicro Technologies, Inc.) were used to regulate pressure and to control flow. The orifice diameter of the restricting capillary was approximately 5 \( \mu m \).

RESULTS AND DISCUSSION

Polydisulfide Studies

The chromatographic separation of Thiokol LP-3 on the BP-10 column is shown in Figure 1. Figure 2 shows separation of the Thiokol LP-3 when the column is replaced with a fused silica open tube with the same internal diameter and length as the column. The polydisulfide was crudely separated in the fused silica open tube, without the benefit
Figure 1. Separation of Thiokol LP-3 on the 12.2-meter BP-10 column. Oven temperature: 100°C. Mobile phase: carbon dioxide. Initial pressure: 102 atm for 15 minutes. Pressure ramp: 3.4 atm/min. Final pressure: 306 atm. Detector gas flows: 240 mL/min H₂, 45 mL/min O₂. Figure published with permission.
Figure 2. Separation of Thiokol LP-3 on the 12.2-meter fused silica tube. Oven temperature: 100°C. Mobile phase: carbon dioxide. Initial pressure: 102 atm for 15 minutes. Pressure ramp: 3.4 atm/min. Final pressure: 306 atm. Detector gas flows: 240 mL/min H₂, 45 mL/min O₂.
of interaction with a stationary phase. The resulting chromatogram mimicked the results obtained when the BP-10 column was used under the same experimental conditions. The BP-10 column separation produced three sets of peaks, each peak being composed of one or more oligomers. The fused silica tube separation lacked the resolution of the column chromatogram, but reproduced the three principle peak clusters.

These results give insight into the retention mechanism of polymers in capillary SFC. The separations observed in the fused silica tube can be attributed to the selective solvation of oligomers as the mobile phase density was ramped. Isobaric conditions did not permit a satisfactory separation to occur. If the initial pressure was too low, there was no sample elution. If the initial pressure was too high, the oligomers coeluted. With isobaric runs at an intermediate pressure, fewer peaks were observed. We attribute the separation achieved without stationary phase in the fused silica tube to the selective solvation of the oligomers by the supercritical fluid. The polydisulfide must precipitate out onto the head of the column. By scanning through the threshold densities of the oligomers with a pressure or density program selective solvation occurs. There are active sites found on fused silica surfaces which can interact with and slow the migration of oligomers, but the number of sites is too small to be wholly responsible for the observed separations.

**MMA/BA Studies: DB-17 Column**

Figure 3 shows the chromatogram resulting from the separation of the 80%/20% MMA/BA copolymer on the 20-meter DB-17 column. After completing initial studies
Figure 3. Separation of MMA/BA on the 20-meter DB-17 column. Oven temperature: 140°C. Mobile phase: carbon dioxide with 0.8 % (v/v) formic acid. Initial pressure: 88 atm. Pressure ramp: 6.8 atm/min. Final pressure: 374 atm. Detector gas flows: 455 mL/min air, 45 mL/min H₂.
on this 20-meter column, the first meter was detached and used in further experiments. This was done to see if the efficiency and the resolution of the separations changed when a shorter column with a lesser amount of active stationary phase surface was used. Figure 4 shows the separation of the MMA/BA copolymer on the 1-meter column. When using either of the DB-17 columns a typical chromatogram contained from 30 to 40 peaks. The fifteen most prominent peaks on the chromatograms of both the 20-meter and the 1-meter columns were selected, and the chromatographic selectivities relative to one another were calculated to ensure that the same peaks were being compared between chromatograms. The height equivalents to a theoretical plate were calculated for each of the peaks using triangulation and are given in Table 6. In general, the 1-meter column was as efficient or more efficient than the 20-meter column. The resolution for the two columns was also determined. Figure 5 shows a comparison of these values. The long column, the less efficient of the two columns, provided superior resolution for oligomers 1-9. In typical chromatographic separations resolution is related to the square root of the total number of plates generated by a column. Although it was less efficient, the longer column still generated a far greater total number of theoretical plates. Table 7 shows the capacity factors and selectivities for the 15 most prominent peaks in the chromatogram for both the 20-meter and 1-meter columns. In Table 8 the efficiency and the resolution of the two columns are compared. If the experimental efficiency of each chromatographic peak on the 20 meter column is divided by 20 and substituted into the resolution equation below
Figure 4. Separation of MMA/BA on the 1-meter DB-17 column. Oven temperature: 140°C. Mobile phase: carbon dioxide with 0.8 % (v/v) formic acid. Initial pressure: 88 atm. Pressure ramp: 6.8 atm/min. Final pressure: 374 atm. Detector gas flows: 455 mL/min air, 45 mL/min H₂.
Figure 5. Chromatographic resolution on the (O) 1 meter DB-17 column and on the (△) 20 meter DB-17 column.
Table 6.  HETP (μm) per peak.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>H, 20m</th>
<th>H, 1m</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2000</td>
<td>1400</td>
</tr>
<tr>
<td>2</td>
<td>550</td>
<td>920</td>
</tr>
<tr>
<td>3</td>
<td>420</td>
<td>680</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>530</td>
<td>180</td>
</tr>
<tr>
<td>6</td>
<td>320</td>
<td>110</td>
</tr>
<tr>
<td>7</td>
<td>290</td>
<td>130</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>120</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>530</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>500</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>210</td>
<td>80</td>
</tr>
<tr>
<td>13</td>
<td>200</td>
<td>70</td>
</tr>
<tr>
<td>14</td>
<td>190</td>
<td>50</td>
</tr>
<tr>
<td>15</td>
<td>180</td>
<td>40</td>
</tr>
</tbody>
</table>
Table 7. Capacity factors ($k'$) and selectivites ($\alpha$) for 20-meter DB-17, 20-meter DB-225, and 1-meter DB-17 columns.

<table>
<thead>
<tr>
<th>Peak</th>
<th>$k'$ 20m DB-17</th>
<th>$k'$ 20m DB-225</th>
<th>$k'$ 1m DB-17</th>
<th>$\alpha$ 20m DB-17</th>
<th>$\alpha$ 20m DB-225</th>
<th>$\alpha$ 1m DB-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>3.30</td>
<td>6.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
<td>8.90</td>
<td>11.10</td>
<td>1.6</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>1.10</td>
<td>13.00</td>
<td>14.80</td>
<td>1.4</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>1.20</td>
<td>13.70</td>
<td>15.50</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>1.40</td>
<td>16.70</td>
<td>18.40</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>1.45</td>
<td>17.70</td>
<td>19.30</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>1.50</td>
<td>19.90</td>
<td>21.50</td>
<td>1.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>1.60</td>
<td>20.90</td>
<td>22.30</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>9</td>
<td>1.80</td>
<td>22.60</td>
<td>24.50</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>1.85</td>
<td>23.90</td>
<td>25.40</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>1.95</td>
<td>25.10</td>
<td>27.10</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>12</td>
<td>2.00</td>
<td>26.30</td>
<td>28.30</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>13</td>
<td>2.10</td>
<td>27.40</td>
<td>29.60</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>14</td>
<td>2.15</td>
<td>28.60</td>
<td>30.70</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>2.20</td>
<td>29.40</td>
<td>31.90</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table 8. A comparison of the number of theoretical plates and resolution observed for the 20-meter DB-17 and 1-meter DB-17 columns. * calculated value

<table>
<thead>
<tr>
<th>Peak pair</th>
<th>( N ) 20m DB-17</th>
<th>( N ) 1m DB-17</th>
<th>( R_s ) 20m DB-17</th>
<th>( R_s ) 1m DB-17</th>
<th>( R_s ) 20m* DB-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>36,000</td>
<td>1,100</td>
<td>9.2</td>
<td>3.2</td>
<td>4.5</td>
</tr>
<tr>
<td>2-4</td>
<td>199,000</td>
<td>4,000</td>
<td>18.0</td>
<td>3.4</td>
<td>6.6</td>
</tr>
<tr>
<td>4-6</td>
<td>62,000</td>
<td>8,800</td>
<td>6.4</td>
<td>3.7</td>
<td>2.6</td>
</tr>
<tr>
<td>6-8</td>
<td>296,000</td>
<td>8,100</td>
<td>10.9</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>8-9</td>
<td>81,000</td>
<td>17,100</td>
<td>3.9</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>9-10</td>
<td>38,000</td>
<td>14,800</td>
<td>1.4</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>10-11</td>
<td>40,000</td>
<td>16,900</td>
<td>1.5</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td>11-12</td>
<td>95,000</td>
<td>12,700</td>
<td>1.0</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>12-13</td>
<td>101,000</td>
<td>13,900</td>
<td>2.3</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>13-14</td>
<td>106,000</td>
<td>21,400</td>
<td>2.1</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>14-15</td>
<td>109,000</td>
<td>23,100</td>
<td>1.5</td>
<td>1.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>
along with the experimental selectivity and capacity factor value for the 1-meter column, then the expected resolution on the 1-meter column can be calculated. This is shown in Table 8. These data show that the experimental resolution obtained on the 1-meter column is always higher than that expected for the larger oligomers found for peak #8 and later. We believe the major cause of this enhanced resolution to be that the separation of the higher molecular weight oligomers is predominately determined by their selective solvation in the supercritical fluid at well defined densities; this is the same mechanism as was described for the polydisulfide polymer. Interactions between the lower molecular weight oligomers and the stationary phase had a greater impact on their resolution. This further illustrates the relationship between the stationary phase and differential solvation in polymer separations. As seen in the polydisulfide studies, stationary phase must be present to achieve anything better than a crude separation of oligomers. The interactions between the stationary phase and oligomers serve to enhance the separation that is initiated by their differential solvation in the mobile phase. At the same time, the presence of too much stationary phase, e.g., in a column that is needlessly long, may diminish the efficiency of the separations because selective solvation of the oligomers is highly important in the retention mechanism, especially for the higher molecular weight compounds.

$$R_s = \left( \frac{\sqrt{N}}{4} \right) \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'}{k'+1} \right)$$  \hspace{1cm} (2)
**MMA/BA Studies: DB-225 Column**

MMA/BA separations were obtained on a DB-225 column. Figure 6 shows a chromatogram resulting from the separation. It resembles those obtained on the 20-meter DB-17 column. The fifteen most prominent peaks on the DB-225 chromatogram were measured and found to have the same relative selectivity, $\alpha$, as the fifteen most prominent peaks observed on the 20-meter DB-17 column. In Table 7 a comparison of the capacity factors ($k'$) and the selectivities ($\alpha$) for the 20-meter DB-225 and the 20-meter DB-17 columns is shown. The elution order of the oligomers was the same regardless of the stationary phase used. These results corroborate that selectivity is a function of the mobile phase. In this case the observed selectivity is due to differential solvation. Oligomer-stationary phase interactions are of lesser importance in determining retention order.

**The Use of Capacity Factors, Separation Factors, and Plate Heights in Gradient Chromatography**

There are some who question the correctness of using capacity factors, separation factors, and plate heights to describe chromatographic data obtained from systems in which a gradient - a thermal gradient in GC, a mobile phase composition gradient in LC, or a pressure gradient in SFC - is used. It is clear from the literature that in certain well-defined situations their use is acceptable. We justify their use in this chapter through the following argument.

The capacity factor in chromatographic theory represents several related values. First, it is an indication of solute equilibrium between stationary and mobile phases. Unquestionably the capacity factor of a substance changes as the solvent strength of the
Figure 6. Separation of MMA/BA on the 20-meter DB-225 column. Oven temperature: 130°C. Mobile phase: carbon dioxide with 0.9 % (v/v) formic acid. Initial pressure: 88 atm. Pressure ramp: 10.2 atm/min. Final pressure: 272 atm. Detector gas flows: 455 mL/min air, 45 mL/min H₂.
mobile phase is altered, both in gradient elution HPLC and in pressure-programmed SFC. The capacity factor also represents the relative difference between the retention time, \( t_R \), of an analyte and the column dead time, \( t_0 \). In this context it may not be common to use the capacity factor as a descriptor of a gradient system, but it should neither be considered as entirely devoid of merit. With the understanding that such a use of the capacity factor cannot give accurate insight into the thermodynamics of the system, the elution order of analytes and the delay between their elution and the column dead time is still a valuable piece of information to the chromatographer examining gradient systems.

It is acceptable to consider selectivity or the separation factor, \( \alpha \), in gradient systems. Snyder has written that "for many practical situations we can assume selectivity \( k_a/k_b \) is independent of \( k' \) (i.e. selectivity does not systematically increase or decrease with changes in solvent strength...)" \(^9\) If selectivity is not affected by gradients, then it seems appropriate to infer that computation of the means by which selectivity is calculated, the capacity factors of the adjacent peaks, is also proper and necessary.

The efficiency and resolution of a gradient system are principally dependent on the rate and the form (shape) of the gradient. Column length and volumetric flow are of secondary importance to the separation. Snyder, again, wrote that "band migration, resolution, and peak width in GE [gradient elution] are largely controlled by the form of the mobile phase gradient or mobile phase program... Other variables such as column length \( L \) and flowrate \( F \) also play a role in gradient elution, but this role is usually subordinate to that of the solvent gradient." \(^{10}\) It is well understood that the imposition of
a gradient on a system results in chromatograms that must be interpreted differently from those obtained in isocratic systems to yield correct values of system efficiency.\textsuperscript{11} By using the equation

\[ N = \left( \frac{t_R}{\sigma_i} \right)^2 \]  \hspace{1cm} (3)

on gradient chromatograms, values of N are usually greatly overestimated.\textsuperscript{11} However, the efficiency data presented in this chapter are valuable not as measures of the absolute efficiency of the systems, but as indicators of how efficiency was affected by varying the column length or the nature of the stationary phase with differential solvation of analyte oligomers as the separation mechanism. As such, it would have been more correct to have labeled them as "relative efficiencies". This oversight should not invalidate their usefulness to interpreting the meaning of our experiments.

The data seem quite clear that differential solvation is responsible for the oligomer separations that were observed. In the experiments conducted the stationary phase, the amount of stationary phase (through manipulation of column length), and the type of stationary phase were varied. All other operating conditions - flow, temperature, initial and final pressures, ramp rate, etc. - were identical. Upon injection, the polymer precipitated out on the head of the column (tube), the mobile phase density being lower than the threshold densities of the oligomers. As the pressure was ramped and the threshold densities of the various oligomers were reached, they were solvated by the
mobile phase and migrated down column. The pressure ramp was essential to the separations. Isobaric trials either resulted in no elution or in unsatisfactory chromatograms, depending on the pressure used. Flow effects could not account for the observations made. Flow cannot render a substance soluble in a mobile phase independent of the solvent strength of the mobile phase. In SFC solvent strength is controlled by altering the mobile phase pressure. The separation cannot be affected by density gradients within the columns. Open tubular columns have no significant pressure drop and cannot, therefore, have density gradients.1 2 Lee has reported that an OTC with dimensions of 50 μm i.d. and a length of 32 meters would produce only a 3% pressure drop at 40°C and 72 atm.1 2 The columns used in the experiments described above were larger in diameter, shorter in length, and operated at higher temperatures than those described by Lee. Any pressure drops that may have existed were undoubtedly less than 3%. Attempts to measure a pressure drop across open tubular columns in our lab have proved fruitless.
List of References


CHAPTER III

AN NMR STUDY OF AOT REVERSE MICELLES IN LIQUIDS AT AMBIENT AND HIGH PRESSURE

INTRODUCTION

Little is known about the behavior of reverse micelles in pressurized liquids. Few studies have examined the effect of pressure on regular micelles\textsuperscript{1-5} or reverse micelles, even though surfactants are used in pressurized systems in a variety of applications.\textsuperscript{6} Most of the work done in this field lies in studies of reverse micelles in supercritical or near supercritical fluids.\textsuperscript{7-21} Although some fundamental observations have been reported, many questions remain unanswered. The effects of pressure on water pools within reverse micelles are unknown. Interfacial water, the intramicellar water that is bound to or closely associated with surfactant head groups and counterions, exhibits unusual characteristics relative to bulk water. These include restricted mobility, abnormal local polarity and viscosity, depressed freezing point, and unusual NMR and IR spectroscopy.\textsuperscript{22-27} Theory suggests that these properties may be affected by external pressure but there are no reports in the literature to support this assumption.

A variety of techniques have been employed in the study of reverse micelles in liquids at ambient pressure. These include dye solubilization followed by UV-vis or fluorescent spectroscopy observation of the probe molecule,\textsuperscript{2,21,24,26,28} IR and FTIR
spectroscopy, photon correlation spectroscopy, light scattering, viscosimetry, ultracentrifugation, small angle neutron scattering, synchrotron small angle x-ray scattering, positron annihilation, and vapor pressure osmometry. Several of these techniques require expensive equipment or yield results that are ambiguous or difficult to interpret. Some give information that is limited to the macroscopic features of the system and leave insights into the microscopic aspects unrevealed. Perturbation of the equilibrium reverse micellar system is problematic in spectroscopic studies that employ molecular probes. Dye solubilization requires the introduction of an additional component to the binary fluid-surfactant system. This affects the critical micelle concentration (CMC) of the system and may alter other properties as well. In addition, the lack of a "universal probe" for all solvent-surfactant systems often necessitates additional work to determine a suitable probe for the system of interest. An analytical method that is easy to use and that can provide information on all solvent-surfactant systems would facilitate studies of reverse micelles and would be of especial interest if it was amenable to high pressure studies.

Nuclear magnetic resonance (NMR) spectroscopy has also been used to study reverse micelles in liquids at ambient pressures. Using NMR nonintrusive studies of micellar size, CMC, aggregation numbers, and conformation have been successfully conducted. Information pertaining to the reverse micellar aqueous core, hydrogen bond formation, and counterion binding has been collected.

In this chapter we describe an NMR sample system that is relatively inexpensive, simple to make, and easy to use in the study of systems at modest pressures. We provide
a thorough description of two solvent-surfactant systems at ambient pressure using NMR spectroscopy. We then examine the effects of high pressure on these same two systems. Finally, we briefly describe our observations of a system in which the solvent is a mixture of benzene and carbon dioxide. This system serves as a bridge to the employment of this technique in the study of surfactant-supercritical fluid systems. The CMC of these solvent-surfactant systems is obtained by observing the chemical shift of water and surfactant protons as a function of changing surfactant concentration at a constant $w_o$, where $w_o$ is the ratio of the water concentration to the surfactant concentration, i.e., $w_o = [\text{H}_2\text{O}]$/[surfactant].

**EXPERIMENTAL SECTION**

**Materials and Methods**

The surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT or Aerosol-OT, Fluka, 98%) was selected for this study. The molecular structure of AOT is shown in Figure 7. Each of the carbon atoms is numbered to facilitate identification of the corresponding bonded protons in the NMR spectra.23,46,47 AOT was purified according to the method of Kotlarchyk38 and stored in a desiccator until used. Purified water (Barnstead Nanopure II) was used. Deuterated chloroform (CDCl$_3$, 99.8 %, Cambridge Isotope Laboratories) and perdeuterated benzene (C$_6$D$_6$, 99.6 %, Cambridge Isotope Laboratories) were used as purchased. SFC-grade carbon dioxide (99.99%, AGA Specialty and Medical Gases Division, Maumee, Ohio) was also used as purchased. Benzene-carbon dioxide solutions were made by filling an Isco (Isco, Lincoln, NE) model 2600 high-pressure syringe pump with the desired mass of C$_6$D$_6$ and by then delivering
Figure 7. The molecular structure of AOT.
the correct mass of CO₂ into the Isco from another syringe pump filled with carbon dioxide. The resulting solution was pressurized to 210 bar and allowed to equilibrate for at least 24 hours before use. Benzene-carbon dioxide solubilities were determined from the work of Kim.⁵⁰ A pressure of 70 bar is required to prevent phase separation of carbon dioxide and benzene at CO₂ mole fractions of 0.8 or greater. Lower pressures are sufficient to maintain one-phase conditions for benzene/CO₂ mixtures with lower CO₂ concentrations. Pressure in the Isco model 2600 was never permitted to drop below 130 bar. All chloroform experiments and all benzene experiments were conducted with w₀ values of 2.0 and 6.5 respectively. The experimental temperature was 40 °C for all studies.

The samples studied were made immediately before each set of NMR trials from a fresh working solution with an AOT concentration of about 200-300 mM. The newly made working solution was sonicated for 30 minutes. Ultrasonification enhances incorporation of water into reverse micelles but does not affect microemulsion stability.⁵¹ The appropriate amount of solution was added with a microliter syringe (Hamilton) to a clean NMR tube, to which then was added the correct amount of the solvent under study. The tubes were sealed, inverted at least six times, and allowed to equilibrate for at least 25 minutes.

**Ambient Pressure NMR Apparatus**

A Bruker AM-250 NMR spectrometer with a multinuclear probe head was used throughout the study. The spectrometer was field-locked on the deuterated solvent being studied prior to data collection to ensure consistent chemical shift readings from sample
to sample. The chemical shift was measured relative to that of the deuterated solvents. Standard NMR tubes (Wilmad Glass Co., Buena, NJ) were used in all ambient pressure experiments.

**High Pressure NMR Apparatus**

As in the ambient pressure studies, the spectrometer was field-locked on the deuterated solvent being studied prior to data collection. The pressures used in these studies ranged from 100 to 250 bar, although most of the work was done at 136 bar. Figure 8 shows the sample apparatus configured for this project. An Isco model 2600 syringe pump was used to pressurize the fluid being studied. Pressure was verified using a Setra (Acton, MA) model 204 pressure transducer. The pressurized fluid was transmitted from the syringe pump to a sapphire NMR tube\(^5\) (Saphikon, Milford, NH) through a 5-m long x 50-μm i.d. piece of fused silica tubing (Polymicro Technologies, Phoenix, AZ). The tubing was coupled to the NMR tube with a titanium 1/4"-1/16" reducing union (Swagelok, Solon, OH). The sapphire tube was held in the reducing union with 85%/15% vespel/graphite ferrules (Alltech, Deerfield, IL). After coupling the pump and the sapphire tube, the tube was placed in a standard NMR spinner that had been slightly modified by the insertion through its bore of a piece of glass tubing 50-mm long x 3-mm i.d., barely larger in diameter than the sapphire tubes. The upper end of the glass tubing was flared to prevent it from slipping through the spinner. This glass sleeve was necessary to prevent vibration of the sapphire tube during data collection. Small rubber o-rings were placed on the sapphire tube immediately above and below the glass sleeve to secure the tube within the spinner. After securing the tube in the spinner, the entire
Figure 8. High pressure sample apparatus.
unit was manually lowered into the spectrometer. The spinner assembly was not spun during data collection. This did not cause a noticeable degradation of spectral quality as determined by comparison of the spectra of spun and unspun liquid standards at ambient pressure.

Figure 9 shows a typical spectrum of 200 mM AOT in C₄D₆ at ambient pressure and with wₒ = 6.5. The observed ¹H NMR peaks were assigned to specific AOT backbone carbon atoms based on the previous work of others.²³,⁴⁶,⁴⁷ The water proton resonances were assigned based on direct NMR observation of two-phase systems which contained both bulk water and the water-saturated solvent. Separate peaks for bulk water and for solvated water were observed simultaneously in the collected spectra of the two-phase samples. The chemical shifts of solvated water protons in chloroform and benzene were 1.55 ppm and 0.40 ppm, respectively. The chemical shift of bulk water protons in either solvent was approximately 4.45 ppm.

RESULTS AND DISCUSSION

Ambient Pressure Experiments: H₂O Protons

The CMC or the onset of micellation is an important characteristic of surfactant-solvent systems. A knowledge of the CMC is essential to the characterization of reverse micellar solutions.³³ At surfactant concentrations below the CMC reverse micelles will not form but premicellar clusters or nuclei may exist. When the wₒ of the system is held constant and the surfactant concentration of the system is increased from trace levels to a value beyond the CMC reverse micelles form. We have observed that formation of
Figure 9. 250 MHz spectrum of 200 mM AOT in C₆D₆ (w₀=6.5) at ambient pressure and 40 °C. The multiplets have been labeled with the number of the carbon atom on the AOT molecule to which the protons are bonded.
reverse micelles produces NMR spectra that exhibit a downfield shift of the water proton chemical shift value as the surfactant concentration of the system increases. Figures 10 and 11 are plots of H$_2$O $^1$H chemical shifts in chloroform and in benzene as functions of AOT concentration.

The CMCs are extrapolated from the first region of dramatic slope change on each chemical shift curve in the two figures. The intercept of tangents drawn through the low concentration data and the region of dramatic slope change is the reported CMC. The change in the slope, d$\delta$/d[AOT], is caused by water molecules in the system passing from a solvent-associated state to a location within the reverse micelles as the interfacial water layer is formed. As the reverse micelles initially forms the H$_2$O molecules engage in the solvation of the sodium counterions and the sulfonate anions.$^{22,30}$ The water involved in these solvation interactions is integral to the development of a hydrogen bond network between the solvating water molecules and the polar head groups of the surfactant molecules. This cooperative hydrogen bonding provides the driving force in reverse micelle formation and stabilization.$^{30,32,42}$ The formation of hydrogen bonds results in a downfield shift of the water proton resonance. This water has been called "bound water"$^{48}$ or interfacial water and exhibits properties different from those of bulk water. As the size of the reverse micelles increases with added AOT at a constant $w_o$ a water core develops and eventually a three dimensional network similar to bulk water (termed "free water"$^{48}$) forms. As the fraction of free water in the reverse micelles increases, the observed chemical shift of water protons $\delta_{obs}$ continues to move downfield toward the chemical shift value of bulk water. $\delta_{obs}$ is a function of the chemical shifts of the bound
Figure 10. Changes in H₂O ³H peak position (ppm) in CDCl₃ (w₀=2.0, ambient pressure, 40°C) as a function of AOT concentration.
Figure 11. Changes in $\delta$H$_2$O $^1$H peak position (ppm) in C$_6$D$_6$ ($\omega = 6.5$, ambient pressure, 40°C) as a function of AOT concentration
water $\delta_B$, the free water $\delta_F$, and their fractional abundances $P_B$ and $P_F$, according to the equation:

$$\delta_{obs} = P_B\delta_B + P_F\delta_F$$

From equation 4, the magnitude of the slope, $d\delta/d[AOT]$, describes the rate of increase of the fraction of free water $P_F$ in the growing micelle as a function of added AOT/H$_2$O at a constant $w_o$ value. The rate at which the free water fraction $P_F$ increases controls the rate of growth of the microemulsion. The observed chemical shift of water protons should approach that of bulk water as the fraction of free water increases, i.e., when $P_F > P_B$, although the ultimate size of the aggregates is governed by system conditions. If $P_F > P_B$ occurs the water proton chemical shifts will level out. This will coincide with the conditions in which the free water predominates the observed chemical shift.

Table 9 shows a comparison of the measured CMCs of AOT in chloroform and benzene from this study and from those using other methods. The observed value for benzene agrees reasonably well with those obtained using other techniques.

The CMC of AOT in chloroform that we observed was over an order of magnitude greater than those obtained using other techniques. The disparity may be due to the tendency of AOT to form premicellar clusters or nuclei consisting of hydrogen-bonded surfactant molecules. In the studies referenced in Table 9 the techniques employed may have been unable to discriminate between premicellar aggregates and reverse micelles, being only able to detect the existence of surfactant aggregates of an indeterminate nature or size. It should also be noted that in the other
Table 9.  Reported CMC values for AOT at ambient pressure.

<table>
<thead>
<tr>
<th>solvent</th>
<th>CMC (mM)</th>
<th>( w_o )</th>
<th>technique(^2)</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroform</td>
<td>0.4 ± 0.1</td>
<td>1.0</td>
<td>TCNQ</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>NR</td>
<td>TCNQ</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>0.8 ± 0.2</td>
<td>1.0</td>
<td>UV</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>6.5 ± 0.4</td>
<td>2.0</td>
<td>NMR</td>
<td>this study</td>
</tr>
<tr>
<td>benzene</td>
<td>2.0</td>
<td>NR</td>
<td>TCNQ</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.1</td>
<td>NR</td>
<td>PA</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>NR</td>
<td>VPO</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>3.3</td>
<td>LS</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>2.2 ± 1.0</td>
<td>4.3</td>
<td>SANS</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 0.6</td>
<td>8.6</td>
<td>SANS</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>2.8 ± 0.5</td>
<td>6.5</td>
<td>NMR</td>
<td>this study</td>
</tr>
</tbody>
</table>

\(^1\) NR: \( w_o \) values were not reported in the literature
\(^2\) Abbreviations for techniques: TCNQ - solubilization of
7,7,8,8-tetracyanoquinodimethane; UV - ultraviolet absorbance of
AOT; NMR - nuclear magnetic resonance; LS - light scattering;
PA - positron annihilation; VPO - vapor pressure osmometry;
SANS - small angle neutron scattering
studies the CMC values for AOT in chloroform were determined by invasive techniques which used dye molecules to monitor the CMC. The presence of such dye molecules has been know to lower the measured CMC. A CMC of 6.3 mM for AOT in chloroform seems more reasonable than the CMC values ranging from 0.4-0.8 mM that are reported in Table 9, based on the failure of indications of hydrogen bonding to appear in the NMR spectra at these lower concentrations.

There were notable differences in the behavior of the water proton peak in the spectra of chloroform/AOT/H₂O and benzene/AOT/H₂O systems. In the chloroform/AOT/H₂O system the chemical shift of water protons advanced in a smooth continuum from the initial value, the chemical shift of water solvated by chloroform, to a chemical shift value approaching that of bulk water. The width of the H₂O ¹H peak gradually increased as the AOT concentration was elevated to a point well beyond the CMC, about 33 mM. The peak width then diminished to a value near that initially observed. This behavior can be observed in Figure 12. Before the CMC interactions of water with surfactant molecules are limited. Once the surfactant concentration reaches the CMC water preferentially interacts with surfactant molecules as reverse micelles are formed. The broadening and the downfield movement of the H₂O ¹H peak suggest that water molecules in the system are relocating in an environment that is highly restrictive to motion. This is the sort of environment experienced in the bound water layer as solvation of counterions and sulfate groups and the formation of the hydrogen bond network in the interfacial water layer takes place.
Figure 12. Changes in peak width (PWHM, in Hz) in CDCl₃ (w₀=2.0, ambient pressure, 40°C) as a function of AOT concentration.
As the AOT concentration increased in benzene the water proton peak moved slowly downfield from the initial chemical shift of water solvated by benzene, 0.4 ppm. Like the chloroform/AOT/H₂O system, this water proton peak dominated the spectra observed at low AOT concentrations. Between AOT concentrations of 2-6 mM the water proton peak became exceptionally broad and diminished markedly in size. Simultaneously in the spectra a very low, broad peak that was difficult to characterize could be observed forming around 2.0 ppm. As the AOT concentration continued to increase the upwelling intensified and narrowed into a discrete peak at around 2.5-3.0 ppm. Figure 13 shows spectra that display this behavior. The AOT concentrations at which this unusual behavior was observed include the range of reported values of the CMC of AOT in benzene. The water proton peak continued to migrate downfield with increasing AOT concentration until it reached the chemical shift value of bulk water. As it moved downfield it continued to narrow and intensify. Figure 14 shows the variation in the H H₂O peak widths as a function of AOT concentration in benzene.

Ambient Pressure Experiments: AOT Headgroup Protons

The plot of the chemical shifts of the C-1 and C-1' protons versus the AOT concentration also yields insights into the changing microenvironment of the reverse micelles. Examples of this behavior can be seen in Figures 15-18. The chemical shifts of these protons decreased with increasing AOT concentration. Significant variation in the chemical shift is observed near the onset of micellation. CMC values extrapolated from these data coincide closely to the observed water proton CMC values. A
Figure 13. AOT in C₆D₆: changes in H₂O ¹H peak position (ppm) and spectral appearance at different surfactant concentrations (ω₀=6.5, ambient pressure, 40°C). (A) 0.6 mM, (B) 2.6 mM, (C) 6.0 mM, (D) 12.0 mM
Figure 14. Changes in peak width (PWHM, in Hz) in C_{6}D_{6} (\omega_{s}=6.5, ambient pressure, 40°C) as a function of AOT concentration.
Figure 15. Chemical shift of the C-1 (in CDCl₃) AOT proton as a function of surfactant concentration.
Figure 16. Chemical shift of the C-1' (in CDCl₃) AOT protons as a function of surfactant concentration.
Figure 17. Chemical shifts of the C-1 (in C₆D₆) AOT protons as a function of surfactant concentration.
Figure 18. Chemical shifts of the C-1' (in C₆D₆) AOT protons as a function of surfactant concentration.
<table>
<thead>
<tr>
<th></th>
<th>H₂O</th>
<th>C-1</th>
<th>C-1'</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroform</td>
<td>6.5 mM (±0.4)</td>
<td>6.4 mM (±0.2)</td>
<td>6.3 mM (±0.1)</td>
</tr>
<tr>
<td>benzene</td>
<td>2.8 mM (±0.5)</td>
<td>2.2 mM (±0.5)</td>
<td>2.3 mM (±0.1)</td>
</tr>
</tbody>
</table>

Table 10. A comparison of CMC values from water, AOT C-1, and AOT C-1' protons at ambient pressure.
comparison of these results can be seen in Table 10. The C-1 and C-1' protons are the surfactant hydrogen atoms nearest the interior of the reverse micelles once they form. The chemical shift of protons on these two carbons should be expected to change appreciably as surfactant molecules move from a solvent-solvated environment to that of a reverse micelle. These observations build on the work of Ueno\textsuperscript{47} who was the first to report that AOT protons have different chemical shift values in the monomeric and the reverse micellar states in chloroform. Since the shifts of the C-1 and C-1' protons can provide information on the solution microenvironment they can also be used to evaluate the CMCs of reverse micelle systems. This is of particular advantage in situations where there is no water proton peak available to monitor, or when it is obscured by other peaks. A good example of this is the AOT/water/propane system. When attempts were made in this laboratory to monitor the CMC of AOT in supercritical propane our efforts were frustrated by the methyl and methylene resonances of propane which occurred at the same frequency as the water proton peak at low AOT concentrations. It was not possible to decouple the propane peaks without suppressing the water proton peak as well.\textsuperscript{55}

\textbf{Ambient Pressure Experiments: AOT C-4,4' Protons}

Another interesting feature of the NMR spectra is seen in the behavior of the peak assigned to the C-4 and C-4' protons of AOT. In chloroform the peak for the C-4 and C-4' protons does not appear until after the AOT concentration exceeds the CMC because it is obscured by the overlap of the water proton peak. In benzene the C-4,4' protons peak is barely distinguishable from background noise at low AOT concentrations. At about the CMC this peak increases significantly in size, becoming over an order of
magnitude larger than it was at pre-CMC AOT concentrations. This may be due to rotation around the succinic acid C-C backbone and the several different surfactant conformations that result. Observed NMR spectra are an average of the various rotamers that exist in an equilibrium that is affected by temperature and the particular solvent employed. Studies of AOT conformations in reverse micelles as a function of temperature and solvent have been made using $^1$H NMR.\textsuperscript{49} Similar investigations have examined the effects of solvent and system $w_o$ on AOT conformation in reverse micelles using $^{13}$C NMR.\textsuperscript{56,57} When the surfactant molecules are involved in reverse micelles, the preferred conformation about the succinic acid C-C backbone is a staggered rotamer which allows for optimal hydrogen bonding between the carbonyl oxygens and water. As the aqueous core of the reverse micelles grows the surfactant-water interface "softens"\textsuperscript{48} and rotation around the ethanic C-C bond becomes more facile. A trans conformation is commonly assumed by the surfactant molecules. From our data it appears the protons on the C-4 and C-4' carbons are somehow shielded from the magnetic field of the spectrometer before the reverse micelle is formed, and only as the rotamer population distribution changes to accommodate the hydrogen bonding of the interfacial water are the C-4 and C-4' protons observed in the spectra.

**Ambient Pressure Experiments: Other Observations**

Other features of the NMR spectra also indicate changes in the system environment as reverse micelles form and their aqueous cores swell. As the AOT concentration is increased from low to high concentrations the AOT hydrogen peaks split into multiplets, some of them very complex. The changes in peak splitting patterns and
peak widths as a function of surfactant concentration have been exploited by others to
shed additional insight into the changing microenvironment of the reverse micelles. In
previous studies by Ueno, Maitra, Magid, and Eicke a measure of ¹H or ¹³C
coupling constants as a function of changing wₐ yielded AOT conformational information.
In earlier work by Wong, Eicke, and Luisi the change in the half linewidths (Δνₛ) of ²³Na AOT counterion or ¹H water spectra with increasing wₐ provided insights into
counterion hydration.

High Pressure Experiments

In some initial experiments with benzene/AOT/water for the pressure range of 100
to 250 bar no difference was found in the behavior of water protons in AOT reverse
micelles.

Differences were observed when systems at ambient and high pressure (136 bar)
were compared. In the benzene/AOT/H₂O system these changes were small. While the
features of the spectra at ambient and high pressure did not differ markedly, the
concentrations at which certain phenomenon occurred changed slightly. Figure 19 shows
a comparison of the water proton chemical shifts obtained in the benzene/AOT/H₂O
system at ambient pressure and at 136 bar. A slight decrease in the CMC and an
increased slope were seen as the change from ambient to high pressure was made. At
136 bar the onset of the CMC is very clearly indicated by the severe broadening of the
water proton peak as described earlier. The observed CMC is slightly lower than that
observed at ambient pressure and is reported in Table 11. Figures 20 and 21 show the
C-1 and C-1' proton chemical shift values as a function of the log of the AOT
Figure 19. $\text{H}_2\text{O}^1\text{H}$ chemical shifts as a function of AOT concentration in C$_6$D$_6$.  ○ - ambient pressure; □ - 136 bar
Figure 20. C-1 AOT proton chemical shifts as a function of surfactant concentration in C₆D₆ at 136 bar.
Figure 21. C-1' AOT proton chemical shifts as a function of surfactant concentration in C$_6$D$_6$ at 136 bar.
Table 11. CMC values for AOT in CDCl$_3$ and C$_6$D$_6$ at ambient pressure and at 136 bar based on H$_2$O $^1$H chemical shift values.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ambient Pressure</th>
<th>136 Bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroform</td>
<td>6.5 mM (±0.4)</td>
<td>72 mM (±13)</td>
</tr>
<tr>
<td>benzene</td>
<td>2.8 mM (±0.5)</td>
<td>2.3 mM (±0.4)</td>
</tr>
</tbody>
</table>
concentration at ambient and high pressure. These plots provide CMC information that is reported in Table 12. The values are consistent with those reported in Table 10. The appearance of certain spectral features at lower AOT concentrations also indicate that the CMC may be lower at high pressure than at ambient pressure. The peak due to the C-4,4' protons appears at lower AOT concentrations than at ambient pressure, although it remains quite small until AOT concentrations near the CMC are reached. Then, as in the ambient pressure spectra, it increases dramatically in size. The transformation of the spectra from singlets to multiplets with increasing AOT concentrations is also observed at slightly lower AOT concentrations in the high pressure spectra. The slope of the curves in Figure 19 indicates that the incorporation of water in reverse micelles takes place more rapidly with an increase in AOT concentration in the pressurized system.

The behavior of AOT in the chloroform/AOT/H₂O system at high pressure is very dissimilar to that displayed at ambient pressure. Figure 22 shows the variation of the water proton chemical shift in the chloroform/AOT/H₂O system at ambient pressure and at 136 bar. An enormous increase in the CMC and a decelerated rate of reverse micelle to microemulsion transition were seen as a change from ambient to high pressure was made. At high pressure the onset of the CMC is somewhat vague. The only region of dramatic slope change has too few data points to permit reliable extrapolation, although an estimate of the CMC was made and is listed in Table 11. Data from the chemical shifts of the C-1 and C-1' protons was similarly ambiguous, indicating only that the environment of the headgroup protons remains quite constant until high surfactant
Figure 22. H$_2$O $^1$H chemical shifts as a function of AOT concentration in CDCl$_3$. O - ambient pressure; □ - 136 bar
Table 12. CMC values for AOT in C₆D₆ at 136 bar.

<table>
<thead>
<tr>
<th></th>
<th>H₂O</th>
<th>C-1</th>
<th>C-1'</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC</td>
<td>2.3 mM</td>
<td>1.3 mM</td>
<td>1.2 mM</td>
</tr>
<tr>
<td></td>
<td>(±0.4)</td>
<td>(±0.4)</td>
<td>(±0.3)</td>
</tr>
</tbody>
</table>
concentrations were reached. The slopes of the water curves in Figure 22 indicate that the incorporation of water in reverse micelles and their conversion to a microemulsion take place more slowly with changing surfactant concentration in the pressurized chloroform system. At the modest pressures studied (100-250 bar) the pressure effects on fluid density, molar volume, polarity, and other of chloroform's intensive properties should be negligible. One explanation may be presented by considering the effects of applied pressure on reverse micelle structures. It has been noted that AOT forms larger and more closely packed reverse micelles in benzene than in chloroform. It has also been observed that small changes in the packing of the polar headgroups can cause significant changes in reverse micellar size and shape and therefore in the extent of hydrogen bonding and the amount of water that may be solubilized. A variety of AOT reverse micelle structures have been observed, depending on system parameters such as the solvent, surfactant, surfactant concentration, temperature, and pH. It may be that some of these structures are more sensitive to system pressure than others. Perhaps the loose, small, reverse micelle structures assumed by AOT in chloroform are more susceptible to the effects of pressure than those preferred in benzene.

**High Pressure-Mixed Solvent Experiments**

A study of the behavior of AOT in benzene-carbon dioxide solutions was made. There were several reasons for undertaking these experiments. Organic solvents modified with significant fractions of liquified gases exhibit mass transfer properties that differ from those of the unaltered solvent. Supercritical fluids at moderate pressures exhibit viscosities 10 to 100 times lower and diffusivities 10 to 100 times greater than those of
liquids. The use of enhanced-fluidity liquids, such as benzene/CO$_2$ provides improved results in many extraction and separation techniques compared to using common liquid solvents. In several recent papers the addition of liquid carbon dioxide to methanol resulted in improvements in time of analysis and separation efficiency in high performance liquid chromatography (HPLC). Since reverse micellar phases have been used in high performance liquid chromatography and in other pressurized applications in which enhanced mass transfer is desirable, surfactant behavior in these reduced viscosity solvents is of interest. Another interesting problem is the inability of AOT to form reverse micelles in supercritical carbon dioxide. While cholesterol, 2,2,3,3,4,4,4-heptafluoro-1-butanol-$d_1$, and 1-butanol-$d_1$ have demonstrated the ability to form aggregates in supercritical CO$_2$, the inability of AOT to do likewise is not well understood. Studies on high fluidity liquid mixtures such as benzene/CO$_2$ may provide information that will help unravel the lack of AOT reverse micelle formation found in supercritical CO$_2$.

Figure 23 contains a plots of H$_2$O $^1$H chemical shifts versus AOT concentration in benzene, 50%/50% benzene/CO$_2$, and 30%/70% benzene/CO$_2$ solutions at 136 bar. The CMC for AOT in the 50%/50% benzene/CO$_2$ mixture was approximately 4.4 mM ($\pm 0.9$ mM) while the CMC for AOT in the 30%/70% benzene/CO$_2$ mixture was about 6.7 mM ($\pm 1.2$ mM). The general appearance of the spectra for water and AOT protons in the 50%/50% benzene/CO$_2$ mixture was similar to that observed for neat benzene at elevated pressures. In contrast, the water proton peak chemical shifts in the 30%/70% benzene/CO$_2$ spectra moved continuously downfield through the AOT concentrations.
Figure 23. $\text{H}_2\text{O}^1\text{H}$ peak chemical shifts as a function of AOT concentration at 136 bar.

- □ - $\text{C}_6\text{D}_6$;
- Δ - 50%/50% $\text{C}_6\text{D}_6/\text{CO}_2$;
- ○ - 30%/70% $\text{C}_6\text{D}_6/\text{CO}_2$
examined. The water proton peak was found at low AOT concentrations at about 0.9 ppm, a value which is well above the chemical shift of that for water solvated by benzene. Additionally, the C-4,4' peaks were prominent in the spectra of all samples in the 30%/70% benzene/CO$_2$ solution regardless of the AOT concentration. From these data, it is expected that AOT in liquid CO$_2$ would have a CMC higher than any of the studied mixtures.

**Conclusions**

The experiments demonstrated that, using $^1$H NMR, the extent of reverse micelle formation can be monitored as a function of hydrogen bonding. CMC information could be obtained from the change in the chemical shift of water protons in the studied systems as a function of surfactant concentration; the chemical shift behavior of the C-1,1' protons also provided CMC information. Pressure did affect the CMC and the rate of reverse micelle growth in the solvents studied, but the manner in which it these were affected was quite different in the two solvents. Increasing the volume fraction of carbon dioxide in benzene/AOT/H$_2$O systems resulted in a slowing of reverse micelle growth.
List of References


CHAPTER IV

CHARACTERIZATION OF A CAPILLARY COLUMN COATED WITH A GLASSY CARBON STATIONARY PHASE FOR SUPERCRITICAL FLUID CHROMATOGRAPHY

INTRODUCTION

As mentioned in chapter 1, the development of new stationary phases is essential to the growth and acceptance of SFC as a technique. It is not just a matter of finding stationary phases that can resist attack from supercritical fluids. Silica and alumina stationary phases and support materials have demonstrated resistance to attack from a variety of supercritical mobile phases including carbon dioxide, pentane/methanol and pentane/isopropanol mixtures, and several different chlorofluoromethanes since the early years of SFC. The principal problem with silica and alumina-based stationary phases is the strong interactions that they can potentially share with large and polar analytes. Hydroxyl groups on silica surfaces act as Lewis acid sites capable of forming donor-acceptor interactions and hydrogen bonds with analytes. Aluminum atoms on alumina surfaces can also act as Lewis acid sites in the formation of donor-acceptor interactions. Some surface deactivation techniques cause the conversion of surface silanols to surface siloxane groups which then act as Lewis base sites. Oxides on alumina surfaces are also Lewis base sites. Attempts to deactivate most common surface groups are at best only partially successful. Silanization, the most common surface treatment for silica-based
stationary phases, is about 50% effective. A principal need in SFC is for stationary phases that will not form hydrogen bonds or donor-acceptor complexes with analytes and that are resistant to attack from supercritical fluids.

Carbon, in several forms, has been employed as a stationary phase in GC and in LC. The use of graphitized carbon in chromatography can provide a nonpolar surface that interacts with either nonpolar or polar molecules exclusively through dispersive interactions. This makes the prospect of graphitic stationary phases appealing to those who practice SFC.

Until recently one drawback to the merging of carbon stationary phases and SFC column technology was the inability to form efficient capillary columns coated with graphitic stationary phases. Capillary columns are popular in SFC because of their greater efficiency, potentially faster analysis times, and lower pressure drops than packed columns. There have been several attempts to make capillary columns for use in GC by coating suspensions of pyrolitic carbon and carbon black particles on tube interiors. The resulting columns exhibited mediocre chromatographic performance due largely to incomplete coverage of the interior wall by the graphitic phase. In situ graphitization of polymeric films is untenable because of the high temperatures required, as will be seen below. A significant step forward was made when polymers capable of forming glassy carbon at relatively low temperatures (LTGC) were discovered. LTGC is a graphite allotrope similar to porous glassy carbon which has demonstrated promise in previous chromatographic studies. LTGC has been successfully exploited in the formation of packed column materials for SFC.
Physical and chemical properties of graphitic compounds

The use of carbon as a stationary phase is almost as old as chromatography itself. This is because carbon possesses many of the properties desired in a stationary phase including chemical inertness, a homogeneous surface, physical strength, and porosity. The extent to which these and other significant properties are exhibited depends on the type of carbon used.

Graphite is a term loosely employed to describe members of a family of carbon allotropes which include charcoal, soot, lampblack, amorphous carbon, carbon black, glassy carbon, randomly oriented graphite, pyrolic graphite, and highly ordered pyrolic graphite. While these substances all employ sp² hybridization and have similar bulk bond lengths, they can be distinguished between on the basis of differences in the fundamental physical aspects of their crystal lattices. The significant varying dimensions are the mean length of the microcrystallite along an axis in the plane of the graphite lattice (Lₐ), the thickness of the stack of hexagonally ordered planes (Lₜ), and the distance between individual planes in the microcrystallites (d₀₀₂).

The adsorbent properties of carbon are a function of its physical structure and surface homogeneity. Carbon allotropes of chromatographic interest exhibit a high degree of porosity and large intraparticulate surface areas. The surface area is directly related to the porosity of the particle since an active carbon particle is extensively riddled with pores of various sizes. Micropores, with diameters of 2 nm and less, and mesopores, with diameters between 2 and 50 nm, provide the bulk of the surface of active carbon particles. Macropores, with diameters in excess of 50 nm, also contribute to the
intraparticulate surface area but are more important as passageways from the exterior of the particle to the micro and mesopores within.\textsuperscript{17} It should also be noted that the mechanical strength of carbon particles is directly related to their porosity.\textsuperscript{17}

Graphitization refers to the process of converting amorphous carbon compounds to three dimensionally ordered substances.\textsuperscript{18} This is generally accomplished by heating the material to be graphitized to temperatures of 2500-3000 °C. During the formation of the various graphitic substances most noncarbon elements are eliminated as gaseous byproducts. The carbon atoms that remain form randomly crosslinked stacks of flat graphitic planes.\textsuperscript{19} Although the lengths $L_\alpha$ and $L_\epsilon$ are small, they are finite. As a consequence graphitic carbons have two types of surfaces. The face of the plane of graphite polyhedra is called the basal plane. The exposed edges of the planes of graphitic carbon atoms are called the edge plane.

The basal plane is thought to be free of unsaturated bonds (with the exception of the delocalized carbon electrons in the graphite structure), functional groups, or ions\textsuperscript{20} although recent work has demonstrated that hydrogen can chemisorb to the basal plane surface.\textsuperscript{21} Nonspecific adsorption of analyte molecules results and is generally attributable to dispersive interactions, although a slight inductive effect can be observed when the adsorbed molecules possess a large dipole moment.\textsuperscript{20}

The edge plane is not nearly as tidy as the basal plane. The random crosslinking of the graphitic planes results in the creation of unpaired electrons and unfilled valence shells.\textsuperscript{19} Oxygen and hydrogen, the most common and also the most abundant contaminants of graphite, can bond at the edge plane and form a variety of surface groups
including hydroxyl, carboxyl, anhydride, quinone, peroxide, aldehyde, and lactone groups.\textsuperscript{19,22} Oxygen and hydrogen can also interact with edge plane carbon atoms and form heterocyclic ring structures that are incorporated into the graphite planes.\textsuperscript{19} The functional groups formed can behave as Lewis acids or bases, depending on their chemical structure, and are capable of strong donor-acceptor interactions that significantly impact adsorption onto the carbon surface. The number of polar sites on the edge plane is relatively small when compared to the number of nonpolar basal plane surface sites. However, the strength and specificity of the interactions that occur between the polar sites and polar molecules can significantly impact observed adsorption behavior.\textsuperscript{23}

**The Use of Carbon in Chromatography**

Most forms of carbon are unsatisfactory for use in chromatography although historically several forms of carbon have been employed as stationary phase materials in gas chromatography (GC) and in liquid chromatography (LC).\textsuperscript{8} Charcoal was widely used as a chromatographic adsorbent through the 1950s.\textsuperscript{6,8} Its popularity has declined because of the difficulty of obtaining consistent chromatographic behavior from one batch of charcoal to the next.\textsuperscript{8,24}

Graphitized carbon black or graphitized thermal carbon black (GTCB) stationary phases continue to demonstrate outstanding performance in the GC separation of oxygenated compounds, sulfur-containing compounds, and geometric isomers.\textsuperscript{24} They can, under the correct conditions, provide the advantages of superior mass transfer and a concomitant lessening of analysis time, increased mobile phase velocities, higher operating temperatures, and improved column stability, i.e., an elimination of stationary
phase bleed, relative to stationary phases that rely on partitioning as the separation mechanism. The formation of GTCB involves heating carbon black at a temperature of 3000°C under an inert gas.23,25 The high temperature causes a reordering of the amorphous carbon black to a more ordered graphitic structure with increased basal and edge plane surfaces. The extent of graphitization is dependent on the type of carbon black used.23 While graphitization introduces a much higher degree of homogeneity to the carbon, it also causes diminished surface area and increased fragility of the material.7,24 These conditions often reduce the usefulness of GTCB in high pressure separation techniques such as high pressure liquid chromatography (HPLC) and SFC applications.

Even with the increased surface homogeneity demonstrated by GTCB stationary phases, surface contaminants can interfere with the effective separation of some analytes. The use of GTCB columns with deactivated surfaces is common. The deactivation has been accomplished by several means. Addition of a small amount of nonvolatile polar liquid (under the operating conditions in use) can minimize the effect of surface groups and improve chromatographic performance.23,26 Phosphoric acid treatments can be used to condition GTCB surfaces prior to the analysis of organic acids.27 Treatment of the graphitic stationary phase with hydrogen at 100°C has been reported to "remove chemisorbed oxygen" (sic), reduce surface acidity, and reduce traces of metals on the surface.24 The elimination of topographic irregularities, which can also affect chromatographic behavior, has been reported by employing a hydrogen treatment at 1000°C.23 Ironically, while this hydrogen treatment has empirically demonstrated
effectiveness in improving chromatographic behavior, hydrogen chemisorbs to carbon surfaces more strongly than oxygen.28,29

Attempts have been made to improve the suitability of GTCB stationary phases for HPLC by improving their physical durability. The heating of benzene vapors at 900° C in the presence of a sample of GTCB caused deposition of a layer of pyrolitic carbon on the GTCB.30,31,32 Graphitization of the resulting material brought slight improvement in chromatographic performance over the original GTCB phase. Increased physical strength of the composite material was not observed.

The construction of a porous glassy carbon (PGC) stationary phase was also a result of efforts to find a more durable carbonaceous stationary phase that could be used in HPLC.12,33 Glassy carbon is hard and brittle, unlike graphitic carbons, and it does not become graphitic at high temperatures.33 It is, however, sufficiently durable to withstand the high pressures of HPLC and SFC. This is attributed to glassy carbon’s structure, which consists of hexagonally arranged sp2 hybridized carbon atoms that form long narrow ribbons that are oriented in a random manner and are extremely intertwined.34 A sketch of the proposed structure of PGC can be seen in Figure 24. The random interconnections of the entangled ribbon-like molecules prevents the structural conversion to a more graphitic structure, even at temperatures in excess of 2500° C.34

The use of graphitic stationary phases in SFC has recently begun to receive attention.13,14,35 In these studies columns packed with a porous glassy carbon material demonstrated the ability to withstand the rigorous conditions employed in SFC and also provide efficient separations of a variety of small polar analytes. Several different carbon
Figure 24. A proposed structure for PGC.
dioxide-modifier systems were characterized in these studies with respect to the importance and extent of various intermolecular interactions between the graphitic stationary phase and the organic modifiers.

In one study the manufacture and brief performance description of a one meter capillary column with an LTGC stationary phase was also described. The advantages of capillary columns over packed columns were discussed briefly above. It is the purpose of this chapter to present the characterization and evaluation of a 30 meter capillary column coated with an LTGC stationary phase. This column is a prototype and is not available commercially. The behavior of the LTGC stationary phase in both GC and SFC was observed. Conclusions about the nature of the LTGC surface and the suitability of the column for chromatography are provided.

**EXPERIMENTAL**

**Preparation of a Low-Temperature Glassy Carbon Column**

A 30 meter wall-coated open tubular column with an inner diameter of 530 μm was prepared by Restek Corporation (Bellefonte, PA) in the following manner. A polymer previously described for the low temperature production of porous glassy carbon (PGC) was deposited as a thin layer of film on the interior wall of a 30 meter piece of Silcosteel® fused silica tubing (Restek) using the static coating method as has been characterized elsewhere. The polymer was graphitized by heating the column gradually to a temperature of 600 °C under a hydrogen atmosphere and holding it at that temperature overnight. This procedure was repeated several times, resulting in a coating of PGC on the interior wall of the column about 8 μm thick.
**Gas Chromatographic System and Materials**

GC characterization work was conducted on an HP 5890A gas chromatograph with a standard Hewlett-Packard 5890 flame ionization detector. A split injector operating at a 40:1 split ratio was used. Ultra high purity helium (Gas Technics, North Royalton, OH) served as the carrier gas. All separations were conducted at 325 °C. The solutes studied were purchased from Aldrich Chemical Co. and were sufficiently pure (96% or better) to be used without further treatment. Standard solutions of the solutes in carbon disulfide at a concentration of 5 mg/mL were used to generate the data examined in these experiments.

**Supercritical Fluid Chromatographic System and Materials**

The same HP 5890A gas chromatograph and FID were used for the SFC experiments described below. Samples were injected using a Valco W-series injection valve fitted with a 200 nL injection loop (Valco Instruments, Houston, TX). An Isco LC-2600 precision syringe pump (Isco, Lincoln, NE) was used to deliver SFC-grade carbon dioxide (99.99%, AGA Specialty and Medical Gases Division, Maumee, Ohio) which was used as purchased. Most trials were conducted at a pressure of 4000 psi (272 atm) and a temperature of 180 °C which resulted in a mobile phase density of 0.39 g/cm³. Mobile phase flow was regulated with either home-made integral restrictors or with short lengths of 15 μm i.d fused silica tubing. Standard solutions of the solutes in carbon disulfide or methylene chloride at a concentration of 25-50 mg/mL were used to generate the data examined in these experiments.
**Data Treatment**

Capacity factors were determined from collected chromatograms. Retention models were then developed from multivariate linear regression analysis of the capacity factor data using SYSTAT software (SYSTAT, Inc., Evanston, IL). The following statistics are deemed necessary for the thorough analysis of the results of multivariate regression analysis:

1. **R** - the coefficient of multiple correlation. This value is analogous to $r$, the correlation coefficient in simple regression analysis. $R$ values range from 0 to 1; as with $r$, the nearer the value to 1, the better the correlation.

2. **s** - the standard deviation from regression. SYSTAT provides standard error of estimate values which conceptually are similar to the standard deviation in that both give a measure of the standard distance. The standard error of estimate is a measure of the standard distance between a regression line and the actual data points.

3. **t-tests** - values generated for each coefficient by the software were compared to published statistical tables at the 95% confidence level. The coefficient was considered significant if its t-test value exceeded the $t_{0.05}$ value.

4. **F-test** - values were generated for each model by the modeling software and compared to published statistical tables at the 95% confidence level. The model was considered significant if its F-test value exceeded the $F_{0.05}$ value.
(5.) \( r_y \) - cross-correlation coefficients give an indication of the uniqueness of the information provided by the independent variables. Independent variables with an \( r_y > 0.8 \) are considered to provide statistically similar information. Conversely, cross-correlation coefficients less than 0.8 indicate that the independent variables provide statistically different information. Values for the independent variables used in the models presented below were always less than 0.7.

In addition to the five variables listed above, \( R^2_a \), the adjusted coefficient of multiple determination was evaluated. Addition of independent variables will generally result in an increase in \( R \) which detracts from its reliability as an indicator of the goodness of fit of the model. \( R^2_a \) can decrease if a model employs too many independent variables because the increase in the fit of the model is offset by a concomitant loss of degrees of freedom. This suggests that \( R^2_a \) is a more accurate indicator than the coefficient of multiple correlation \( R \) as a measure of the goodness of fit of a particular model.39

RESULTS AND DISCUSSION

Retention Mechanisms in Adsorption Chromatography:

A Preliminary Discussion

Retention in GC, LC, and in SFC is most commonly achieved through one of two means: either the solute partitions into and out of a liquid film coated on a support material or on the interior wall of a column, or the solute adsorbs onto and desorbs off of a solid surface.7 Each technique offers distinct advantages over the other. Partitioning
is a common means of retention but its theory does not describe solute behavior on graphitic surfaces and hence will not be considered here.

The theory behind analyte retention on adsorptive surfaces in SFC and a discussion of the parameters that affect retention has been described in detail elsewhere.\textsuperscript{14,36} The relationship between retention and LTGC is described by the equation

$$\ln k' = A + B\Omega + C\pi^* + D\beta + E\alpha$$

(5)

where $A$ is a constant, $\Omega$ is the dispersive energy parameter and $B$ is its coefficient, $\pi^*$ is a measure of solute dipolarity and $C$ is its coefficient, $\beta$ describes the Lewis basicity of a solute and $D$ is the Lewis basicity term coefficient, and $\alpha$ is the Lewis acidity term with $E$ the term coefficient. Previous studies have shown that in SFC retention on LTGC relies principally on dispersive interactions between the stationary phase and analytes.\textsuperscript{14,36} Solute dipolarity/polarizability is of lesser importance and Lewis acid-base characteristics have the least effect on observed retention in SFC. The work of others has also identified that the importance of each parameter and the magnitude of its coefficient depends on characteristic differences between the different types of adsorptive stationary phases.\textsuperscript{36}

**Factors Affecting Retention: Gas Chromatography**

Prior to shipping the LTGC column to Ohio State University, Restek successfully used it for the GC separation of, in order of elution, carbon monoxide, carbon dioxide, methane, ethane, ethene, and ethyne at an operating temperature of 60 °C.\textsuperscript{40} The elution order of the C$_2$ hydrocarbons varies from several published in other studies. On charcoal the order ethyne < ethene < ethane was previously observed.\textsuperscript{41,42} It should be noted that
this elution order coincides with the molecular weights and the boiling points of the analytes. A similar pattern was obtained on a graphitized carbon black stationary phase and also on a carbon molecular sieve stationary phase. As will be elaborated below, this difference might serve as a preliminary indication that the retention mechanism of the Restek column is not the same as that which is normally expected of a graphitic stationary phase.

Our GC studies further illuminated the unexpected behavior of the Restek column. When attempts were made to elute simple nonpolar or slightly polar solutes at 60 °C the efforts were unsuccessful. It was found that an operating temperature of 325 °C or better was necessary to make the elution of simple analytes such as benzene possible. Even at this temperature many of the solutes tested were permanently retained by the column and did not elute. To a certain extent the need for a temperature in excess of 60 °C was anticipated. It is common on other graphitic stationary phases to have to use operating temperatures well above the boiling points of the analytes examined. Temperatures in excess of 325 °C were difficult because of a 400 °C maximum temperature ceiling on the gas chromatograph. Some of the composite graphite-vespel ferrules used in system connections became crumbly and of dubious worth after prolonged exposure to temperatures in excess of 375 °C. At temperatures below 325 °C retention times increased drastically - if, in fact, the analytes would elute at all - and peak shape deteriorated severely.

The analytes used in the GC studies are listed in Table 13 along with characteristic properties of each analyte that may affect their retention on the LTGC stationary phase.
Capacity factors are also listed for each analyte in Table 13. These substances were selected because of their successful performance in previous characterization studies. Substances that were tested but that would not elute are listed in Table 14.

Several general trends distinguish the permanently retained solutes from those that eluted. Molecular size affected retention. Larger molecules were more retained than smaller molecules. The C₅ through C₉ members of a homologous alkane series eluted from the column with a retention order based on carbon number, i.e., the larger the alkane the greater the retention time. The C₁₀ member of the series, decane, did not elute. The solutes in a homologous series of alkylbenzenes behaved similarly. The C₆ through C₉ members of the family showed increased retention as the number of carbon atoms in the solute increased. C₆ and C₉ alkylbenzene isomers were retained about the same as the straight chain alkylbenzene solute with a similar carbon number, e.g., ethylbenzene, styrene, and m-xylene had similar retention times. The C₁₀ and C₁₁ alkylbenzene compounds, butyl- and pentylbenzene, did not elute. Several solutes with either nitrile or amine functional groups eluted, as did pyridine. Alkyl-substituted anilines would not elute. All analytes with an oxygen atom in either a carbonyl or hydroxyl group did not elute. Benzyl alcohol was the lone exception, and it has been noted by others doing similar studies that this solute behaves in a manner inconsistent with that of other alcohols.

The elution of the alkane and alkylbenzene series in order of carbon number is neither unprecedented nor unexpected on a graphitic stationary phase. There are, however, published reports of those analytes which would not elute on the Restek column.
Table 13. Capacity factors and other parameters of the analytes used in GC experiments. *** Indicates that no literature value was available.

a Capacity factor values. 

b Heat of adsorption (kJ/mole) of analytes on graphitized thermal carbon black. 

c Heat of vaporization (kJ/mol) values of analytes at 25 °C. 

d Boiling point (°C). 

e Molar volume (cm³/mole) calculated from molar mass and density data. 

f Solute dipolarity-polarizability parameter. 

** Hydrogen bond acceptor basicity. 

<table>
<thead>
<tr>
<th>Compound</th>
<th>(k^{12})</th>
<th>(H_{\text{ads}})</th>
<th>(H_{\text{vap}})</th>
<th>BP</th>
<th>(V_m)</th>
<th>(\pi^*)</th>
<th>(\beta^b)</th>
<th>(\alpha^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aniline</td>
<td>18.8</td>
<td>54.4</td>
<td>55.8</td>
<td>184.0</td>
<td>91.2</td>
<td>0.73</td>
<td>0.50</td>
<td>0.26</td>
</tr>
<tr>
<td>benzene</td>
<td>1.5</td>
<td>41.0</td>
<td>33.8</td>
<td>80.1</td>
<td>89.4</td>
<td>0.59</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>benzonitrile</td>
<td>17.0</td>
<td>***</td>
<td>***</td>
<td>191.1</td>
<td>103.1</td>
<td>0.90</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>4.4</td>
<td>***</td>
<td>***</td>
<td>205.5</td>
<td>103.8</td>
<td>0.99</td>
<td>0.52</td>
<td>0.39</td>
</tr>
<tr>
<td>carbon disulfide</td>
<td>0.0</td>
<td>***</td>
<td>27.5</td>
<td>46.3</td>
<td>60.4</td>
<td>0.51</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td>6.4</td>
<td>45.2</td>
<td>41.0</td>
<td>131.7</td>
<td>101.7</td>
<td>0.71</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>cyclohexane</td>
<td>1.7</td>
<td>36.4</td>
<td>33.0</td>
<td>80.7</td>
<td>108.1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>cyclopentane</td>
<td>0.5</td>
<td>36.0</td>
<td>28.5</td>
<td>49.3</td>
<td>94.0</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>9.7</td>
<td>53.1</td>
<td>42.1</td>
<td>136.2</td>
<td>122.5</td>
<td>0.53</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>fluorobenzene</td>
<td>1.7</td>
<td>38.1</td>
<td>33.5</td>
<td>84.7</td>
<td>93.9</td>
<td>0.62</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>heptane</td>
<td>4.8</td>
<td>52.3</td>
<td>36.6</td>
<td>98.4</td>
<td>146.6</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>hexane</td>
<td>1.8</td>
<td>43.5</td>
<td>31.6</td>
<td>68.7</td>
<td>130.7</td>
<td>-0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>mesitylene</td>
<td>19.7</td>
<td>62.8</td>
<td>47.5</td>
<td>164.7</td>
<td>139.2</td>
<td>0.47</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>nonane</td>
<td>27.6</td>
<td>61.9</td>
<td>46.4</td>
<td>150.8</td>
<td>178.7</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>octane</td>
<td>11.7</td>
<td>56.1</td>
<td>41.5</td>
<td>125.7</td>
<td>162.6</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>pentane</td>
<td>0.6</td>
<td>37.2</td>
<td>26.4</td>
<td>36.1</td>
<td>115.2</td>
<td>-0.08</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>23.0</td>
<td>59.8</td>
<td>46.0</td>
<td>159.2</td>
<td>139.4</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>pyridine</td>
<td>4.9</td>
<td>42.3</td>
<td>40.2</td>
<td>115.2</td>
<td>80.86</td>
<td>0.87</td>
<td>0.64</td>
<td>0.00</td>
</tr>
<tr>
<td>styrene</td>
<td>10.2</td>
<td>***</td>
<td>41.5</td>
<td>145.1</td>
<td>115.0</td>
<td>0.55</td>
<td>0.18</td>
<td>0.00</td>
</tr>
<tr>
<td>toluene</td>
<td>4.2</td>
<td>50.2</td>
<td>37.8</td>
<td>111.0</td>
<td>106.4</td>
<td>0.55</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>m-xylene</td>
<td>10.1</td>
<td>67.8</td>
<td>42.6</td>
<td>139.1</td>
<td>122.3</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 14. Solutes that would not elute under GC conditions.

<table>
<thead>
<tr>
<th></th>
<th>$H_{ads}$</th>
<th>$H_{vap}$</th>
<th>BP</th>
<th>$V_m$</th>
<th>$\pi^*$</th>
<th>$\beta$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetophenone</td>
<td>54.4</td>
<td>58.3</td>
<td>202.1</td>
<td>117.4</td>
<td>0.90</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>biphenyl</td>
<td>***</td>
<td>54.0</td>
<td>255.0</td>
<td>155.2</td>
<td>1.18</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>1-bromononane</td>
<td>***</td>
<td>***</td>
<td>201.0</td>
<td>191.1</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>64.0</td>
<td>49.8</td>
<td>183.3</td>
<td>156.0</td>
<td>0.49</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>cis-stilbene</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>cyclohexanone</td>
<td>46.9</td>
<td>45.1</td>
<td>155.7</td>
<td>103.6</td>
<td>0.76</td>
<td>0.53</td>
<td>0.00</td>
</tr>
<tr>
<td>cyclopentanone</td>
<td>***</td>
<td>42.7</td>
<td>130.6</td>
<td>88.5</td>
<td>0.76</td>
<td>0.52</td>
<td>0.00</td>
</tr>
<tr>
<td>decane</td>
<td>69.9</td>
<td>51.4</td>
<td>174.1</td>
<td>194.9</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>N,N'-diethylaniline</td>
<td>***</td>
<td>***</td>
<td>216.3</td>
<td>160.4</td>
<td>0.86</td>
<td>0.43</td>
<td>0.00</td>
</tr>
<tr>
<td>2,4-dimethylphenol</td>
<td>***</td>
<td>***</td>
<td>211.0</td>
<td>118.9</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>hexanol</td>
<td>50.2</td>
<td>61.9</td>
<td>157.5</td>
<td>124.82</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>o-nitrotoluene</td>
<td>***</td>
<td>***</td>
<td>222.0</td>
<td>118.0</td>
<td>0.97</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>pentanophenone</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>164.2</td>
<td>0.83</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>pentylbenezene</td>
<td>***</td>
<td>52.9</td>
<td>202.2</td>
<td>172.5</td>
<td>0.47</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>phenol</td>
<td>54.4</td>
<td>55.7</td>
<td>181.8</td>
<td>89.0</td>
<td>0.72</td>
<td>0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>3-phenyl-1-propanol</td>
<td>***</td>
<td>***</td>
<td>235.0</td>
<td>135.1</td>
<td>0.60</td>
<td>0.55</td>
<td>0.60</td>
</tr>
</tbody>
</table>
being successfully separated on other carbon stationary phases and at lower operating
temperatures than those that were necessary in this study. The separation of decane from
other straight chain alkanes at 150°C on a GTCB stationary phase has been observed.\textsuperscript{20}
The separations of series of alcohols ranging from C\textsubscript{1} to C\textsubscript{10} on graphitized carbon black
stationary phases have been reported.\textsuperscript{20,54} The C\textsubscript{1} through C\textsubscript{4} alcohols were separated at
91°C, the C\textsubscript{5} through C\textsubscript{10} alcohols at 250°C, and the C\textsubscript{1} through C\textsubscript{9} series by using a
programmed temperature run from 50-180°C. GTCB stationary phases have also been
used in the separation of phenol and the three cresol isomers and in the isolation of
aniline, N-methylaniline, and N,N'-dimethylaniline; both were performed at temperatures
below 200°C.\textsuperscript{53} In one report ortho-, meta-, and para-terphenyl were separated on a
20 cm GTCB column at 360 °C.\textsuperscript{54} These compounds are one phenyl group larger than
biphenyl which was unsuccessfully attempted on the Restek column.

Multivariate linear regression analysis of the data was performed in an attempt to
clarify those factors most significant to retention. Models were generated using the
capacity factors and the solute parameters listed in Table 13. Several variables in
addition to those described in eqn 5 were considered during analysis of the data. These
include the heat of adsorption (H\textsubscript{a}), the heat of vaporization (H\textsubscript{v}), and the boiling points
of the analytes. Capacity factors for the analytes listed in Table 13 were used as the
dependent variables. The other parameters listed in Table 13 were used as independent
variables. Values for H\textsubscript{a}, H\textsubscript{v}, BP, and V\textsubscript{m} were scaled before inclusion in the modeling
studies by dividing them by the largest listed value for each. This was done so that
comparisons of the model coefficients would be valid. The \(\pi^*\) values used in the
modeling studies were adjusted by subtracting the published value of pentane (-0.08) from each. This again was done to bring the $\pi^*$ values used in closer agreement with other parameters used in these studies to improve the validity of comparison between parameter coefficients. Modeling was done twice, once with all of the listed variables, and once without consideration of the $\alpha$ values. Only two of the examined solutes have nonzero $\alpha$ values and these became points of high leverage during the calculations, resulting in models that were "bent" to fit these two points. As a consequence the results of the second modeling series are those presented below. All of the possible combinations of the other independent variables were tested. An appreciable number of the resulting models were discarded for one of two reasons:

1. the model contained combinations of two or more independent variables with high degrees of cross-correlation ($r_{ij} > 0.7$), indicating that the independent variables conveyed similar information. This included the following combinations: $r_{ij}$ for $H_a$ and $H_v$ - 0.856; $r_{ij}$ for $H_a$ and BP - 0.850; $r_{ij}$ for $H_v$ and BP - 0.995.

2. One or more of the coefficients had t-test values with an absolute value less than two, or, the tolerance of the coefficient (the ratio of the coefficient and its standard deviation) did not exceed 0.1. The disregarding of coefficients that do not exceed these two criteria is recommended by statistical experts at SYSTAT. Models were also discarded if the t-test value of one or more coefficients was not statistically significant at the 95% confidence level.
Table 15. Retention models for Restek column using gas chromatography. These data were obtained from multivariate analysis of the capacity factors of the analytes and their various parameters listed in Table 13. Oven temperature: 325 °C. Carrier gas: Helium. Flow rate: 7 cm³/min.

<table>
<thead>
<tr>
<th>model #</th>
<th>ln k' =</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>9.592 Hᵥ + 1.543 Ω - 6.090</td>
</tr>
<tr>
<td>2.</td>
<td>3.666 BP + 4.350 Ω + 1.102 π* - 3.848</td>
</tr>
<tr>
<td>3.</td>
<td>11.317 Hᵥ - 0.557 π* - 5.995</td>
</tr>
<tr>
<td>4.</td>
<td>5.172 BP + 2.318 Ω - 2.834</td>
</tr>
<tr>
<td>5.</td>
<td>10.665 Hᵥ - 5.772</td>
</tr>
</tbody>
</table>
Statistics for the models presented in Table 15.  

<table>
<thead>
<tr>
<th>model #</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>t: #1&lt;sup&gt;c&lt;/sup&gt;</th>
<th>t: #2&lt;sup&gt;d&lt;/sup&gt;</th>
<th>t: #3&lt;sup&gt;e&lt;/sup&gt;</th>
<th>t&lt;sub&gt;05&lt;/sub&gt;&lt;sup&gt;g&lt;/sup&gt;</th>
<th>R&lt;sup&gt;h&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;&lt;i&gt;i&lt;/i&gt;&lt;/sub&gt;</th>
<th>s&lt;sup&gt;j&lt;/sup&gt;</th>
<th>F&lt;sup&gt;k&lt;/sup&gt;</th>
<th>F&lt;sub&gt;95&lt;/sub&gt;&lt;sup&lt;l&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>16</td>
<td>13</td>
<td>23.47</td>
<td>5.04</td>
<td>***</td>
<td>2.16</td>
<td>0.993</td>
<td>0.984</td>
<td>0.156</td>
<td>475.6</td>
<td>3.81</td>
</tr>
<tr>
<td>2.</td>
<td>18</td>
<td>14</td>
<td>6.65</td>
<td>5.82</td>
<td>2.97</td>
<td>2.14</td>
<td>0.992</td>
<td>0.980</td>
<td>0.173</td>
<td>283.4</td>
<td>3.34</td>
</tr>
<tr>
<td>3.</td>
<td>16</td>
<td>13</td>
<td>22.70</td>
<td>3.16</td>
<td>***</td>
<td>2.16</td>
<td>0.989</td>
<td>0.974</td>
<td>0.202</td>
<td>282.1</td>
<td>3.81</td>
</tr>
<tr>
<td>4.</td>
<td>18</td>
<td>15</td>
<td>19.35</td>
<td>6.21</td>
<td>***</td>
<td>2.13</td>
<td>0.987</td>
<td>0.970</td>
<td>0.213</td>
<td>276.4</td>
<td>3.68</td>
</tr>
<tr>
<td>5.</td>
<td>16</td>
<td>14</td>
<td>18.36</td>
<td>***</td>
<td>***</td>
<td>2.14</td>
<td>0.980</td>
<td>0.957</td>
<td>0.259</td>
<td>337.2</td>
<td>4.60</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of analytes tested.  
<sup>b</sup> Number of degrees of freedom, N = n - (number of independent variables) - 1.  
<sup>c</sup>d<sup>f</sup> Absolute value of the t-test for the 1st, 2nd, 3rd, and 4th coefficients of the model. Shaded data indicate coefficients that are not statistically significant based on t-test values.  
<sup>g</sup> t-test value from statistical tables for the 95% confidence level.  
<sup>h</sup> Coefficient of multiple correlation.  
<sup>i</sup> Adjusted coefficient of multiple determination.  
<sup>j</sup> Standard error of estimate.  
<sup>k</sup> F-test value for model equation.  
<sup>l</sup> F-test value from statistical tables for the 95% confidence level.
Figure 25. A plot of $\ln k'_\text{obs}$ versus $\ln k'_\text{pred}$ by GC model #1. $r = 0.99$
All viable models with $R^2$ values that exceed 0.95 can be seen in Table 15. Statistics for the models are presented in Table 16. The F-test values are statistically significant at the 95% confidence level for all of the models presented. As stipulated above, all of the coefficients are statistically significant at the 95% confidence level. Estimates of the adjusted coefficient of multiple correlation range from about 0.96 to 0.99 which indicates a high degree of goodness of fit for the corresponding models. A plot of observed retention versus retention predicted by model #1 can be seen in Figure 25. The correlation coefficient of the line in Figure 25 is very high, $r = 0.99$, indicating that the model does an excellent job of predicting retention for the solutes used in the GC studies. Plots of observed retention versus predicted retention for the other four models also resulted in very good fits with correlation coefficients of 0.98 to 0.99 for each.

The magnitude and the sign of each of the coefficients in the models reveals information about retention on the Restek column. The A term in each model is an indication of the offset necessary during the multivariate linear regression to generate a y-intercept that approaches zero.\textsuperscript{16} The retentive nature of the stationary phase is indicated by their negative values; the magnitude of the retention is directly proportional to the absolute value of the A term.\textsuperscript{36} The magnitude of the other coefficients is directly related to the significance of the contribution of their particular parameter to retention. A negative coefficient indicates that the larger a particular parameter is for an analyte the greater its effect on lessening retention. A positive coefficient signifies that the larger that a parameter is for a solute the greater is its contribution to retention.
Dispersive interactions are those principally responsible for retention on graphitic stationary phases in GC.\(^6\) The observed retention behavior of the alkane and alkylbenzene series is more closely related to the polarizability of the molecules than their molecular weight.\(^{20}\) The energy of the dispersive interactions between two molecules and its dependence on their polarizabilities is described by the equation\(^5\)

\[
\varepsilon = -\frac{3}{2} \left( \frac{\alpha_1 \alpha_2}{r^6} \right) \left( \frac{I_1 I_2}{I_1 + I_2} \right)
\]

where \(\varepsilon\) is attractive interaction energy between two atoms resulting from dispersion forces, and \(\alpha\) and \(I\) are the polarizability and the first ionization energy of the atoms respectively. A linear relationship between retention and the heat of adsorption on graphitic thermal carbon black has been demonstrated.\(^{58}\)

On the Restek column the modeling studies indicate that retention of the studied solutes was most influenced by their heat of vaporization. This is emphasized by the large coefficients for the \(H_v\) parameter in models \#1, \#3, and \#5. The adjusted coefficient of multiple determination for model \#5, which models solute retention exclusively on their \(H_v\) values, does not differ much from the \(R^2\) values for models \#1 and \#3 which use a second parameter. Contributions are made to solute retention by the molar volume (\(\Omega\)) term in model \#1. Solute dipolarity/polarizability terms played a minor role in retention in model 3#. Tendencies towards Lewis base behavior did not seem to contribute to retention in the solutes studied.
The boiling point and the heat of vaporization of nonpolar or slightly polar substances are also closely related. This is indicated not only by the high degree of cross-correlation in the GC experiments ($r_{ij}$ for $H_v$ and BP: 0.995) but also in a more general sense by the Hildebrand rule:

$$\Delta H_{298}^v (\text{cal/mole}) = -2950 + 23.7 \ T_b + 0.020 \ T_b^2$$  

(7)

which predicts that the heat of vaporization for a nonpolar or slightly polar substance at 25 °C is directly proportional to its boiling point $T_b$.

Although $H_v$ or BP coefficients were the largest in the five models, this does not necessarily mean that solute vaporization or boiling point are the factors that control retention on the Restek column. Most of the solutes studied in the GC experiments are nonpolar or slightly polar and therefore are capable of dispersion interactions and little else. For these solutes the energies correlated to their BP or $H_v$ seem to be better indicators of the energy of the dispersive interactions with the LTGC surface than $H_a$ values that were obtained from studies on a surface that may be dissimilar to that in the Restek column. It can be seen in Table 13 that the values of the heat of adsorption and the heat of vaporization are similar for all of the analytes examined in these studies; the heat of adsorption value is never more than 1.5 times greater than the heat of vaporization value for the same solute. The $H_a$ values used are the result of observations made on GTCB, a surface with relatively few active sites. Given the similarity of the energies involved, it is thought that dispersive interactions control retention in the Restek column.
but that these interactions are better modeled by the lower energies conveyed by the solute heat of vaporization values.

**Factors Affecting Retention: Supercritical Fluid Chromatography**

Initial SFC experiments on the Restek column began rather inauspiciously. After numerous unsuccessful attempts to elute neat pentane or carbon disulfide had been made on the 30 meter column, it was decided that the retentive nature of the stationary phase made it necessary to use a shorter length of column. A three meter section of the column was used for some initial trials, but it, too, was too long. The use of one meter sections of the column made it possible to elute numerous analytes of interest.

Several ongoing problems made the SFC studies of the one meter Restek column difficult. The first was caused by the large diameter of the column. Most capillary columns used in SFC have inner diameters of 50-250 μm, less than half the diameter of the Restek column. While using the Restek column at linear flow rates of 2 cm/sec or greater the volume of carbon dioxide exiting the restrictor was so great that it was generally difficult or impossible to keep the flame in the FID lit. By adjusting the flame gas ratio it was possible to work at linear flow rates around or slightly greater than 2 cm/sec, but with an attendant sacrifice in detector sensitivity. In addition, flows of 2 cm/sec and less are rather slow and may contribute to band broadening. Conversely, when smaller restrictors were used slower linear flow rates were a consequence but this also resulted in fewer detector problems. Peak broadening, probably due in part to lateral diffusion of the analytes in the slow moving mobile phase, was also a result.
A second, more serious problem was that of increasing retention times with column use. We observed that within 50-60 injections on a given one meter piece of the Restek column it became more retentive. The nature of this problem is unclear. It is possible that traces of analytes that were moderately to totally retained accumulated on the LTGC surface and eventually formed a molecular film with retention characteristics of its own. Prolonged heating of the column (i.e., overnight) at 400 °C did not correct the problem. Another potential source of this behavior may be suggested by the continuous struggle with plugged restrictors. This is not an uncommon problem in SFC but in the experiments with the Restek column the restrictor tips were often fouled with a black particulate. Usual sources of restrictor plugging - injectors, fittings, dirty sample vials, insoluble particulate material in solid analytes, etc. - were examined and repaired or controlled as potential contributors to the problem. It has been noted that the hardness of glassy carbon is directly related to the hydrogen-carbon atomic ratio. The greater the amount of hydrogen in glassy carbon the softer it becomes. It is possible that the flow and pressure conditions within the column, coupled with vibration from the GC oven fan, may have caused small pieces of the stationary phase to spall from the LTGC surface. In each place that this happened a new surface would present itself to analytes migrating down the column. The solvents that were used during the SFC studies, carbon disulfide, methylene chloride, and pentane were more affected by this change than many of the larger solutes that were tested. This resulted in diminishing solute capacity factors with time. Since it is the capacity factors that serve as the dependent variables in the retention modeling studies, this made it extremely difficult to both sample many analytes and to do
repetitive trials of single analytes. In an attempt to solve both of these problems, single injections of analytes were made on two separate one meter pieces of the Restek column. The results from both columns were subject independently to multivariate regression analysis. The results from both columns indicated similar possible retention mechanisms for the Restek column in SFC.

A slightly wider range of analytes were examined in the SFC studies than in the GC experiments. The analytes examined are listed in Table 17 along with their various parameters and their capacity factors. As in the GC experiments these analytes were selected based on their successful performance in previous characterization studies. Those analytes which would not elute in the SFC studies are listed in Table 18.

There were several obvious trends in the retained and the unretained solutes. The enhanced solvating power of the supercritical mobile phase made it possible to elute larger molecules from the Restek column than in GC. Examples of this are the C_{10} and C_{12} compounds decane, 1,2,4,5-tetramethylbenzene, biphenyl, and hexanophenone. Larger solutes, such as benzophenone (C_{13}), the stilbene compounds (C_{14}), hexadecane (C_{16}), and eicosane (C_{20}) were permanently retained. The stronger mobile phase also made it possible to elute more polar molecules such as the phenones and several nitro compounds from the column but methanol and carboxylic acids were permanently retained. While phenol would elute, chlorophenol isomers would not.

As with the GC results, the behavior of some solutes in the SFC experiments was at variance with that observed elsewhere. The separation of a homologous series of alkanes from C_{10} to C_{32} on a capillary column coated with LTGC has been previously
Table 17. Capacity factors and parameters of analytes used in SFC experiments. Values in bold face are estimates based on linear regression analysis of values from other members of homologous series.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$k'$</th>
<th>$H_{ads}$</th>
<th>$H_{vap}$</th>
<th>BP</th>
<th>$V_m$</th>
<th>$\pi^*$</th>
<th>$\beta$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetophenone</td>
<td>3.91</td>
<td>54.4</td>
<td>58.3</td>
<td>202.1</td>
<td>117.4</td>
<td>0.90</td>
<td>0.049</td>
<td>0.06</td>
</tr>
<tr>
<td>anisole</td>
<td>0.97</td>
<td>56.1</td>
<td>46.8</td>
<td>153.8</td>
<td>108.8</td>
<td>1.15</td>
<td>0.32</td>
<td>0.00</td>
</tr>
<tr>
<td>benzene</td>
<td>0.13</td>
<td>41.0</td>
<td>33.8</td>
<td>80.1</td>
<td>89.4</td>
<td>0.59</td>
<td>0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>benzonitrile</td>
<td>2.66</td>
<td>***</td>
<td>***</td>
<td>191.1</td>
<td>103.1</td>
<td>0.90</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td>biphenyl</td>
<td>13.45</td>
<td>***</td>
<td>54.0</td>
<td>255.0</td>
<td>155.2</td>
<td>1.18</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>bromobenzene</td>
<td>1.28</td>
<td>49.0</td>
<td>44.5</td>
<td>156.2</td>
<td>105.0</td>
<td>0.79</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>butylbenzene</td>
<td>1.82</td>
<td>64.0</td>
<td>49.8</td>
<td>183.3</td>
<td>156.0</td>
<td>0.49</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>butyrophenone</td>
<td>8.23</td>
<td>***</td>
<td>***</td>
<td>222.0</td>
<td>145.2</td>
<td>0.85</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td>0.76</td>
<td>45.2</td>
<td>41.0</td>
<td>131.7</td>
<td>101.7</td>
<td>0.71</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>decane</td>
<td>0.77</td>
<td>69.9</td>
<td>51.4</td>
<td>174.1</td>
<td>194.9</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ethylbenzene</td>
<td>0.60</td>
<td>53.1</td>
<td>42.1</td>
<td>136.2</td>
<td>122.5</td>
<td>0.53</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>fluorobenzene</td>
<td>0.15</td>
<td>38.1</td>
<td>33.5</td>
<td>84.7</td>
<td>93.86</td>
<td>0.62</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>heptane</td>
<td>0.01</td>
<td>52.3</td>
<td>36.6</td>
<td>98.4</td>
<td>146.6</td>
<td>-0.02</td>
<td>-0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>hexanophenone</td>
<td>19.04</td>
<td>***</td>
<td>***</td>
<td>265.2</td>
<td>184.0</td>
<td>0.81</td>
<td>0.45</td>
<td>0.06</td>
</tr>
<tr>
<td>iodobenzene</td>
<td>2.66</td>
<td>55.6</td>
<td>50.0</td>
<td>188.3</td>
<td>111.0</td>
<td>0.81</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>mesitylene</td>
<td>0.80</td>
<td>62.8</td>
<td>47.5</td>
<td>164.7</td>
<td>139.2</td>
<td>0.47</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>2-nitroanisole</td>
<td>12.64</td>
<td>***</td>
<td>***</td>
<td>277.0</td>
<td>122.3</td>
<td>1.15</td>
<td>0.57</td>
<td>0.00</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td>3.87</td>
<td>59.4</td>
<td>55.0</td>
<td>210.8</td>
<td>102.2</td>
<td>1.01</td>
<td>0.39</td>
<td>0.00</td>
</tr>
<tr>
<td>o-nitrotoluene</td>
<td>4.84</td>
<td>***</td>
<td>***</td>
<td>222.0</td>
<td>118.0</td>
<td>0.97</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>nonane</td>
<td>0.41</td>
<td>61.9</td>
<td>46.4</td>
<td>150.8</td>
<td>178.7</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>octane</td>
<td>0.19</td>
<td>56.1</td>
<td>41.5</td>
<td>125.7</td>
<td>162.6</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>pentanophenone</td>
<td>12.45</td>
<td>***</td>
<td>***</td>
<td>248.5</td>
<td>164.2</td>
<td>0.83</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>pentylbenezene</td>
<td>3.03</td>
<td>67.6</td>
<td>52.9</td>
<td>202.2</td>
<td>172.5</td>
<td>0.47</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>phenol</td>
<td>1.94</td>
<td>54.4</td>
<td>49.8</td>
<td>181.8</td>
<td>88.9</td>
<td>0.72</td>
<td>0.33</td>
<td>0.61</td>
</tr>
<tr>
<td>propiophenone</td>
<td>6.06</td>
<td>***</td>
<td>***</td>
<td>218.0</td>
<td>132.8</td>
<td>0.87</td>
<td>0.49</td>
<td>0.06</td>
</tr>
<tr>
<td>propylbenzene</td>
<td>1.04</td>
<td>59.8</td>
<td>46.0</td>
<td>159.2</td>
<td>139.4</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
<tr>
<td>pyridine</td>
<td>0.96</td>
<td>42.3</td>
<td>40.2</td>
<td>115.2</td>
<td>80.7</td>
<td>0.87</td>
<td>0.64</td>
<td>0.00</td>
</tr>
<tr>
<td>styrene</td>
<td>1.09</td>
<td>***</td>
<td>41.5</td>
<td>145.1</td>
<td>115.0</td>
<td>0.55</td>
<td>0.18</td>
<td>0.00</td>
</tr>
<tr>
<td>toluene</td>
<td>0.34</td>
<td>50.2</td>
<td>37.8</td>
<td>111.0</td>
<td>106.4</td>
<td>0.55</td>
<td>0.18</td>
<td>0.00</td>
</tr>
<tr>
<td>1,2,4,5-tetramethylbenzene</td>
<td>2.50</td>
<td><strong>6.6</strong></td>
<td><strong>51.6</strong></td>
<td>196.8</td>
<td>157.8</td>
<td>0.43</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>1,2,4-trimethyl benzene</td>
<td>1.44</td>
<td><strong>60.7</strong></td>
<td><strong>47.1</strong></td>
<td>169.4</td>
<td>137.3</td>
<td>0.47</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>m-xylene</td>
<td>0.65</td>
<td><strong>54.9</strong></td>
<td>42.6</td>
<td>139.1</td>
<td>122.3</td>
<td>0.51</td>
<td>0.12</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 18. Solutes that would not elute under SFC conditions.

<table>
<thead>
<tr>
<th>Solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetic acid</td>
</tr>
<tr>
<td>aniline</td>
</tr>
<tr>
<td>benzoic acid</td>
</tr>
<tr>
<td>benzophenone</td>
</tr>
<tr>
<td>cis-stilbene oxide</td>
</tr>
<tr>
<td>3-chlorophenol</td>
</tr>
<tr>
<td>4-chlorophenol</td>
</tr>
<tr>
<td>1,1'-diphenylethylene</td>
</tr>
<tr>
<td>eicosane</td>
</tr>
<tr>
<td>hexadecane</td>
</tr>
<tr>
<td>methanol</td>
</tr>
<tr>
<td>N-methylaniline</td>
</tr>
<tr>
<td>N,N'-diethylaniline</td>
</tr>
<tr>
<td>o-chloroaniline</td>
</tr>
<tr>
<td>octanoic acid</td>
</tr>
<tr>
<td>p-cresol</td>
</tr>
<tr>
<td>propanoic acid</td>
</tr>
<tr>
<td>trans-stilbene</td>
</tr>
<tr>
<td>trans-stilbene oxide</td>
</tr>
<tr>
<td>triethylamine</td>
</tr>
</tbody>
</table>
performed in this laboratory. This was done at 80 °C with mobile phase densities ranging from 0.23-0.76 g/cm³. The separation of naphthalene, biphenyl, fluorene, phenanthrene, and pyrene, C₁₂ to C₁₆ compounds, was performed on the same column at a temperature of 200 °C and at mobile phase densities of 0.13-0.40 g/cm³. This suggests, among other things, that the inability of some solutes to elute from the Restek column cannot be attributed to a lack of solubility in the mobile phase.

As in GC, multivariate linear regression analysis of the SFC data was performed in an attempt to clarify those factors that most significantly impact retention. The acceptance or rejection of the resulting models was based on the same criteria used for the GC experiments. Models with two or more independent variables with a high degree of cross-correlation (rₓᵧ > 0.7) or with a coefficient that was not statistically significant (tₓᵧ < t₉₅) were discarded. Those independent variables with high degrees of cross-correlation include Hₓ and Hᵧ (rₓᵧ = 0.742), Hₓ and BP (rₓᵧ = 0.752), Hₓ and Vₓ (rₓᵧ = 0.810), and Hᵧ and BP (rₓᵧ = 0.977). The models that meet the described stipulations and with Rₓ² terms greater than 0.85 are listed in Table 19.

Statistics for the models are presented in Table 20. The F-test values are statistically significant for at the 95% confidence level for both of the models presented. Estimates of the adjusted coefficient of multiple correlation range from 0.87 to 0.89 which indicates an adequate goodness of fit. A plot of observed retention versus retention predicted by model #1 can be seen in Figure 26. The correlation coefficient for the line in Figure 26 is 0.94, a reasonably good fit. The correlation coefficient for a similar plot
Table 19.  Retention models for SFC.

<table>
<thead>
<tr>
<th>model #</th>
<th>( \ln k' = )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6.556 BP + 1.371 ( \pi^* ) - 4.775</td>
</tr>
<tr>
<td>2.</td>
<td>8.514 BP - 2.105( \Omega ) - 3.606</td>
</tr>
</tbody>
</table>
Table 20. Statistics for the models presented in Table 19.  

<table>
<thead>
<tr>
<th>Model #</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>t: #1&lt;sup&gt;c&lt;/sup&gt;</th>
<th>t: #2&lt;sup&gt;d&lt;/sup&gt;</th>
<th>t&lt;sub&gt;0.05&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</th>
<th>R&lt;sup&gt;f&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;&lt;sub&gt;a&lt;/sub&gt;&lt;sup&gt;g&lt;/sup&gt;</th>
<th>s&lt;sup&gt;h&lt;/sup&gt;</th>
<th>F&lt;sup&gt;i&lt;/sup&gt;</th>
<th>F&lt;sub&gt;95%&lt;/sub&gt;&lt;sup&gt;j&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>29</td>
<td>10.41</td>
<td>3.82</td>
<td>2.045</td>
<td>0.943</td>
<td>0.882</td>
<td>0.545</td>
<td>116.6</td>
<td>3.33</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>29</td>
<td>14.75</td>
<td>3.16</td>
<td>2.045</td>
<td>0.935</td>
<td>0.866</td>
<td>0.557</td>
<td>116.6</td>
<td>3.33</td>
</tr>
</tbody>
</table>
Figure 26. A plot of $\ln k'_{\text{observed}}$ versus $\ln k'_{\text{predicted}}$ by SFC model #1. $r = 0.94$
using model #2 is 0.93. This demonstrates that despite the rather low $R^2$ values of the models the predicted data still correlates strongly with the empirical results.

Previous SFC studies of retention on LTGC have demonstrated that dispersive interactions are those of principal importance with significant contributions also being made by solute dipolarity/polarizability. On the Restek column the solute boiling point and dipolarity were the most influential on the retention of the solutes examined. Both models in Table 19 contain boiling point coefficients that are positive and that are larger than the either of the coefficients of the other terms. SFC model #1 contains a $\pi^*$ coefficient that is 21% the size of the BP coefficient. This suggests that solute dipolarity or polarizability may play a significant role in SFC retention on the Restek column.

As in the GC results the significance of solute boiling point to retention in SFC may not be exactly what it seems at first glance. A recapitulation of the facts behind the interpretation of the GC data is as follows. In those studies there was a very strong correlation between the boiling point, the heat of vaporization, and the heat of adsorption values. Heat of vaporization values can be estimated from the boiling points of nonpolar and slightly polar compounds using the Hildebrand rule (eqn 6), thus illustrating the close relationship between the two. As was pointed out above $H_a$ and $H_v$ were quite similar for most of the studied solutes, generally within a factor of 1.5. The relationship between $H_a$ and dispersive interactions has been previously noted. It was inferred from all of these facts that dispersive interactions are at least partially responsible for retention on the Restek column during GC but that the energy of the interactions is more correctly modeled by the energy levels provided by the $H_{vap}$ values, possibly because there are
significant differences in the LTGC surface of the Restek column and the GTCB used in the $H_s$ studies.

This line of reasoning may also help to explain the results of the SFC modeling. In the SFC results there is a very high degree of cross-correlation between $H_{vap}$ and BP ($r_{ij}=0.977$) which indicates that the information conveyed by these two independent variables is statistically similar. There are also strong correlations between $H_{ads}$ and $H_{vap}$ ($r_{ij}=0.742$) and $H_{ads}$ and BP ($r_{ij}=0.752$), again confirming the similarity of the information provided by the three variables. This may indicate that, similar to the GC results, during SFC the energy levels conveyed by $H_{ads}$ and $H_{vap}$ values do not satisfactorily describe the strength of dispersive interactions between the solutes studied and the Restek LTGC. This information may be more accurately conveyed by the boiling points of the analytes. The boiling point of a substance is reflective of the associative forces between its atoms or molecules. Thus, there is not only a correlation between the boiling point of a substance and the strength of the dispersive interactions it can enter into, but also a relationship between the boiling point and all of the other interactions that can occur between the substance's molecules such as dipole-dipole, hydrogen bonding, and other donor-acceptor interactions. The boiling point may provide information about dispersive interactions between a surface and a solute, and it may also give some indication that there are additional, stronger solute-surface interactions occurring.

There may also two other, more mundane but none the less trivial, potential explanations for the dominance of the BP term in the SFC models. First, there are nearly 22% more solutes with boiling point values than either $H_s$ or $H_v$ values, 32 BPs versus
25 $H_v$ values. This number increases to over 31% if the estimated values are not counted, 32 BPs to 22 $H_v$ values. It is possible that this biased the modeling with respect to the BP terms. Second, there is less similarity between the BP values of the different solutes than exists between most of the $H_v$ values. Small errors in either the observation or the calculation of these values could result in a "blurring" of distinctions between two or more different analytes. The greater differences between the BP values would make them less susceptible to this sort of error.
A Proposed Explanation of the Observations

There are several factors, physical and chemical, which, acting alone or in concert may be responsible for the highly retentive behavior displayed by the Restek column. The relatively thick layer of stationary phase - 8 \( \mu \text{m} \) - coupled with the porous nature of the LTGC may have permitted analyte migration into areas where mobile phase flow was either slow or stagnant. The observed retention of the analyte would then be a function of both the solute-LTGC adsorption-deadsorption kinetics and the diffusivity of the solute in stagnant mobile phase. The likelihood of this occurring is questionable.

More important is the question of available surface area on the stationary phase. The greater the surface area, the more retentive the stationary phase will behave. A high surface area relative to the LTGC stationary phases characterized in previous SFC studies would help explain the excessive retentiveness of the Restek column.

Stationary phase deposition inhomogeneities may have contributed to the excessive retentiveness of the Restek column. Although the LTGC film thickness was such that the underlying support should have been well-covered, it is impossible to guarantee that a film of stationary phase coated the entire interior wall of the capillary. Silcosteel, the material the column is made of, consists of fused silica tubing sheathed in stainless steel which is generally opaque to visible light. This made it difficult to visually inspect the column. Attempts at making columns in our lab were frustrated by an inability to achieve a satisfactory coating of LTGC within the capillary, obtaining instead columns with an interior coating of alternating stripes of stationary phase and bare fused silica support. It may be that the Restek column suffered from a similar affliction. However, the
exposed fused silica surfaces would not be nearly as retentive as the behavior observed in the GC and SFC experiments. This condition would be more likely to be characterized by poor peak shapes.

The method of preparing the LTGC may also be responsible for the observed retention behavior. Previous methods of coating the LTGC on a substrate included gradual graphitization of the initial polymer under an argon atmosphere. The Restek column went through the graphitization process under a hydrogen atmosphere at a maximum temperature of 600 °C. Normally during graphitization oxygen, hydrogen, nitrogen, and other volatiles leave the carbon as gaseous by-products. If this were done under a hydrogen atmosphere, conditions would favor a diminished loss, or perhaps even a net gain, in the fraction of hydrogen present in the carbon. An additional complication may also result from the reduction of surface oxides not eliminated by the graphitization process. Reducing these oxides would result in the formation of hydroxyl groups which would behave chromatographically like the siloxyl groups that cause poor chromatographic performance on silica-based stationary phases. The graphitization conditions employed by Restek may favor these reduction reactions even though they may not occur under normal laboratory conditions. Neither the GC nor the SFC models seem to confirm this assumption about the presence of hydroxyl groups on the LTGC surface within the Restek column. Although the Lewis acid-base character of the analytes used in the GC and SFC models was of no significance, a relationship between retention and solute acidity was noted during the SFC experiments. Four carboxylic acids, acetic, propanoic, octanoic, and benzoic, were permanently retained by the stationary phase.
Pyridine and three positional isomers of cyanopyridine were tested. The capacity factors and pKₐ values of pyridine and of 2-, 3-, and 4-cyanopyridine are listed in Table 21. A plot of the natural log of the capacity factor versus pKₐ can be seen in Figure 27. The direct relationship between solute acidity and retention can be clearly seen in both and may possibly be linked to the presence of hydroxyl groups on the LTGC surface. The pKₐ of hydroxyl protons in RCH₂OH and R₃COH groups, the most likely to be found on a graphitic surface as a consequence of carbonyl reduction, is from 16 to 17 relative to water. The behavior of these groups as Lewis bases could explain the observed retention behavior of the cyanopyridines. It may also help explain the highly retentive nature of the Restek column.
Table 21. Capacity factors and $pK_a$ values for pyridine and cyano-substituted pyridines.

<table>
<thead>
<tr>
<th></th>
<th>$k'$</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cyanopyridine</td>
<td>4.95</td>
<td>-0.26</td>
</tr>
<tr>
<td>3-cyanopyridine</td>
<td>3.49</td>
<td>1.45</td>
</tr>
<tr>
<td>4-cyanopyridine</td>
<td>2.96</td>
<td>1.90</td>
</tr>
<tr>
<td>pyridine</td>
<td>0.96</td>
<td>5.17</td>
</tr>
</tbody>
</table>
Figure 27. A plot of \( \ln k' \) versus \( pK_a \) for pyridine compounds.
Conclusions

The need for new stationary phases in SFC has been discussed as have the advantages of capillary columns. In principle the Restek column could become a valuable tool to practitioners of SFC. However, several severe flaws in the present column render it of little or no value other than for the separation of permanent gases in GC. The column diameter must be reduced by a factor of two to ten before it will receive much acceptance for use in SFC. Restek must ensure that the LTGC coating within the silcosteel tube is uniform rather than the striated deposition that was commonly observed in this lab. An alternative graphitization process to that employed during the manufacture of the examined column should be considered. It is possible that the use of hydrogen, rather than argon or another inert gas, rendered the resulting LTGC surface highly retentive and difficult to use. Determinations of the surface area should be made since it is likely that variations in the graphitization technique affect the resulting LTGC surface area. The origins of the extreme retentiveness should be ascertained and corrected.
List of References


28. Ref. 17, p. xi.


40. Private Communication from Restek Corp.


45. Larkins, W. C., Ph. D. Thesis, The Ohio State University, Columbus, Ohio, 1993.


55. Dr. P. Flory, SYSTAT Inc.: private communication.


60. Engel, T. M.; Olesik, S. V., unpublished data.

BIBLIOGRAPHY


Chester, T. L., private communication.

Chester, T. L.; Pinkston, J. D.; Raynie, D. E. Anal. Chem. 1992, 64, 153R.


Cui, Y.; Olesik, S. V. Submitted for publication in *Anal. Chem.*


Eicke, H.-F. *J. Colloid Interface Sci.* 1979, 68, 440.


Engel, T. M.; Olesik, S. V., unpublished data.


Giddings, J. C. *Sep. Sci.* 1966, 1, 73.

Giddings, L. D.; Olesik, S. V., unpublished data.


Larkins, W. C., Ph. D. Thesis, The Ohio State University, Columbus, Ohio, 1993.


Marti, E.; Giddings, L. D.; Olesik, S. V., unpublished data.


Neter, J.; Wasserman, W.; Kutner, M. In Applied Statistical Models; Richard E. Irwin, Inc.: Homewood, IL 1985, 2nd Ed.


The Supelco Reporter; Supelco, Inc.: Bellefonte PA, 1993, Vol. XII, No. 3., p. 3.


Wells, M. A. Biochemistry 1974, 13, 4937.


