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Studies of enhanced-fluidity solvents for chromatography and extraction

Cui, Yi, Ph.D.
The Ohio State University, 1992
STUDIES OF ENHANCED-FLUIDITY SOLVENTS
FOR CHROMATOGRAPHY AND EXTRACTION

DISSERTATION

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

By

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The Ohio State University
1992

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Department of Chemistry

Advisor
To my wife, my son and my parents
ACKNOWLEDGEMENTS

It hasn't been easy.

First of all, I would like to thank my research advisor Dr. Susan Olesik for her guidance during the four years of graduate studies. The graduate school days and nights could have been much longer without her patience and friendship. I learned a lot from her. I won’t forget the help from every group member: Tina Engel, Lars Pekay, Curt Miller, Jed Mellott, Jerry Woodruff, Lu Giddings, Bill Larkins, Stephen Lee, Jesusa Abad, Isabelle Souvignet, Ellen Pineda, Michael Rider, Don Kenny, Tricia Reighard, and Jeff Kinzer. I’d also like to thank two undergraduate students, Wendy Flory and Mark Lada, for working with me. I will miss all of them. Thanks also go to Dr. Jim Coe, Dr. Alan Marshall and their research groups for the cooperation interactions.

There are two special friends I’d like to thank, Dr. S. J. Yao and Dr. J. T. Wang, for their help, guidance and friendship.

Finally, thank you to my wife, Winnie, my son, Jonathan, and my parents for their support.
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Studies in analytical chemistry including liquid and supercritical fluid technologies, separations, and extractions performed with Dr. Susan V. Olesik.
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CHAPTER I

INTRODUCTION

ENHANCED-FLUIDITY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

In the world of separation, chromatography is probably the most important technique. On the analytical scale, chromatography has three major subdivisions: liquid chromatography (LC), gas chromatography (GC); and supercritical fluid chromatography (SFC). An overall comparison of these three chromatographic techniques is summarized in Table 1. The tremendous ability of GC to separate and analyze volatile compounds is widely appreciated. However, only 20% of known organic compounds can be satisfactorily separated by GC, because the other 80% are either insufficiently volatile to pass through the column or thermally unstable under typical GC conditions. LC, on the other hand, is not limited by these factors. High performance liquid chromatography (HPLC), distinguished from classical LC and thin layer chromatography, has shown continuing growth and many subdivisions have been developed, such as reversed-phase HPLC, normal-phase HPLC, and size exclusion chromatography. In general, HPLC has longer analysis times and lower column efficiencies than GC because the rate of solute diffusion in liquids is approximately three orders of magnitude slower than in gases. Also, liquids have viscosities that are typically 100 times greater than those of gases. Accordingly, a higher pressure drop is often required in HPLC to achieve a given linear
Table 1. Comparison among GC, HPLC, and SFC Techniques.

<table>
<thead>
<tr>
<th>Technique*</th>
<th>Solvent viscosity (cP)</th>
<th>Solute diff. coef. (cm²/s)</th>
<th>Velocity range (cm/s)</th>
<th>Efficiency range (N)</th>
<th>Speed of analysis (N/s)</th>
<th>Elution time range (min)</th>
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<td>HPLC (packed)</td>
<td>$10^{-1}$-$1$</td>
<td>$10^{-6}$-$10^{-5}$</td>
<td>0.1-0.4</td>
<td>$5,300$-$8,500$</td>
<td>14-35</td>
<td>0.5-60</td>
</tr>
<tr>
<td>SFC (packed)</td>
<td>$10^{-2}$-$10^{-1}$</td>
<td>$10^{-4}$-$10^{-3}$</td>
<td>0.5-1.5</td>
<td>$3,500$-$5,100$</td>
<td>42-83</td>
<td>0.3-30</td>
</tr>
<tr>
<td>SFC (open tubular)</td>
<td>$10^{-2}$-$10^{-1}$</td>
<td>$10^{-4}$-$10^{-3}$</td>
<td>0.5-4.0</td>
<td>$20,000$-$137,000$</td>
<td>11-13</td>
<td>5.0-90</td>
</tr>
<tr>
<td>GC (open tubular)</td>
<td>$10^{-3}$-$10^{-2}$</td>
<td>$10^{-2}$-$10^{-1}$</td>
<td>15-50</td>
<td>$64,000$-$112,000$</td>
<td>93-180</td>
<td>1.5-60</td>
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* HPLC (packed): 10-cm column length with 5 µm packing. SFC (packed): 10-cm column length with 5 µm packing. SFC (open tubular): 10-m column length with 50 µm i.d. GC (open tubular): 30-m column length with 300 µm i.d.  
  b Calculated for a solute with $k' = 5$. 
velocity. Mixtures are often used as mobile phases in HPLC to adjust the solvent strength. In addition, increasing mobile phase fluidity, f, or decreasing viscosity (\( f = \eta^{-1} \)) of the mobile phase, will increase efficiency. For example, Snyder showed that in liquid-solid chromatography a 2.5-fold decrease in mobile phase viscosity caused approximately a two-fold increase in column efficiency (1). Liquid mobile phases with low viscosities are therefore preferred in liquid-solid chromatography. However, typical liquid mobile phase viscosities vary from only 0.30 to 1.0 cP, and the possible list of solvents to choose from is shortened due to the solvent strength requirements of the separation. One attempt to lower mobile phase viscosity in HPLC involved elevating the temperature of the mobile phase, sometimes even higher than the boiling point of the solvent (2). However, at high temperature the analysis of thermally unstable solutes is problematic.

Supercritical fluid chromatography (SFC) comes in as a combination of GC and HPLC. SFC provides the advantage of higher efficiency in a shorter analysis time than in HPLC because solute diffusion rates in supercritical fluids are intermediate between those of gases and liquids (Table 1). Also the pressure drop across the column for a given velocity is less in supercritical fluids than in liquids due to the gas-like viscosities of supercritical fluids. As with other techniques, SFC does have disadvantages. SFC cannot be used for the separation of highly polar compounds or larger molecules due to the low solubility of these compounds in supercritical fluids. Even the addition of organic modifiers (5-10%) to supercritical mobile phases does not offer the solvent strength range observed with liquid mixtures in HPLC.
In Chapter II and III, some of the SFC advantages are provided to HPLC by attempting HPLC separations with low viscosity, or high fluidity mobile phases. These solvents were prepared by dissolving large proportions of low viscosity fluids in the liquid mobile phase to achieve more efficient separation in shorter analysis time than standard HPLC. The most important parameters in these studies are viscosity of the mobile phase and diffusivity of the solute in the mobile phase. In general, solute diffusivity and solvent viscosity are related and affect both analysis time and efficiency.

**Dependence of Diffusion Coefficients on Viscosity**

The relationship that best describes diffusion coefficient in non-associating as well as associating solvents such as alcohols is (3,4):

\[
D_{12} \eta^p = AT \tag{1}
\]

where \(D_{12}\) is the binary infinite dilution diffusion coefficient of the solute, \(\eta\) is the solvent viscosity, \(T\) is the absolute temperature, and \(p\) and \(A\) are terms which are characteristic of the solute. The \(p\) and \(A\) terms have been found to be linear functions of the inverse solute radius (3). Typical values of \(p\) range from 0.5 to 1.

**Variation of Separation Time with Viscosity**

The separation time is related to the linear velocity through the following relation (5):
\[ t = 16 R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{(1 + k')^3}{k'^2} \right) \left( \frac{H}{u} \right) \]  \tag{2}

where \( R_s \) is the chromatographic resolution, \( \alpha \) is the separation factor, \( k' \) is the capacity factor, \( H \) is the plate height, and \( u \) is the average linear velocity. By combining Darcy’s Law, equation [3], and the Kozeny-Karman description of specific permeability, equation [4], then equation [5] is obtained which relates velocity and viscosity.

\[ U = \frac{B^o \Delta P}{\varepsilon \eta L} \]  \tag{3}

\[ B^o = \frac{d_p^2 \varepsilon^3}{180 (1 - \varepsilon)^2} \]  \tag{4}

\[ u = \frac{d_p^2 \Delta P \varepsilon^2}{180L \eta (1 - \varepsilon)^2} \]  \tag{5}

where \( B^o \) is the specific permeability, \( \Delta P \) is the pressure drop across the column, \( \varepsilon \) is interparticle porosity, \( \eta \) is mobile phase viscosity, \( L \) is the column length, and \( d_p \) is the particle diameter. If the eq [5] is substituted into eq [2], the following expression is obtained:

\[ t = 2880 R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{(1 + k')}{k'^2} \right) \frac{HL \eta (1 - \varepsilon)^2}{\Delta P d_p^2 \varepsilon^2} \]  \tag{6}

From eq [6], it is clear that a given column with a defined interparticle porosity, column length and particle size will display decreased separation time if either the mobile phase viscosity or the column plate height is lowered. In actuality, the column plate height also
varies with mobile phase viscosity. Typically decreased viscosity causes the plate height also to decrease. A multiplexed decrease in separation time is therefore expected.

**Variation of Plate Height with Viscosity**

Equation [7], the Knox equation, shows the variation of the plate height as a function of linear velocity for a packed column.

\[
H = Au^{1/3} + B/u + C_s u + C_m u
\]  

where \( A \) measures the dispersion due to analyte molecules having different flow paths in a packed bed, \( B \) measures dispersion caused by longitudinal diffusion, \( C_s \) measures the band dispersion due to slow mass transfer kinetics of the adsorption/desorption process in the stationary phase, and \( C_m \) measures the band dispersion due to diffusion in the stagnant mobile phase inside a microparticle of the chromatographic packing material.

In typical HPLC separations, the linear velocity used is such that the \( C \)-terms are the major cause of band dispersion in the column. If the packing particles are spherical, the following expression describes the variables that control the \( C_m \) term (6).

\[
C_m = \frac{\theta (k_o + k' + k_o k')^2 d_p^2}{30 k_o (1 + k_o)^2 (1 + k')^2 D_m}
\]  

where \( \theta \) and \( k_o \) are the stationary packing material structure parameters, \( k' \) is the capacity factor, \( d_p \) is the packing particle diameter, and \( D_m \) is the binary diffusion coefficient of the solute. Since the diffusion coefficient of the solute is inversely related to solvent viscosity, from eq [8], the \( C_m \) term is expected to decrease with decreasing mobile phase viscosity. Over the limited range of viscosities possible with common liquid mobile
phases, this is the experimentally-observed trend (7). When slow mass transfer kinetics at the stationary phase occurs, $C_s$ can be predominated. Equation [9] shows that $C_s$ is inversely proportional to the rate constant, $k_d$, of the solute desorption from the stationary phase (5).

$$C_s = \frac{2k'}{(1 + k_o)(1 + k')^2 d_p k_d} \quad [9]$$

**Goals of Research**

The expected impacts of adding low viscosity fluids to the liquid mobile phase are better efficiency and faster speed of analysis comparing to standard HPLC. Since the mobile phase strength is also one of the important concerns for the separation system, a complete solvent strength study of enhanced-fluidity mixtures needs to be done as well. In SFC, the interactions between supercritical fluids and the stationary phase are weak and usually negligible. With enhanced-fluidity mobile phases, however, attention needs to be paid to the solvent effect to the stationary phase surface. Comparison between SFC and enhanced-fluidity HPLC is helpful to understand the differences and improvements.

In Chapter II and III, experiments are designed and done to achieve higher efficiency in shorter analysis time using enhanced-fluidity mobile phases. Two different stationary phases, a porous glassy carbon (PGC) and octadecyl polysiloxane (ODS) were used. Methanol/CO$_2$ and methanol/water/CO$_2$ mixtures were shown as mobile phases. The strong molecular interaction abilities have made methanol and water two of the most commonly used solvents in HPLC. CO$_2$, on the other hand, is one the basic supercritical fluids SFC is based on. By dissolving large amount of compressed CO$_2$ in methanol and
methanol/water mixtures, a series of solvent strength studies were also undertaken to understand their chromatographic behaviors.

**COMPARISON OF ENHANCED-FLUIDITY EXTRACTION AND SUPERCRITICAL FLUID EXTRACTION**

Within the past ten to twenty years, supercritical fluid extraction (SFE) has been investigated in both academic and industrial laboratories. SFE uses supercritical fluids, most often carbon dioxide, to replace traditional liquid extraction techniques. As described by McHugh and Krukonis (8), a sharp increase in the cost of traditional energy-intensive separation techniques, more stringent environmental concerns, and increased performance demands on new materials which traditional processing techniques cannot meet, are the most direct causes of SFE development. Much of the recent research on SFE using supercritical CO₂ has demonstrated advantages over liquid extraction methods, such as Soxhlet extraction. For example, nontoxic CO₂ is capable of complete extractions of some chemicals as efficient as Soxhlet extraction in shorter time. However, as discussed previously, the low solubility of polar and large compounds in CO₂ and weaker solvent strength of CO₂ are disadvantages of this technique. Desorption of solutes from the extraction matrix and solute diffusion away from the surface to the extraction fluid are the major steps in the extraction process. Supercritical CO₂ may improve the extraction rate only when solute diffusion is the limiting step. Since most of the extraction samples, especially those from environment, often contain water, SFE with neat CO₂ or CO₂ with low fraction of different organic modifiers is problematic.
Based on the solvent strength studies shown in Chapter II and III, it is proposed in Chapter IV that the same kind of enhanced-fluidity solvent mixtures should be able to perform better extractions than SFE. In Chapter IV, initial experiments on enhanced-fluidity liquid extraction are described and compared to SFE with supercritical CO₂.

**CHROMATOGRAPHIC STUDIES OF FULLERENES**

In 1985, a group of chemists from Rice University led by Richard E. Smalley published their theoretical proposal on a soccerball-shaped all carbon molecule, C₆₀ (9). The search for this "perfect" compound was unsuccessful until 1990 when two physicists who were studying interstellar dust surprisingly announced their discovery: a red benzene extract from their specially prepared carbon soot contained not only C₆₀ but also C₇₀ (Figure 1). Suddenly, Kratschmer, Huffman and their colleagues opened up a whole new world to scientists (10,11).

The fullerenes were first made by expensive laser-vaporization cluster-beam apparatus. It has been replaced by the technique of Huffman and Kratschmer which involves evaporating graphite electrodes in an atmosphere about 100 torr of helium (12). The resulting black soot is collected and dissolved in organic solvents, such as benzene. Shortly after the discovery of C₆₀ and C₇₀, the observations that even-numbered carbon clusters in the range C₃₀ - C₄₀₀ were reported. The structures of these fullerenes have been theoretically predicted. Calculations have been made to understand the physical properties, including electron energies (13,14,15), the optical spectra (14), vibration modes (16,17,18), and electric and magnetic properties (19,20).
Figure 1. Molecular structures of fullerene $C_{60}$ and $C_{70}$. 
To characterize all possible fullerene compounds in the soot experimentally, organic solvent extractions are usually the first steps. Further separation and purification of each component in the extract are necessary.

**Different Chromatographic Systems**

When the fullerene separation project, presented in Chapter IV started early in 1991, a normal-phase liquid chromatography system, which included a packed silica column and hexane and pentane as mobile phases was used. Poor resolution of $C_{60}$ and $C_{70}$ was observed. It was found later that bare silica surface might cause degradation of fullerene molecules. The first baseline separation of $C_{60}$ and $C_{70}$ was achieved by use of an in-house capillary C$_{18}$ column. Figure 2 shows the chromatogram and Figures 3 and 4 show the respective mass spectrum of each peak. Because identification and characterization of different fullerenes are often limited by separation and purification procedures, various chromatographic techniques have been reported in the past year to separate fullerenes. For high efficiency separations different types of HPLC columns were used. Early chromatographic separations involved use of alumina or silica stationary phases (21,22,23). However, the fullerene capacity factors on these columns were low and degradation of the fullerenes was occurring when silica was used. Hawkins *et al.* (24) reasoned that a $\pi$-acid type HPLC stationary phase would work well for the separation of the $\pi$-basic fullerenes. To test this, they separated the fullerenes on a Pickle phenylglycine column which contains dinitrobenzamide groups on the surface of the stationary phase. With hexane as the solvent they obtained a separation factor, $\alpha = k'_{C_{70}}/k'_{C_{60}} = 2.25$. Cox *et al.* (25) used a dinitroanilinopropyl silica column (300 Å pore
Figure 2. Chromatogram of the separation of C_{60} and C_{70} on a 60 cm x 320 μm i.d. home-packed C_{18} capillary column. Detection wavelength: 330 nm; injection volume: 200 nL; mobile phase: hexane; inlet pressure: 34 atm.
Figure 3. Negative ion laser desorption Fourier transform mass spectrum of $C_{60}$ peak.
Figure 4. Negative ion laser desorption Fourier transform mass spectrum of C\textsubscript{70} peak.
size, 5 μm particles) with a gradient from n-hexane to 60% methylene chloride to achieve baseline separation of C\textsubscript{60} and C\textsubscript{70}. Other attempts to enhance the separation of the fullerenes involved the use of a carbon stationary phase (26), multi-legged phenyl phases (27), and a size exclusion stationary phase (28). However, the most commonly-used HPLC stationary phase is ODS. Both monomeric and polymeric ODS phases are successfully used for the separation of fullerenes (26,29,30). Polymeric ODS is known to exhibit selectivity based on the nonplanarity of the molecule in addition to the expected dispersive interactions with ODS. However, Jinno (26) suggested that C\textsubscript{60} and C\textsubscript{70} are so bulky that their retention characteristics on monomeric and polymeric should be similar.

All of the previously reported fullerene separations with ODS as the stationary phase used a nonpolar solvent such as hexane or a gradient of hexane with methylene chloride as the mobile phase. Under these conditions the separation mechanism cannot be classified as a classical normal or reversed-phase separation. Both the stationary phase and the mobile phase are nonpolar. The fullerenes are separated solely by small differences in solubility in the nonpolar solvent and the nonpolar stationary phase. Fullerene solubility was the reason for the common preference of such nonstandard HPLC conditions. Fullerenes are most soluble in aromatic solvents (5 mg/mL); slightly soluble in hexane, pentane, chloroform, and diethyl ether (21).

In Chapter V, chromatographic studies are shown by comparing the separation of C\textsubscript{60} and C\textsubscript{70} fullerenes on different reversed-phase HPLC columns. More polar mobile phases, such as methanol and diethyl ether mixtures, were tried for the first time and
better separations were observed. Also the specific type of molecular level interactions involved in the separation of these fullerenes was evaluated. A linear solvation energy relationship was used to understand these different interactions between fullerenes and stationary phases.

**LITERATURE CITED**


CHAPTER II

ENHANCED-FLUIDITY HPLC USING A POROUS GLASSY CARBON AS STATIONARY PHASE

INTRODUCTION

In Chapter I, high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) were discussed and compared. To improve the performance of HPLC, a low viscosity or high fluidity mobile phase can be a choice. On the other hand, to increase the mobile phase solvent strength in SFC, stronger solvent strength is expected without losing advantages of low viscosity and high solute diffusivity. The first attempt to combine some of the advantages of SFC with HPLC was to dissolve large amounts of compressed gas in the liquid. Carbon dioxide (CO₂) and methanol were chosen because they are commonly used and very well studied in SFC and HPLC, respectively. A porous glassy carbon (PGC) stationary phase was used in this study because PGC had been just characterized its capabilities for SFC (1). Since enhanced-fluidity HPLC system is more like SFC, it would be interesting to do a comparison under different conditions.

Gilbert, Knox, and Kaur (2) first introduced PGC as a stationary phase for HPLC in 1982. The same group extensively characterized the material later (3). PGC became commercially available at the time when this project was about to start. As a stationary
phase, PGC has many desirable characteristics. It has an average spherical particle size of 7 μm, 80% porosity, high surface area (150 m²/g), and a sponge-like structure resistant to shearing forces. PGC is strongly hydrophobic. It is stable to acids, bases, high temperature and high pressure. Recently, PGC has been successfully applied in LC analyses of aromatics (4), structural and configurational isomers (5,6,7), and pharmaceuticals (8,9,10). The chromatographic behavior of PGC as a stationary phase has been compared to that of ODS-silica and a uniform surface evidenced by the absence of active sites (3). The retention of solutes on PGC is determined primarily by the electronic interactions between the π electrons of the graphitic carbon with the solute’s electron cloud.

Mixtures of methanol and CO₂ were used as the mobile phase in this study. Methanol is often used as a mobile phase in HPLC. Its molecular structure has been studied. CO₂, on the other hand, is the mobile phase of SFC and it has been very well characterized under different temperature and pressure. Another reason for the choice of these two solvents is that methanol and CO₂ are highly miscible over a wide range of pressures and temperatures. Chang and Rousseau reported their studies on methanol/CO₂ systems in 1985 (11).

In this chapter, the effect of lowering the mobile phase viscosity on the separation efficiency and the column permeability is described. The variation in solvent strength of the mixtures is also discussed. With this information the retention mechanism on PGC was further characterized by studying the thermodynamics of solute transfer and monitoring the variations in solute capacity factors with solute polarity.
EXPERIMENTAL SECTION

Column

A packed capillary column containing porous glassy carbon adsorbent (Hypercarb PGC, Shandon Scientific Ltd, England) was used in this investigation. The column was prepared by slurry packing the solid support in methanol. The PGC slurry was placed in a 5 mm i.d. x 15 cm stainless steel tube and then pushed into a 318 μm i.d. x 30 cm fused silica tube (Polymicro Technologies) with methanol pressurized to ca. 270 atm. The 7 μm diameter PGC particles were held in the fused silica column with a microbore end fitting (U-434, Upchurch Scientific Inc., Oak Harbor, WA) that contained a 2 μm frit (C407, Upchurch Scientific Inc.). A 16 μm i.d. fused silica linear restrictor was used to control the flow rate of the slurry during the packing process.

Instrumentation

The chromatographic system used in this study consisted of Hewlett-Packard 5790A gas chromatograph, an ISCO LC-2600 syringe pump (ISCO, Lincoln, NE), a Valco W-series high-pressure injection valve (Valco Instruments, Houston, TX) with a injection volume of 60 nL, and a Spectroflow 757 UV/Vis absorbance detector (Kratos Analytical Instruments, Ramsey, NJ). The detector excitation wavelength was 210 nm. A 16 μm i.d. x 35 cm fused silica tube was placed after the detector to control the linear flow rate in the column. The flow restrictor is a necessary part of the chromatographic system. The pressure along the column must be maintained above a minimum pressure to avoid having the mixture separate into two phases (liquid-gas). For example, at 25°C the methanol/CO₂ mixture separates into two phases at pressures lower than 54 atm. The
solvent strength of the methanol/CO₂ mixtures was determined by monitoring the solvatochromic shift of various dyes. A Varian DMS-100 UV-Vis spectrophotometer was used to measure the spectra of these dyes.

**Materials**

The test mixture employed in this study was a methanol solution containing 10 mg/mL dichloromethane, 1 mg/mL each of 4-methylphenol, 3,5-dimethylphenol, and 2,4,6-trimethylphenol, and 0.4 mg/mL each of quinoline, naphthalene, and β-naphthol. Dichloromethane eluted with the solvent and therefore was assumed to be unretained. All compounds were obtained from Aldrich Chemical Company (Milwaukee, WI.) with purity levels of ≥ 96%. Supercritical fluid grade CO₂ was obtained from Scott Specialty Gases (Plumsteadville, PA). Methanol/CO₂ mixtures were prepared by mixing known volumes of CO₂ and methanol at 272 atm and 25 °C with two high-pressure syringe pumps. Each methanol/CO₂ solution was equilibrated for at least 12 hours.

**Density Measurements**

The densities of the methanol/CO₂ mixtures were measured with a DMA512 density meter (Anton Paar USA, Inc., Warminster, PA.) and a TU16D constant temperature bath (Techne Inc., Princeton, NJ). The density meter was calibrated at each pressure and temperature using CO₂ and ethane as reference fluids.

**RESULTS AND DISCUSSION**

**Porosity of Column Packing**
The interparticle porosity, $\epsilon$, of the slurry-packed PGC column was found to be $\epsilon = 0.30$ using Darcy's law, equation [1], and the Kozeny-Carman relationship, equation [2]:

$$u = \frac{B^o \Delta P}{\epsilon \eta L}$$  \[1\]

$$B^o = \frac{d_p^2 \epsilon^3}{180 (1-\epsilon)^2}$$  \[2\]

where $B^o$ is the specific permeability, $\Delta P$ is the pressure drop across the column (Table 2), $\epsilon$ is interparticle porosity, $\eta$ is mobile phase viscosity, $L$ is column length, and $d_p$ is particle diameter. The viscosity of methanol under the experimental pressure conditions was calculated using the estimation technique of Lucas (12) and the well-documented viscosity of methanol at atmospheric pressure (13):

$$\frac{\eta}{\eta_{sl}} = \frac{1 + D (\Delta P_r / 2.118)^4}{1 + C \omega \Delta P_r}$$  \[3\]

where $\eta$ is the mobile phase viscosity at the desired pressure, $P$; $\eta_{sl}$ is the viscosity of the saturated liquid at its vapor pressure, $P_{vp}$; $\Delta P_r = (P - P_{vp})/P_c$, $P_c$ is the critical pressure; $\omega$ is the acentric factor; and $A$, $C$, and $D$ are constants that depend on the critical temperature of the mobile phase (14). This viscosity estimation technique is expected to be valid to within $\pm 5\%$ (14). The interparticle porosity was then determined since all other variables in equations [1] and [2] were experimentally measured. By propagating the error in the variables described in Darcy's Law, the error in the calculated porosity, $\epsilon$, is $\pm 0.03$. 
Table 2. Pressure Drop across the Column and Elution Time of nonretained Solute for Different Mobile Phase Compositions.

<table>
<thead>
<tr>
<th>Mole Fraction of CO₂</th>
<th>ΔP (atm)</th>
<th>t₀ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.3</td>
<td>6.4</td>
</tr>
<tr>
<td>0.10</td>
<td>87.1</td>
<td>5.6</td>
</tr>
<tr>
<td>0.20</td>
<td>84.9</td>
<td>5.0</td>
</tr>
<tr>
<td>0.30</td>
<td>82.4</td>
<td>4.4</td>
</tr>
<tr>
<td>0.40</td>
<td>78.7</td>
<td>3.8</td>
</tr>
<tr>
<td>0.50</td>
<td>77.0</td>
<td>3.2</td>
</tr>
<tr>
<td>0.60</td>
<td>74.7</td>
<td>2.6</td>
</tr>
<tr>
<td>0.70</td>
<td>72.2</td>
<td>2.2</td>
</tr>
<tr>
<td>0.80</td>
<td>70.3</td>
<td>1.8</td>
</tr>
<tr>
<td>0.90</td>
<td>67.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Typical values of interparticle porosity for well-packed columns are in the range \( \epsilon = 0.35-0.50 \). The porosities of stable packed beds of particles range from 0.259 upward (15) with the closest-packed arrangement of spheres having an \( \epsilon = 0.26 \). The PGC packing consists of spherical particles with an average diameter of 7 \( \mu \)m. The experimentally-measured porosity therefore is very similar to the closest-packed arrangement.

**Viscosity of Methanol/Carbon Dioxide Mixtures**

Having determined the porosity of the chromatographic bed, the average viscosity of the methanol/CO\(_2\) mixtures at 25 °C and 170 atm were experimentally determined. The linear velocity and the pressure drop across the column were measured and viscosities were calculated using equations [1] and [2]. Figure 5 shows the resultant average viscosities of the methanol/CO\(_2\) mixtures. The viscosity of the mixture at 170 atm and 25 °C varies from 0.57 to 0.09 cP for the composition range of 0 to 1 mole fraction of CO\(_2\). These experimentally determined values of viscosity were compared to values calculated using the empirical equation of mixture viscosities proposed by Grunberg and Nissan (16) at inlet pressure. Due to the nature of the mixing equation, the calculated viscosities differed most (ca. 30%) from the measured viscosities at the intermediate composition values. Since pressure drop across the column is inevitable, the viscosities at the inlet and outlet of the column are slightly different, and viscosity varies nonlinearly along the column. Therefore the average viscosities as measured by the Darcy’s Law were more relevant than the empirically determined viscosities.
Figure 5. Experimental average viscosities of methanol/CO₂ mixtures across the column at 25 °C and 170 atm, and diffusion coefficients ($D_m$) of benzene (19) for the same mobile phase compositions.
**Band Dispersion and Analysis Time**

Figure 6 shows the variation of the plate height, $H$, versus linear velocity, $u$, for 4-methylphenol at 170 atm inlet pressure and 25 °C for three different methanol/CO$_2$ mixtures. The most obvious change is that the curves shift toward higher velocities with increasing carbon dioxide. Therefore if speed of analysis ($H/u$) is important, the 0.40 mole fraction of carbon dioxide mixture has an obvious advantage over lower proportions of carbon dioxide. The same curve shift was found as a function of viscosity for all the test solutes. Similar advantages of enhanced speed of analysis are often ascribed to SFC when comparing SFC to HPLC (17).

The increased speed of analysis with decreasing viscosity can be understood by considering the variation in the optimum linear velocity, $u_{opt}$, as a function of viscosity. For the purpose of demonstration, the Knox equation (18), which describes the relationship of plate height and mobile phase linear velocity, shown below is used:

$$H = Au^{1/3} + B/u + Cu$$  \[4\]

where $H$ is the plate height or column dispersivity, $A$ measures dispersion caused by multifold paths in the column, $B$ measures dispersion caused by diffusion along the flow axis, and $C$ measures the dispersion caused by mass transfer resistances in both the mobile phase and stationary phase. In packed columns, $B = \gamma D_m$, where $\gamma$ is the obstruction factor and $D_m$ is the diffusion coefficient of the analyte in the mobile phase. Under practical conditions in liquid-solid HPLC, the main contribution to $C$ is band dispersion in the stagnant mobile phase inside the porous packing. Under these circumstances, $C = f(k')/D_m$, where $k'$ is the capacity factor, a measure of the retention
Figure 6. Variation of plate height of 4-methylphenol at 25 °C and 170 atm as a function of mobile phase linear velocity for different mobile phase compositions: (*) methanol; (▲) 0.20 CO₂ mole fraction; and (+) 0.40 CO₂ mole fraction in the mobile phase.
of solute. By taking the derivative of plate height equation, equating that to zero, and substituting in the definitions of $B$ and $C$, the $u_{opt}$ is found to be:

$$u_{opt} \propto \frac{D_m}{\sqrt{f(k')}}$$  \[5\]

As shown later, the capacity factors of the test solutes varied minimally from 0 - 0.40 mole fraction of CO$_2$ in the mobile phase. The diffusion coefficients shown in Figure 5 are those of benzene determined by Sassiat et al. (19). Since most of the test solutes are of comparable size to benzene, the variation of the test solutes’ diffusion coefficients with mixture composition should be comparable to that observed for benzene.

From Figure 6, the $u_{opt}$ would be expected to shift to higher velocities for mixture compositions $> 0.40$ mole fraction of CO$_2$ and therefore the speed of analysis would also continue to increase. However, this was not the trend observed. Figure 7 shows a plot of retention time as a function of mixture composition at 25°C and 170 atm with a constant length flow restrictor (i.e. the linear velocity was increasing with decreasing viscosity). The holdup time, $t_o$, as measured by dichloromethane, decreased with increasing proportions of carbon dioxide. The retention time for all the test compounds increased for CO$_2$ mole fractions $> ca. 0.50$ with the exception of naphthalene. Figure 8 shows that for mixtures containing more than 0.50 mole fraction of CO$_2$, the solutes’ capacity factors increased drastically. The capacity factor of naphthalene increased more slowly with added CO$_2$ than the other solutes. This experiment shows that with the exception of naphthalene the gain in speed of analysis ($H/u$) will not continue over the entire methanol/CO$_2$ composition range because the time of analysis increases when the
Figure 7. Variation of solute retention times with mobile phase composition at 25 °C and 170 atm: (△) dichloromethane; (+) 4-methylphenol; (▲) 3,5-dimethyl phenol; (♦) 2,4,6-trimethylphenol; (▼) quinoline; (♦) naphthalene; (■) β-naphthol.
Figure 8. Variation of solute capacity factors with mobile phase composition. Compound identifications are given in Figure 7.
mole fraction of CO₂ in the mobile phase is greater than 0.50. The cause of the increased time of analysis and increased capacity factors for mixture composition > ca. 0.50 CO₂ mole fraction is probably the corresponding decrease in solvent strength over the same composition range. The cause of the solvent strength decrease is described in the next section of this chapter.

Snyder and others (20,21) showed that the plate height was also lowered by decreasing the viscosity of the solvent. The cause of the lowered plate height was a corresponding decreased $C$ coefficient in the Knox equation. However, in this study as illustrated in Figure 6, the variation of the mobile phase viscosity had minimal effect on the plate height.

Figure 9 shows the variation of the plate height for all of the test compounds as a function of linear velocity in a mixture of 0.20 CO₂ mole fraction in the mobile phase at 170 atm and 25°C. On the high velocity portion of the curve which is controlled by the $C$ coefficient, the curve’s slope decreases as the capacity factor of the compound increases (see Figure 8 for capacity factor ordering of solutes). The same trend in the curve’s slope as a function of compound capacity factor was observed for the other methanol/CO₂ mixtures. If the band dispersion in the mobile phase was controlling the $C$ coefficient as was the case in Snyder’s experiments, then the slope would increase with capacity factor (22). One possible contribution to the $C$ coefficient that has this type of dependence on the capacity factor is the term that correspond to slow mass-transfer kinetics at the solid adsorbent surface (23). In most liquid-solid chromatographies using silica stationary phases such as silica gel and bonded phases, the mass transfer kinetics
Figure 9. Plate height versus linear velocity for all solutes in 0.20 mole fraction of CO₂ at 25 °C and 170 atm. Compound identifications are given in Figure 7.
term is small and often negligible.

To summarize, the analysis time in HPLC decreased to levels comparable to that in SFC by using methanol/CO₂ mixtures as the mobile phase. Figure 10 shows sample chromatograms of the test solutes. However, no decrease in plate height was found with decreased mobile phase viscosity as is found in other liquid chromatographies in which the stagnant mobile phase term is the limiting factor. Instead, slow mass transfer kinetics at the PGC stationary phase may be the predominant contribution to band dispersion in this system.

**Solvent Strength**

Retention in liquid-solid chromatography is most commonly described using Snyder’s adsorption model (24). This model assumes that the interaction of the solute with the mobile phase and the solid stationary phase can be described by a series of equilibrium steps. The adsorption step is assumed to involve competition between solvent and solute molecules for a finite number of surface sites (24). Using this model, the retention of analytes is described by the following expression:

\[
\ln k' = \ln V_a + \alpha (S_o - A_s \epsilon)
\]  

where \(k'\) is the capacity factor of the solute, \(V_a\) is the adsorbent’s surface volume, \(\alpha\) is a term which describes the surface activity, \(S_o\) is the standard adsorption energy of the solute, \(A_s\) is the area required by the solute when it is adsorbed on the surface, and \(\epsilon\) is eluotropic solvent strength of the mobile phase solvent per unit surface area. Equation [6] shows that the retention of solutes, \(\ln k'\) should decrease linearly with the solvent.
Figure 10. Chromatograms at 25 °C and 170 atm. (A) with methanol as the mobile phase; (B) with 0.50 CO₂ mole fraction in the mobile phase. (1) 4-methylphenol; (2) 3,5-dimethylphenol; (3) 2,4,5-trimethylphenol; (4) quinoline; (5) naphthalene; (6) β-naphthol.
strength of the mobile phase. Therefore a means of measuring the solvent strength of
the mobile phase was sought.

Many empirical solvent polarity parameters have been introduced and have
shown varying degrees of success as predictive tools (25,26). One commonly used
method is to monitor the solvatochromic shift of the absorption spectrum of a dye as a
function of polarity. Pyridinium-N-phenoxide betaine is the most commonly used
solvatochromic dye. The variation of the $\pi - \pi^*$ transition energy of this dye has been
designated as the $E_{30}$ solvent strength scale. Early in the development of this solvent-
strength scale, concern was voiced that the measured transition energies were not
necessarily a measure of a solvent’s polarity (27). For example, hydrogen bonding
between the solvent and the dye also caused similar shifts in the absorption spectrum of
betaine. Deye et al. recently suggested that Nile Red ($E_{NR}$) be used instead of betaine
(28). They predicted that Nile Red’s solvatochromic shift would be less susceptible to
hydrogen bond interactions. Other advantages of Nile Red were that it does not degrade
in acidic solutions and is soluble in nonpolar solvents such as supercritical fluids. Using
$E_{NR}$ values for methanol/CO$_2$ mixtures determined by Deye et al. (28), the dependence
of $\ln k'$ versus the transition energy of Nile Red was determined (Figure 11). The
retention of all the test solutes increased with the measured transition energy, which for
Nile Red corresponds to a decrease in the measured solvent strength. Only naphthalene’s
retention increased as a linear function of Nile Red’s transition energy. The variation
in retention of the other solutes with the transition energy of Nile Red was nonlinear.
Figure 11. Variation of capacity factors with Nile Red transition energy, $E_{NR}$. Compound identifications are given in Figure 7.
To understand further the important solvent properties that were affecting the retention other solvent strength parameters were considered.

Of the numerous methods to determine solvent strength, the Kamlet-Taft solvatochromic parameters ($\pi^*$, $\alpha$, and $\beta$) are unique in that they provide information on the specific type of molecular-level interaction that is controlling the retention (29). The $\pi^*$ parameter is a measure of solvent strength due to dipole-dipole or dipole-induced dipole interactions. The $\alpha$ parameter measures the hydrogen bond donation capability, and the $\beta$ parameter measures the ability of a molecule to accept a hydrogen bond. Kamlet-Taft solvent strength parameters have been used in a broad range of applications to describe the behavior of solvents or solutes. For examples, the measurement of solute solubility in water (30), the partitioning of solutes in HPLC (31), the adsorption of gases (32) and liquids (33) onto solids were all well described by these parameters.

Kamlet et al. (34) also determined that $E_{30}$ was linearly related to their parameters. From the published values of $E_{NR}$ (29), $\pi^*$, and $\alpha$ (35,36), the following linear relationship was found between $E_{NR}$ and the Kamlet-Taft parameters:

$$E_{NR} = 53.9 - 6.77\pi^* - 3.93\alpha$$

$n = 21, r = 0.986, \text{ std. dev.} = 2.0$

The $\beta$ parameter was not used in this model because Nile Red does not have the ability to donate a hydrogen atom for hydrogen bond formation. However, one interesting point is that Nile Red does accept hydrogen bonds which is opposition to Deye and Berger's (28) supposition.
Ortho-nitroanisole is one of the dyes commonly used to determine the $\pi^*$ of a solvent (31). The $\pi^*$ parameter for the methanol/CO$_2$ mixtures was determined from the solvatochromic shift of o-nitroanisole and the following equation:

$$
\pi^* = \frac{(\nu_{\text{max}} - \nu_o)}{s}
$$

where $\nu_{\text{max}}$ is the frequency, in wavenumbers, of the absorbance maximum of the dye when dissolved in the solvent of interest; $\nu_o$ is the frequency of the dye's absorption maximum in a reference solvent, typically cyclohexane; and $s$ is a proportionality constant that limits values of the $\pi^*$ to a range of 0 to 1 for common solvents. Values of $\nu_o$ and $s$ were obtained from reference (31).

Figure 12 shows the variation of $\pi^*$ with methanol/CO$_2$ composition. Included in this plot are the experimentally determined values of $\pi^*$ as well as available literature values of $\pi^*$ for low proportions of added CO$_2$ (37). The $\pi^*$ parameter of the mixture decreases approximately linearly with addition of CO$_2$. Using the experimental values of $\pi^*$, published values of $E_{NR}$ and equation [8], the $\alpha$ parameter values for the methanol/CO$_2$ mixtures were determined. Figure 13 shows that the $\alpha$ parameter varies nonlinearly with volume fraction of CO$_2$.

The values of the $\beta$ parameter for the mixtures were determined by measuring the solvatochromic shift for 4-nitroaniline relative to that of $N,N$-dimethyl-4-nitroaniline (38). Hexamethylphosphoramide was used as the reference compound with its solvatochromic shift used to determine the $\beta = 1$ value. Figure 12 shows that the $\beta$ parameter varies
Figure 12. $\pi^*$ values for different methanol/CO$_2$ mixtures at 25 °C and 170 atm: experimental values; (+) literature values (37).
Figure 13. Experimental $\alpha$ and $\beta$ values for different methanol/$\text{CO}_2$ mixtures at 25°C and 170 atm.
nonlinearly with volume fraction of CO$_2$ with the curve being quite similar to that found for the $\alpha$ parameter.

The similar variation of the $\alpha$ and $\beta$ parameters with mixture composition can be easily understood when the structures and polarity of methanol and CO$_2$ are considered. Methanol is both a hard Lewis acid (hydrogen bond donor) and a hard Lewis base (hydrogen bond acceptor) using the Pearson acid-base classification (39). The amphiphilic behavior causes the observed self-association of liquid methanol. Methanol has less than 3% monomers present at 25°C. Hard acids prefer to complex with hard bases. Since CO$_2$ is a hard, weak Lewis acid and methanol can act as a hard Lewis base, this criterion for acid-base complexation was met. However, that criterion is not sufficient to cause significant complexation. To determine whether two compounds will form an acid-base complex, Pearson suggested that the following qualitative rule be considered (40):

$$\log K = S_A S_B + \sigma_A \sigma_B$$  \hspace{1cm} [9]

where $K$ is the equilibrium constant for the complex, AB; $S_i$ is a measure of the strength of the acid-base interaction; and $\sigma_i$ is the Pearson hardness-softness factor. The strength of the interaction is therefore also highly important. CO$_2$ is a very weak acid. Significant complexing of CO$_2$ with methanol is therefore unlikely. Also the shape of the plots of the $\alpha$ and $\beta$ parameters versus percent CO$_2$ show that the predominant effect of increasing proportions of CO$_2$ is hydrogen bond breaking. If hydrogen bonds were not broken with increasing CO$_2$ and only simple dilution occurred, then the $\alpha$ and $\beta$ values would decrease linearly with the volume fraction of CO$_2$. Instead, as shown in Figure
13, significant hydrogen bond breakage occurs with increasing CO\textsubscript{2} to the extent that the \(\alpha\) and \(\beta\) values are invariant until the CO\textsubscript{2} mole fraction in the mixture is greater than ca. 0.50.

**Retention Mechanism**

Figure 14 shows the variation of the solute retention as a function of the \(\pi^*\) parameter. Naphthalene is the only compound with retention that was linearly related to the \(\pi^*\) parameter. Alternatively, Figure 15 shows the variation of solute retention as a function of the \(\alpha\) parameter for the methanol/CO\textsubscript{2} mixtures. Interestingly, for all solutes except naphthalene the retention is a linear function of the \(\alpha\) parameter with a correlation coefficient \(\geq 0.991\). The dependence of the solute retention on the \(\beta\) parameter is also approximately linear with a correlation coefficient of 0.997. A possible retention mechanism that describes the observed retention behavior is discussed below.

PGC is expected to be a highly polarizable solid with chemical properties similar to graphite. When forming intercalation complexes, graphite shifts its electron density to accommodate the electron distribution of a molecule or an ion (41). The solutes used in this study were phenolic compounds, which are Lewis acids, and two weak Lewis bases, quinoline and naphthalene. For all the solutes except naphthalene, the retention increases with the increased H-bond donating or H-bond accepting ability of the mobile phase. This linear dependence of the retention on the \(\alpha\) and \(\beta\) parameters could be interpreted as being due to the formation of Lewis acid-base complexes with the PGC surface. The PGC surface may shift its electron distribution to accommodate either acidic and basic solutes. For the phenols the retention increases in order of increasing
Figure 14. Effect of $\pi^*$ on capacity factors at 25°C and 170 atm. Compound identifications are given in Figure 7.
Figure 15. Effect of $\alpha$ on capacity factors at 25°C and 170 atm. Compound identifications are given in Figure 7.
acidity of the compound which would indicate that the PGC surface was behaving like a Lewis base toward the phenolic molecules. Similar acid-base complexation was recently observed in PGC/SFC (42). Since quinoline was the strongest Lewis base studied, retention order information on more basic solutes was unavailable to corroborate the proposed mechanism of adsorption for basic compounds. However, other studies have reported that strong bases are strongly retained on PGC (43).

This proposed mechanism does not explain why naphthalene’s retention is not correlated with the Lewis acidity/basicity parameters, but instead is correlated with the $\pi^*$ parameter. The linear $\pi^*$ dependence indicates that the retention of naphthalene is mostly driven by interactions between the $\pi$-electron cloud of the naphthalene and that of the delocalized electron cloud of the PGC surface and that this interaction is different in some way to the interactions of the other solutes. As shown in Figure 8, naphthalene’s retention varies linearly with volume fraction of the mixture, while the retention of the other molecules does not. The adsorption sites that interact with naphthalene and the phenolic molecules, respectively, may be chemically or structurally different.

This possibility can be more easily understood by considering published information on the structure of porous glassy carbon (PGC). PGC is known to be macroscopically amorphous, but have microscopic order. On the 100-1000 Å scale, PGC appears as a randomly distributed interlacing of thin carbonaceous ribbons (44). At even higher magnification however, the ribbons appear to have graphitic-like crystalline structure. The crystalline surface of these ribbons is termed the basal-plane of PGC and
is expected to be similar to the basal plane of graphite (45). These planes of hexagonally-packed sp² carbons have finite lengths which indicates that edge-planes exist in PGC (43, 45). Therefore, PGC has at least two different types of adsorption sites: basal-plane and edge-plane. For a PGC, such as the Hypercarb used in this study, which was prepared at high temperatures > ca. 2000 °C, the proportion of basal-plane carbon is much higher than that of edge-plane carbon; however, the proportion of edge-plane may still be as high as ca. 10-20%. The two different types of adsorption sites also differ in reactivity. Recent studies found the edge-plane sites are more reactive than the basal-plane sites (46). Edge-plane sites are expected to have dangling bonds (43) which are likely candidates for the Lewis acid/basic sites. Alternatively, the basal plane with its crystalline graphitic structure is flat on the molecular scale and is expected to interact strongly with polyaromatic hydrocarbons, such as naphthalene, predominately by charge-transfer between the π-electron clouds of the polyaromatic hydrocarbon and the PGC.

The large difference in retention behavior of β-naphthol and naphthalene versus π* and α can also be explained by the combination of these two mechanisms. Variation of the retention of β-naphthol with solvent composition is predominately controlled by acid-base complexing of the phenol group with the Lewis basic sites of the surface, but the absolute value of the capacity factor, k', shows that interaction with the basal-plane also is probably involved in its retention. The competition between these two mechanisms is supported by thermodynamic data in the succeeding sections.

An alternate retention mechanism is that instead of solutes interacting with two different types of adsorption sites on the carbon, the two different mechanisms are caused
simply by the molecules interacting differently with the same sites on the PGC. For example the polyaromatic hydrocarbons without heteroatoms could lie down against the flat PGC surface with all carbon atoms in the molecule interacting with the PGC surface; and the heteroatom sites could be causing the molecules to interact with the PGC with the molecules configured approximately perpendicular to the surface. The mechanism that involves two different adsorption sites rather than this mechanism is favored because: 1) the presence of the two different reaction sites on PGC is well documented, and 2) in comparing the retention of β-naphthol to the retention of naphthalene as a function of solvent composition, if the major interaction difference was conformation of the solute molecule with respect to the surface, then averaging effects would be observed in the retention behavior. That is, it seems reasonable that the heteroatoms would be capable of interacting with the surface at interaction angles other than exactly perpendicular. Therefore, under these conditions, the molecule would probably have some of both mechanisms occurring at many interaction sites. The plot of retention of β-naphthol versus the α parameter seems to be close to a simple addition of the curve for the interaction of monosubstituted phenolic compound with the curve from naphthalene curve, which implies minimal averaging occurred. However, to discern conclusively which mechanism is valid, nonchromatographic means of monitoring the adsorption interaction should be studied.

Variation of $k'$ with Temperature

The effect of temperature on retention under constant pressure conditions is illustrated in Figure 16. For a 0.40 CO$_2$ mole fraction mixture at 170 atm, the tempera-
Figure 16. Variation of capacity factors with temperature in 0.40 CO₂ mole fraction at 25 °C and 170 atm. Compound identifications are given in Figure 7.
ture was varied from 343 to 463 K. This temperature range spans both the liquid and supercritical regions of the phase diagram for this mixture. Using the estimation technique of Chueh and Prausnitz (47), the critical temperature of this mixture was approximately 433 K.

At low temperatures, ln $k'$ decreases linearly as temperature increases until just past the critical temperature of the solvent. After that point, ln $k'$ increases with temperature. Yonker et al. (48) reported similar variation of ln $k'$ with temperature for liquid and supercritical at constant pressure. The following expression describes the variation of ln $k'$ with temperature.

$$
(\partial \ln k' / \partial T)_p = \frac{1}{RT^2} \left[ \Delta H_i^\infty (T_0) + \int_{T_0}^T \Delta C_p^m dT \right] + \frac{1}{V_m} (\partial V_m / \partial T)_p \tag{10}
$$

where $\Delta C_p^m$ is the change in heat capacity of the solute associated with the transfer from the mobile phase to the stationary phase; $\Delta H_i^\infty$ is the enthalpy of transfer; $T$ is absolute temperature; and $V_m$ is the molar volume of the mobile phase. The differential term in eq. [11] is the volume expansivity and contributes negligibly to the variation in ln $k'$ with temperature in most liquid chromatographies. However, with the high fluidity mobile phases, the volume expansivity term was significant. For the 0.40 CO$_2$ mole fraction mixture in LC and SFC, the volume expansivity was responsible for ca. 20-30%, and 70% of the change in retention with temperature, respectively.

By rearranging eq. [11] and noting that $\Delta H_i^\infty$, the average enthalpy of transfer is defined as:
the following equation is obtained:

\[ \Delta H_i^\infty = \Delta H_i^\infty (T_o) + \int_{T_o}^{T} \Delta C_{pi}^\infty \, dT \]  

[11]

Therefore, by measuring the mixture densities as a function of temperature and then plotting \((\ln k' - \ln V_m)\) versus \(1/T\), the average enthalpy of solute transfer from mobile phase to stationary phase at infinite dilution, \(\Delta H_i^\infty\), was determined for supercritical and liquid conditions. The free energy associated with the transfer equilibrium was also determined using the expression below:

\[ \Delta G_i^\infty = -RT \ln K = -RT \ln \left( \frac{V_{net}}{SN_0 V_m} \right) \]  

[13]

where \(K\) is the partition coefficient; \(V_{net}\) is specific net retention volume; \(S\) is the specific surface area of adsorbent; and \(N_0\) is standard surface coverage of adsorbed solute. The entropy of transfer, \(\Delta S_i^\infty\), was then determined from:

\[ \Delta S_i^\infty = \frac{(\overline{\Delta H_i^\infty} - \Delta G_i^\infty)}{T} \]  

[14]

The specific surface area of the PGC adsorbent used in the calculation was 150 m²/g (49) and the Kemball-Reideal standard surface state was used for \(N_0\) (50). Table 3 shows the resultant \(\Delta H_i^\infty\), \(\Delta G_i^\infty\), and \(\Delta S_i^\infty\) of the test solutes; the solutes are listed in order of increasing retention. For both SFC and LC, the enthalpy of transfer is small. Similar
Table 3. Thermodynamics of Solute Transfer from Mobile Phase to Stationary Phase for a 0.40 CO₂ Mole Fraction Mixture.

<table>
<thead>
<tr>
<th>Solutes</th>
<th>$\Delta H_i^{\infty}$ Kcal/mol</th>
<th>$\Delta G_i^{\infty}$ kcal/mol$^a$</th>
<th>$\Delta S_i^{\infty}$ cal/(mol K)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC SFC</td>
<td>LC SFC</td>
<td>LC SFC</td>
</tr>
<tr>
<td>4-methylphenol</td>
<td>-2.4 -6.9</td>
<td>-13.1</td>
<td></td>
</tr>
<tr>
<td>2,4-dimethylphenol</td>
<td>-2.8 1.7 -7.0 -8.6</td>
<td>12.2 23.2</td>
<td></td>
</tr>
<tr>
<td>2,4,6-trimethylphenol</td>
<td>-3.1 1.0 -7.1 -8.7</td>
<td>11.7 21.9</td>
<td></td>
</tr>
<tr>
<td>quinoline</td>
<td>-3.2 2.0 -7.3 -8.9</td>
<td>12.0 24.6</td>
<td></td>
</tr>
<tr>
<td>naphthalene</td>
<td>-3.3 -1.4 -7.3 -8.8</td>
<td>11.7 16.7</td>
<td></td>
</tr>
<tr>
<td>$\beta$-naphthol</td>
<td>-3.5 2.1 -7.8 -9.3</td>
<td>12.5 25.7</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Data taken at 170 atm and 343 and 443 K for LC and SFC, respectively. $^b$ Standard deviation for $\Delta H_i^{\infty}$ and $\Delta G_i^{\infty}$ is $\leq 0.1$ kcal/mol and for $\Delta S_i^{\infty}$ is $\leq 0.5$ cal/(mol K).
low values of $\Delta H^\circ$ for PGC-SFC with other mobile phases were recently reported (1). In SFC, the transfer process from the mobile phase to the stationary phase was endothermic for all the solutes studied except for naphthalene; while in LC, the transfer process was exothermic. In LC an increase in $\Delta H^\circ$ is correlated with increased retention of the solutes. However, no such trend is apparent in SFC data. The free energy of transfer, $\Delta G^\circ$, in both SFC and LC was strongly negative. Therefore, the retention mechanism is controlled by the positive entropy of transfer in SFC. In LC, the entropy of transfer remains the predominant cause of retention, but the enthalpy of transfer does contribute to the resultant negative free energy.

Typically in an adsorption process, the $\Delta S^\circ$ associated with solute transfer from the mobile phase to the stationary phase is negative. That is, solutes typically lose degrees of freedom on adsorption. However, for this system the solutes gain entropy with adsorption. Possible causes of this entropy gain could be: 1) the breakup of an ordered solvent shell upon adsorption, 2) rearrangement of an adsorbed surface layer, or 3) increased entropy of the adsorbent due to chemical adsorption interactions such as swelling phenomena. This chromatographic analysis of the thermochemistry cannot discriminate among these possibilities, which represent both physical and chemical adsorption mechanisms. However, no macroscopic indications of adsorbent swelling was observed, and therefore the aforementioned possibilities seemed more likely. Also, two interesting points should be noted. In SFC, increased ordering occurs in the transfer process compared to that in LC for all solutes. In addition, naphthalene adsorbs with a negative enthalpy of transfer and a smaller positive entropy of transfer than the other
molecules. This difference is most acute under SFC conditions. Naphthalene's variant behavior compared to that of the other molecules further supports the hypothesis that naphthalene has a different adsorption mechanism than the other molecules.

**Variation of $k'$ with Pressure**

The effect of pressure on retention in HPLC is less studied than in GC and SFC. Because liquids have low compressibilities, the capacity factor varies minimally in liquids unless a very large range of pressure is studied. The high fluidity liquids have compressibilities higher than common liquid solvents. Therefore, the variation in capacity factor as a function of pressure was monitored for fluid-modified HPLC over a range of reasonable operating pressures. The effect of pressure variation on the retention of quinoline for various methanol/$\text{CO}_2$ mixtures is shown in Figure 17. At low proportions of $\text{CO}_2$, the capacity factor varied minimally with pressure change. Measurable change in retention with pressure variation only occurred when high proportions of $\text{CO}_2$ were added. The enhanced variation in capacity factor as function of pressure is a function of compressibility. The isothermal compressibility varies from $6.95 \times 10^{-5} \text{ atm}^{-1}$ for methanol to $50 \times 10^{-5} \text{ atm}^{-1}$ for a methanol/ $\text{CO}_2$ mixture with 0.90 $\text{CO}_2$ mole fraction.

**CONCLUSIONS**

In summary, the methanol/$\text{CO}_2$ liquid mobile phases have lower viscosities than those of pure liquids, which results in increased diffusivity and smaller column pressure drop. In this study, the addition of a low viscosity, weakly interacting fluid, such as
Figure 17. Effect of pressure on capacity factors of quinoline for different mobile phase compositions at 25 °C: (+) 0.20 CO₂; (▲) 0.30 CO₂; (●) 0.60 CO₂; (▼) 0.90 CO₂ mole fraction in the mobile phase.
CO₂, to methanol caused decreased time of analysis for all test compounds studied. The decreased time of analysis found for this system was comparable to that found in SFC which is not surprising since the viscosity of liquid CO₂ is only ca. 20% greater than that of supercritical CO₂ at the same pressure.

For the case of naphthalene, the time of analysis decreased continuously with increased CO₂. For the other test solutes a minimum time of analysis was found when CO₂ mole fraction in the mobile phase was ca. 0.50. This composition is also the one above which the capacity factors of all the test solutes increases with increasing CO₂. The increase in capacity factor for naphthalene was much less than that of the other compounds. Increased retention was attributed to diminished solvent strength, Lewis acidity/basicity, over the same composition range.

The efficiency of the column did not vary with mobile phase viscosity as in previously cited liquid-solid chromatographic studies. This invariance of efficiency was attributed to slow mass-transfer kinetics at the solid adsorbent which is unaffected by mobile phase viscosity. However, for other stationary phases such as bonded phases or other solids such as silica gel, which display fast mass transfer kinetics, the added advantage of higher efficiencies with increasing CO₂ is expected.

To account for the significant difference found between the retention behavior of naphthalene and the other test compounds, two different retention mechanisms are proposed which involved different interaction sites in the PGC surface. Further characterization of the mobile phase-stationary phase interaction was undertaken. The effect of temperature variation on the capacity factor of the compounds over a
temperature range that scanned through both supercritical and liquid conditions of the mixture was described. Also, the effect of pressure on the capacity factor was monitored to determine the effect of the increased compressibility of the mobile phase on retention.

**LITERATURE CITED**


42. Engel, T. M.; Olesik, S. V. unpublished results.


CHAPTER III

REVERSED-PHASE HPLC USING ENHANCED-FLUIDITY MOBILE PHASES

INTRODUCTION

In Chapter II, it was shown that liquid mobile phases with enhanced fluidity could provide HPLC some of the advantages of SFC, such as decreased time of analysis without losing the significant power of liquids which is high solvent strength (1). To produce these enhanced-fluidity solvents, large proportions of low viscosity gases are dissolved in common eluents used for HPLC. The previous chapter was a demonstration of the advantage of using methanol/CO₂ mixtures as the eluent in porous glassy carbon HPLC.

In this chapter the use of enhanced-fluidity solvents in reversed-phase HPLC will be described. The most common eluents used in HPLC are mixtures of water with methanol or acetonitrile. There are substantial drawbacks to the use of methanol/water mixtures as eluents. The methanol/water mixture viscosity is larger than that of the components. At 50% (wt/wt) methanol (or 0.56 mole ratio of methanol/water), the mixture viscosity is approximately twice the viscosity of water. The variation in viscosity with composition can cause substantial variation in linear velocity during composition gradients. In addition, high viscosities will lower efficiencies and increase
the time of analysis. This chapter describes the use of methanol/water/carbon dioxide mixtures as eluents for reversed-phase HPLC. It was hypothesized that the addition of substantial portions of carbon dioxide (CO$_2$) to the mixture would decrease the mixture viscosity without affecting the solvent strength substantially.

The theory involved in the use of enhanced-fluidity solvents in HPLC was described in Chapter I and II. In reversed-phase liquid chromatography the predominant contribution to band dispersion is commonly diffusion in the stagnant mobile phase inside the porous packing. Using the Knox equation (2), the $C$ term, which describes the mass transfer resistances in the mobile phase, is $f(k')D_m$ where $k'$ is the capacity factor and $D_m$ is the binary diffusion coefficient of the analyte in the mobile phase and $u_{opt} \propto D_m/\sqrt{f(k')}$. If by lowering the viscosity the diffusion coefficients of the solutes can be increased without a substantial increase in $k'$, then the time of analysis should decrease and efficiency should increase in reversed-phase separations by addition of carbon dioxide to the mobile phase. The mobile phase system chosen for analysis includes a constant methanol/water mole ratio of 2.3 with carbon dioxide added to it in proportions that varies from 0 to 0.5 mole fraction. This methanol/water mixture is commonly used as the eluent in HPLC separations and its viscosity is ca. 1.5 times that of water (3).

**EXPERIMENTAL SECTION**

**Column Preparation**

A packed capillary column containing Adsorbosphere octadecyl polysiloxane coated 5 $\mu$m silica particles with 80 Å pore size (Alltech Associates, Inc.) was used in
this study. The column was prepared by slurry packing the particles in acetonitrile with 1% Triton X-100 (4). The ODS particles were held in the fused silica column with a microbore end-fitting (U-434, Upchurch Scientific Inc., Oak Harbor, WA) that contained a 2-μm frit (C407X, Upchurch Scientific Inc.). The ODS slurry was placed in a 5-mm i.d. x 15-cm stainless steel tube (used as a reservoir) and then pushed into a 320-μm i.d. x 30-cm fused silica tube (Polymicro Technologies). The pressure was increased gradually up to ca. 300 atm then maintained at that pressure for at least two hours. The column was depressurized over night by turning off the pump and allowing the whole system to gradually approach atmospheric pressure.

**Chromatographic System**

The chromatographic system used in this study was similar to that used in the previous study of enhanced-fluidity HPLC (1). The instrumentation included an ISCO LC-2600 syringe pump (ISCO, Lincoln, NE), a Valco W-series high-pressure injection valve (Valco Instruments, Houston, TX) with an internal injection volume of 60 nL, and a Spectroflow 757 UV/Vis absorbance detector (Kratos Analytical Instruments, Ramsey, NJ). The detector wavelength was 254 nm. The end of the chromatographic column was connected with a zero dead volume fitting to a piece of 100 μm i.d. fused silica tubing. The polyimide coating was removed from a 5 mm length of this tubing to create the detection cell. A 5 or 10-μm i.d. fused silica restricting tube was placed after the detector to control the linear velocity in the column. As in supercritical fluid chromatography, the flow restrictor is a necessary part of the chromatographic system. In addition in enhanced-fluidity HPLC, the column pressure must be maintained above
a minimum pressure to avoid having the mobile phase mixture separate into two phases (liquid-gas). For example, for 0 and 0.5 mole fractions of CO$_2$ the necessary column outlet pressure was 61 atm and 82 atm, respectively (5). The column inlet pressure was maintained at 204 atm throughout the study. Sodium nitrite, methylene chloride, and acetone were considered as possible nonretained markers. The retention times of all three compounds were the same across the whole mixture composition range. Acetone was used as the nonretained marker because the concentration needed to provide an observed response was least.

Materials

The analyte test mixture was a methanol solution containing 0.4 mg/mL naphthalene and 0.5 mg/mL each of phenanthrene and pyrene. All solvents and analytes were obtained from Aldrich Chemical Company (Milwaukee, WI.) with purity levels of $\geq$ 98%. Supercritical fluid grade CO$_2$ was obtained from Scott Specialty Gases (Plumsteadville, PA). Methanol/water/CO$_2$ mixtures were prepared by using two syringe pumps. A 2.3 mole ratio mixture of methanol and water of a given volume was placed in a syringe pump. The liquid CO$_2$ was maintained at 272 atm and room temperature in another syringe pump. From the known density of CO$_2$ under these conditions, the requisite volume of CO$_2$ was added to the methanol/water mixture in the other syringe pump to make a given solution composition. The final solution was pressurized to 204 atm, and equilibrated at 25 °C for at least 12 hours.
RESULTS AND DISCUSSION

Analyte Diffusion Coefficients

Two models were used to predict the binary diffusion coefficients of the analytes in these enhanced-fluidity mixtures, the modified Wilke-Chang (6) (equation 1) and Perkins-Geankoplis (7) (equation 3). Both theories have been successful in predicting diffusion coefficients in mixed liquids.

\[
D_{Am} = 7.4 \times 10^{-8} \frac{\left[ \sum \phi_i M_i \right]^{1/2}}{\eta_m V_A^{0.6}} \tag{1}
\]

\[
\phi M = \sum (x_i \phi_i M_i) \tag{2}
\]

where \(D_{Am}\) is the diffusion coefficient of solute A in mixture m, \(\eta_m\) is the mixture viscosity, \(V_A\) is the molar volume of solute A at its normal boiling temperature, \(T\) is temperature, \(x_i\) is mole fraction, \(\phi_i\) is the Wilke-Chang association constant of solvent component i and \(M\) is molecular weight of solvent component i.

\[
D_{Am} \eta_m^{0.8} = \sum x_i D_{Ai} \eta_i^{0.8} \tag{3}
\]

where \(x_i\) is mole fraction of i in the mixture; \(\eta_i\) is the viscosity of pure i, and \(D_{Ai}\) is the diffusion coefficient of A in pure component i. The viscosities of the methanol/water/CO\(_2\) mixtures (Figure 18) were calculated using the Teja-Rice method (8,9,10). The viscosities of methanol and water were corrected for operating pressures using the estimation technique of Lucas (11). The viscosity of CO\(_2\) at various pressures was obtained from reference (12). The accuracy of the viscosity predictions using the pressure-modified Teja-Rice method was estimated by comparison with experimentally
Figure 18. Calculated Diffusion coefficients of naphthalene (Δ), phenanthrene (♦), pyrene (+), and viscosities (○) of methanol/water/CO₂ mixtures at 25 °C and 204 atm.
measured viscosities of methanol/CO₂ mixtures (1). The viscosity predictions were within 5% of the experimental values.

To evaluate the accuracy of each model for predicting the diffusion coefficients of solutes in enhanced-fluidity liquids, experimental diffusion coefficients methanol/CO₂ mixtures (13) were compared to the predictions. The predicted diffusion coefficients were within 5% of the experimental values. Figure 18 shows the predicted average diffusion coefficients for the analytes as a function of solvent composition calculated using equations 1 and 3. The calculated diffusion coefficient of naphthalene varied from 1.2 x 10⁻⁵ to 2.7 x 10⁻⁵ cm²/s when mole fraction of CO₂ varied from 0 to 0.5.

**Evaluation of Column Bed Quality**

Before the properties of reversed-phase separations using enhanced fluidity mobile phases were determined, the integrity of the column bed was evaluated. The flow resistance parameter, \( \varphi \), is often used to evaluate the quality of the packing structure in the chromatographic column. The flow resistance parameter is defined as:

\[
\varphi = \frac{\Delta P t_o d_p^2}{\eta L_c^2}
\]

where \( \Delta P \) is the pressure drop along the column, \( t_o \) is the dead time of the column, \( d_p \) is the packing particle diameter, \( \eta \) is the eluent viscosity, and \( L_c \) is the column length. A well-packed column of spherical particles should have a dimensionless \( \varphi \) value between 500 and 1000 (14). Higher values of the flow resistance parameter are indicative of crushed packing or the presence of too many fines in the column packing. The slurry-packed ODS column with methanol mobile phase had a flow resistance parameter of 775.
which is well within the acceptable range for a well-packed column. Figure 19 shows the variation of the flow resistance parameter with mobile phase composition. The flow resistance parameter decreased with increased percent CO₂ in the mobile phase. With added carbon dioxide the mobile phase viscosity decreases. Therefore, to achieve a lower flow resistance parameter, the pressure drop necessary to obtain a given velocity must be lowered.

Another characteristic which is commonly used to evaluate columns is the total porosity, \( \varepsilon_T \), which is defined as the following equation:

\[
\varepsilon_T = \frac{F_v t_o}{r_c^2 L_c \pi}
\]

where \( t_o \) is the dead column time, \( F_v \) is the volumetric flow-rate, \( L_c \) is the column length, and \( r_c \) is the column radius. With a mixture of water and methanol, the slurry-packed ODS column was found to be \( \varepsilon_T = 0.723 \). The total porosity is the sum of the column interparticle porosity, \( \varepsilon_a \), and intraparticle porosity, \( \varepsilon_i \). Estimates of \( \varepsilon_a \) and \( \varepsilon_i \) can be determined by non-chromatographic methods, e.g. mercury porosimetry. However, porosities measured with Hg may not accurately represent the pore structure observed by the analytes in the column. The column interparticle porosities were determined with the modified Kozeny-Carman equation:
Figure 19. Dependence of the flow resistance on the mole fraction of CO$_2$ in the mobile phase.
where \( \varphi \) is the column flow resistance and \( \Psi^2 \) is a structural factor which is equal to 1 for spherical packing (15). The intraparticle porosity was then determined by difference, \( \varepsilon_T - \varepsilon_u \). Both experimental values of interparticle porosity, \( \varepsilon_u = 0.394 \), and intraparticle porosity, \( \varepsilon_i = 0.329 \) correspond well with those reported by other groups for similar well-packed ODS columns (15,16,17).

**Retention**

The expected gains in chromatographic efficiency and lowering of the time of analysis is predicated on the assumption that the capacity factor will not increase significantly with added carbon dioxide. If the addition of carbon dioxide substantially lowers the eluent strength for reversed phase separations, then the expected gains will be minimized. Figure 20 shows the change in the capacity factors \( (k') \) versus the mole fraction of added \( \text{CO}_2 \) in the mobile phase. For all three analytes, the retention decreased with added \( \text{CO}_2 \). From 0-0.3 mole fraction \( \text{CO}_2 \) in the mobile phase, capacity factors decreased 38%, 51%, and 54% for naphthalene, phenanthrene, and pyrene, respectively. However, over the range of 0.3 - 0.50 mole fraction \( \text{CO}_2 \), the analyte retention remained fairly constant.

**Solvent Strength Measurement**

To better understand the eluent strength of the methanol/water/\( \text{CO}_2 \) mixtures a study of solvent strength variation with added \( \text{CO}_2 \) was undertaken. Figure 21 shows measured Kamlet-Taft parameters, \( \pi^*, \alpha, \) and \( \beta \) for the mixed methanol/water/\( \text{CO}_2 \).
Figure 20. Variation of capacity factors with the mole fraction of CO$_2$ in the mobile phase. (△) naphthalene, (◇) phenanthrene, (+) pyrene.
Figure 21. Variation of experimentally measured Kamlet-Taft parameters with the mole fraction of CO$_2$ in the mobile phase. The standard error for $\pi^*$ (□), $\alpha$ (○), and $\beta$ (◇) are ±0.02, ±0.06, and ±0.04 respectively.
eluent. The specific details of the solvatochromic measurements were described in Chapter II. These methods are also similar to those used by Kamlet and Taft et al. (18) and Carr et al. (19). Both $\pi^*$ and $\alpha$ decrease slowly when the percentage of CO$_2$ in the mobile phase increased while $\beta$ increased with added CO$_2$. In Chapter II, we measured the solvent strength variation that occurred when 0 to 1 mole fraction of CO$_2$ was added to methanol. The addition of CO$_2$ had negligible effect on the measured $\alpha$ and $\beta$ values until more than 0.6 mole fraction of CO$_2$ was added. For CO$_2$ mole fraction higher than 0.60, the observed decrease in the $\alpha$ and $\beta$ parameters was similar. The minimal change in the hydrogen bond acidity or basicity was believed to be caused by carbon dioxide functioning as a structure breaker, lowering the extent of self-association in methanol and, therefore, maintaining the lewis acidity and basicity of the mixture. In the same methanol/CO$_2$ mixture, the $\pi^*$ value decreased by ca. 50% over the 0 - 0.60 CO$_2$ mole fraction range. The solvent strength variation in the methanol/water/carbon dioxide system is much different with added carbon dioxide than the methanol/carbon dioxide system. $\alpha$ and $\pi^*$ decrease by approximately 10% over the range of 0 - 0.5 mole fraction CO$_2$ and the $\beta$ value increased with added CO$_2$ with a marked increase in H-bond basicity observed between 0.2 and 0.40 mole fraction of added CO$_2$. Liquid carbon dioxide has solvent strength parameters of $\alpha = 0.05$, $\pi^* = 0.07$, and $\beta = 0.23$ at 170 atm and 25 °C (Figure 21). Again the carbon dioxide is decreasing the hydrogen bond association in the solvent. Otherwise the solvent strength would decrease monotonically with added carbon dioxide. Methanol ($\beta = 0.62$) is a much stronger lewis base than CO$_2$ or water ($\beta = 0.18$). Therefore, monomeric methanol is probably being released
from hydrogen-bond self-association or methanol-water hydrogen bond association to cause the observed increase in basicity.

Sadek et al. (20) first showed that solute hydrogen-bond basicity and to a lesser extent solute dipolarity were important in establishing retention behavior in reversed-phase separations using ODS. Hydrogen-bond acidity variation did not seem to impact the measured retention. Large positive $\beta$ values for solutes indicated substantial retention on ODS. Accordingly, changing to mobile phases with increased basicity should decrease solute retention.

The observed increase in $\beta$ over the same eluent composition region where the substantial decrease in retention occurs may mean that hydrogen-bond basicity is important in controlling the retention in this reversed-phase ODS system similar to what Sadek et al. described in their system. However, solvent polarity is not the only factor typically involved in the retention of PAH compounds on reversed-phase stationary phases. The shape or structure of the stationary phase also affects the retention of PAH molecules (21, 22). It is shown later in this chapter that the ordering of the stationary phase is also substantially affected by the addition of CO$_2$ to the eluent.

**Chromatographic Efficiency**

The separation of naphthalene, phenanthrene, and pyrene was complete for all of the mobile phase compositions studied. The column efficiency was measured using an eluent composition which varied from 0 to 0.5 mole fraction CO$_2$ with the mole ratio of methanol/water maintained at 2.3. The inlet pressure was maintained at constant inlet pressure of 204 atm, and temperature, 25 °C, conditions. The maximum mobile phase
velocity was limited by the minimum pressure necessary to keep the eluent mixture one phase after the column. As described in the experimental section, with increasing proportions of CO$_2$ in the mobile phase higher post-column pressure was necessary. Therefore, the maximum possible linear velocity decreased with added CO$_2$. As Figure 22-24 show linear velocities higher than typically used in HPLC were readily achieved with the chosen experimental conditions. If higher linear velocities than those in Figure 22-24 are desired, this is readily achieved by increasing the column inlet pressure. The column inlet pressure at 204 atm was chosen, however, to standardize the study.

Figures 22-24 show the variation in the reduced plate height versus reduced velocity for the three analytes under different mobile phase compositions. Each data point is an average of 2-3 values to ensure the precision of the results. Using equation 7, these values were also corrected for the band dispersion contribution caused by the connecting tubing which included the detector volume (see experimental section).

$$\sigma_T^2 = \frac{\pi^2 r_T^6 L_T u_T}{24 D_m}$$

$\sigma_T^2$ is the connecting tube volumetric variance, $r_T$ is the tube radius, $L_T$ is the tube length, $u_T$ is the linear velocity within the tube, and $D_m$ is the solute binary diffusion coefficient. The contribution to band dispersion caused by the interconnecting tubing caused 2% error in the band dispersion at the lowest linear velocities and 5% error at the highest velocity studied. Other extracolumn sources of error contributed < 1 %. No effort was taken to correct the measured peak widths for such small errors. The curves for all three analytes are very similar. With increasing CO$_2$ in the eluent, the reduced plate height
Figure 22. Reduced plate height of naphthalene versus reduced linear velocity. (□) methanol/water; (▼) 0.1 CO₂ mole fraction; (◇) 0.2 CO₂ mole fraction; (+) 0.3 CO₂ mole fraction; (▲) 0.4 CO₂ mole fraction; (○) 0.5 CO₂ mole fraction in the mobile phase.
Figure 23. Reduced plate height of phenanthrene versus reduced linear velocity. (□) methanol/water; (♀) 0.1 CO₂ mole fraction; (◊) 0.2 CO₂ mole fraction; (+) 0.3 CO₂ mole fraction; (△) 0.4 CO₂ mole fraction; (○) 0.5 CO₂ mole fraction in the mobile phase.
Figure 24. Reduced plate height of pyrene versus reduced linear velocity. (□) methanol/water; (▼) 0.1 CO₂ mole fraction; (◇) 0.2 CO₂ mole fraction; (+) 0.3 CO₂ mole fraction; (▲) 0.4 CO₂ mole fraction; (○) 0.5 CO₂ mole fraction in the mobile phase.
decreased across the entire velocity range studied. However, the decrease in plate height was marked at high velocities. For example, at a reduced velocity of 2, the reduced plate height for naphthalene changed from 4 (or $H = 0.02$ mm) to 2 (or $H = 0.01$ mm) for a change in eluent composition of 0 to 0.5 mole fraction CO$_2$; however for a reduced velocity of 10, the reduced plate height for naphthalene decreased from 11 to 4 for the same eluent composition change.

To better understand the chromatographic band dispersion process involved in this system, the data in Figures 22-24 were fit to the reduced Knox equation (2). The reduced Knox equation is defined as:

$$h = A \nu^{1.3} + B/\nu + C\nu$$  \[8\]

where $h$ is the reduced plate height, and $\nu$ is the reduced linear velocity. The $A\nu^{1.3}$ describes the band dispersion from flow anisotropy in the mobile phase, and $C\nu$ describes the band dispersion caused by mass transfer between the mobile and stationary phases. $B/\nu$ describes the dispersion caused by axial molecular diffusion, and it contributes to the total band dispersion only when $\nu$ is smaller than 2. When B term was included in the fitting equation, it was found to be statistically insignificant because the low velocity region was not sampled in this study. Therefore, the final equation used to fit the experimental data included only the flow anisotropy and mass transfer terms at $\nu > 2$. Figure 25 and 26 show the change in coefficients $A$ and $C$ with added CO$_2$ in the mobile phase.

Typical values of the constant $A$ for a well packed-column is about 1, with values greater than 2 or 3 indicating a poorly packed column. (2,23). For all three analytes,
Figure 25. Variation of flow anisotropy, A term with the mole fraction of CO₂ in the mobile phase. (△) naphthalene, (◊) phenanthrene, (+) pyrene. The average standard error is ±0.1.
Figure 26. Variation of mass transfer resistance, C term with the mole fraction of CO$_2$ in the mobile phase. (Δ) naphthalene, (◊) phenanthrene, (+) pyrene. The average standard error is ±0.04.
the constant $A$ varied from 1-1.5 with no trend with composition change observed. The
last constant in the Knox equation is the $C$ term, the sum of resistance to mass transfer
and the kinetics of analyte desorption from the stationary phase. In Figure 26, the $C$
terms of all three analytes are plotted versus mole fraction CO$_2$ added to the mobile
phase. With no added CO$_2$, the $C$ values are ca. 0.75 - 0.85. Karlsson and Novotny
(24) recently reported a $C$ value of 0.37 for a 265 $\mu$m i.d. microcolumn packed with
5$\mu$m ODS coated particles. They also showed that the value of the measured $C$ term
increased with the internal diameter of the microcolumn. The internal diameter of the
column used in this study was larger, 320 $\mu$m. However, it is highly doubtful that the
small difference in column internal diameter would cause the $C$ value to double. A more
logical cause of the differences in the measured $C$ values is the different mobile phases
used in each study. Karlsson and Novotny used acetonitrile/water and we used
methanol/water. The viscosity difference between the mixture and the pure components
is much larger for the methanol/water mobile phase than in the acetonitrile/water mobile
phase. In addition, as more CO$_2$ was added to the methanol/water system, the $C$ terms
for all the analytes decreased dramatically to values as low as $C = 0.08$ for naphthalene
with 0.5 mole fraction CO$_2$. The $C$ terms also did not vary significantly among the
analytes studied which indicates that the trend in $C$ term was not caused by capacity
factor variation.

The most likely causes of the large $C$ coefficient when the methanol/water
mixture was the eluent include: unaccounted for extra-column band broadening such as
injection profile, column quality, or slow kinetics associated with the reversible binding
of the PAH compounds by the stationary phase. It is believed that the primary cause of the large $C$ coefficient is slow kinetics. Using the theoretical model of Guiochon et al. (25), the band dispersion caused by the injection profile was calculated to be less than 1% of the total measured variance and was therefore considered negligible. The quality of the column was documented early in this chapter and other results that are described later will further substantiate its integrity.

Equation 9 is the relationship derived by Horvath and Lin (26,27) which describes the dispersion in the stagnant mobile phase inside the pores of the particles ($C_{stag}$) and the kinetics of desorption at the stationary phase ($C_{kin}$).

$$C = C_{stag} + C_{kin} = \frac{\theta (k_o + k' + k_o k')^2}{30 k_o (1 + k_o)^2 (1 + k')^2} + \frac{2k'D_m}{(1+k_o)(1+k')^2 d_p^2 k_d}$$

$D_m$ is the binary diffusion coefficient of the analyte molecule, $\theta$ and $k_o$ are stationary phase structure parameters, $k_d$ is the desorption rate constant and $d_p$ is particle diameter of the column packing. Almost all of the variable in equation 9 are experimentally measurable. The stationary phase structure parameters of Horvath and Lin were used to predict the band broadening caused by diffusion in the stagnant mobile phase of the pores. Then this value was subtracted from the total $C$ value to provide an estimate of the proportion of the band broadening caused by slow desorption kinetics, $C_{kin}$. The estimated diffusion coefficients described previously were then substituted into the expression for $C_{kin}$ to allow the calculation of the desorption rate constant, $k_d$. This calculation is used to show the order of magnitude changes in the rate constant.
variation of the desorption rate constant as a function of added CO₂ to the mobile phase was listed in Table 4. The rate constant increased significantly with added CO₂.

The plate height contribution caused by slow desorption kinetics is inversely related to the desorption rate constant and the particle size. Horvath and Lin (27) predicted significant band dispersion contributions from kinetic resistance for ODS particle sizes less than 6 µm. Others have also reported the significant of kinetic resistance in reversed-phase HPLC (28). However, the cause of the low desorption rate was not discussed. When methanol/water mobile phases are used, the evidence below indicates the major cause of slow desorption kinetics is the formation of a polar hydrogen bonded layer of water or water/methanol on the stationary phase surface. When increasing quantities of CO₂ are added to the methanol/water eluent, structure breaking of the hydrogen bonded layer occurs and the desorption rate constant increases.

Figure 27 shows data that supports this hypothesis. β-naphthol and naphthalene have similar capacity factors across the entire eluent composition range. For example, for the eluent with methanol/water mole ratio of 2.3, the capacity factors of naphthalene and β-naphthol were 0.75 and 0.28, respectively. However, the reduced plate height of each chromatographic band was different as shown in Figure 27. The slope of the β-naphthol curve, which mainly represents the effect of the C term contributions, is smaller than that of naphthalene. The hydroxyl on β-naphthol may allow ready movement of naphthol through the structured layer above the ODS phase while naphthalene has more difficulty.
Table 4. Calculation of Rate Constants of the Desorption Kinetics.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mole Fraction of CO₂</th>
<th>Calculated $C_{stag}$</th>
<th>Observed $C$ (i.e., $C_{stag} + C_{kin}$)</th>
<th>Calculated $k_d$ (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.00</td>
<td>0.091</td>
<td>0.73</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.084</td>
<td>0.50</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.077</td>
<td>0.47</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.075</td>
<td>0.31</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.078</td>
<td>0.17</td>
<td>209</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.00</td>
<td>0.12</td>
<td>0.86</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.11</td>
<td>0.59</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.10</td>
<td>0.51</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.095</td>
<td>0.37</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.10</td>
<td>0.23</td>
<td>152</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.00</td>
<td>0.14</td>
<td>0.84</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.13</td>
<td>0.60</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0.12</td>
<td>0.44</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.11</td>
<td>0.35</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>0.40</td>
<td>0.11</td>
<td>0.25</td>
<td>137</td>
</tr>
</tbody>
</table>
Figure 27. Plots of reduced plate height of naphthalene (□) and naphthol (○) versus reduced linear velocity when methanol/water mixture was used as mobile phase.
Another way of studying the possible existence of the hydrogen bonded layer is to look at the band dispersion of the same compound on the same column and stationary phase in the presence and absence of such a surface layer. Acetonitrile is also often used as an organic modifier in reversed-phase separations. Acetonitrile like methanol is a small molecule, with low viscosity, and complete miscibility with water. However, it does not participate in hydrogen bonding interactions. Figure 28 shows a comparison of the measured efficiency of phenanthrene using the same column with a 1.03 mole ratio acetonitrile/water eluent and the 2.3 mole ratio methanol/water eluent. The capacity factors of phenanthrene in acetonitrile/water and methanol/water system were 2.30 and 1.71, respectively. The marked difference in the plate height between the two solvents is clearly an increased $C$ coefficient for the methanol/water system.

Our hypothesis of the existence of the hydrogen-bonded surface layer that is broken by increasing proportions of carbon dioxide is also compatible with the predominance of other published information in stationary phase composition, structure and polarity. Yonker et al. (29) proposed a ternary component model of the stationary phase which was composed of a combination of silica substrate, bonded organic moiety and solvated layer consisting of mobile phase components. For the methanol/water composition used in this study, Yonker et al. (29) proposed a "brush"-like solvated stationary phase structure in which the $C_{18}$ chains become erect and minimal intermolecular interactions between individual chains occurs. Also, at this composition, the individual $C_{18}$ chains were believed to be highly solvated by the methanol in the system which resulted in liquid-like motion of the $C_{18}$ chains and partitioning into the
Figure 28. Plots of reduced plate height of phenanthrene versus reduced linear velocity in methanol/water (□) and acetonitrile/water (○) mixtures.
chain structure as part of the retention mechanism. However, more recent NMR studies of ODS motion in methanol/water solutions comparable to those used herein shows that the ODS chain motion under these conditions is highly hindered. Gilpin et al. (30) showed that the $^{13}$C NMR spectrum of ODS solvated with 60/40 v/v methanol/water mixture (0.67 mole ratio), was very similar to that of unsolvated ODS. This was contrasted to the NMR spectrum of ODS solvated with pure methanol which had line shapes that were liquid-like. If a highly order hydrogen-bonded surface layer of water and methanol were sheathing the ODS, then the observed hindered chain motion could be rationalized. This proposed structure is also compatible with the present understanding of the microstructure of the methanol/water solvent. For solutions of methanol/water where the methanol concentration is significantly higher than that of water, such as the mixture used in this study, two different statistical mechanical simulations (31,32) predicted that hydrophobic solvation of the methanol molecules cause ca. sixteen self-associated water molecules to form a cage around the methyl portion of the alcohol; while the hydroxyl portion of the alcohol forms hydrogen bonds with only ca. 2.6 water molecules. Similar engagement of the ODS phase by associated water or a water/methanol sheath may be causing the observed low desorption rate constant for naphthalene and the interaction with ODS when solvated in the 2.3 methanol/water mole ratio eluent.

**Time of Analysis**

In the previous section, improvements in efficiency were demonstrated. However, instead of simply improving efficiency of a separation, a more practical goal
is to increase resolution per unit time and therefore decrease the time of analysis. If the time of analysis, \( t \), is defined as the retention time of the last eluting peak, then equation 10 shows the relationship between experimentally controlled chromatographic variables and \( t \) for the separation of two analytes.

\[
\begin{align*}
    t &= 16 R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k'}{k'}^3 \right) \left[ \frac{H}{u} \right] \\
\end{align*}
\]

where \( t \) is time of analysis, \( R_s \) is resolution, \( \alpha \) is separation factor, \( k' \) is capacity factor of the peak, \( H \) is the plate height, and \( u \) is the mobile phase linear velocity. Once the chromatographic system is chosen (stationary phase, solvent, solute, etc.), capacity factors and the separation factor are constants. Therefore, when \( R_s \) is fixed for a required separation, the ratio of \( H \) to \( u \) must be minimized to decrease time of analysis not \( H \) itself.

Figure 29 show the variation of \( H/u \) versus \( u \) for the methanol/water mixture and for the methanol/water mixture with 0.5 mole fraction CO\(_2\) added for naphthalene. When methanol/water was the eluent, the \( H/u \) ratio reached a minimum value for a linear velocity of 0.15 cm/s or reduced linear velocity of ca. 5. The \( H/u \) ratio for the eluent with 0.5 mole fraction CO\(_2\) does not reach a minimum until linear velocities of 0.4-0.5 are reached. Also, the mobile phase which has 0.5 mole fraction of CO\(_2\) had shorten the analysis time by factors of 2.5 and 8 at linear velocity of 0.15 \((v = 5)\) and 0.35 \((v = 17)\) cm/s, respectively. If a specific analysis time is required, the low \( H/u \) value at high velocity for the eluent with added CO\(_2\) will allow longer column lengths to be used.
Figure 29. Variation of the ratio of plate height to linear velocity with the mole fraction of CO$_2$ in the mobile phase for naphthalene. ($\square$) 0.5 mole fraction of CO$_2$ in the mobile phase; (○) methanol/water mixture as mobile phase.
Figure 30. Variation of observed number of plates per unit time, $N/s$ ($\circ$) and the ratio of plate height to the mobile phase linear velocity, $H/u$ ($\triangle$) with the mole fraction of CO$_2$ in the mobile phase. Vertical lines show typical $N/s$ values in HPLC and SFC.
without losing resolution. Because the permeability of the column is much higher when CO₂ is added to the mobile phase, very long packed-capillary columns should be possible at workable inlet pressures for the separation of highly complicated samples.

Finally, Figure 30 shows the variation in the $H/u$ and $N/time$ (number of theoretical plates per unit time) ratios as a function of added CO₂ for naphthalene at a reduced linear velocity of 5. The range of $N/time$ for standard packed HPLC and packed SFC with 5 μm particles is also marked. Clearly, as expected the use of enhanced fluidity mobile phases moves the measured $N/time$ into a range larger than found in standard HPLC and common for SFC.

**LITERATURE CITED**


CHAPTER IV
COMPARISON OF SUPERCritical FLUID
EXTRACTION AND ENHANCED-FLUIDITY EXTRACTION

INTRODUCTION

Because of more and more environmental concerns, as well as longer extraction time spent using traditional techniques, supercritical fluid extraction has been developed. Among many supercritical fluids, carbon dioxide (CO₂) is the most commonly used solvent in SFE because it is nontoxic and has low critical pressure and temperature range. Based on recent reports, SFE with CO₂ competes well with liquid extraction in extraction recovery of some organic contaminants, such as polynuclear aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (1). In some of the cases, supercritical CO₂ can achieve much faster extraction rate because of the fast solute diffusivity in supercritical fluids than in liquids.

When organic compounds are desorbed from a matrix, such as soil, the solute mass transfer from matrix surface to liquid solvents is slow. Steinberg (2) estimated that the release of 1,2-dibromoethane from undisturbed soils took more than 20 years. Some pesticides take days to be removed from undisturbed soils (3). The desorption of most of the organic compounds from soils to liquids is characterized as having an initial fast step followed by a much slower approach to equilibrium conditions. Recent results
showed that the initial fast step could be the solute desorption. The limiting step is the removal of the solute away from the surface of the adsorbent through the thin water film that surrounds the adsorbent particle. Another slow step is diffusion of the solute out of the interior micropore structure of the adsorbent (4). Since the diffusivity of solutes in supercritical fluids is 1-2 orders of magnitude higher than that in liquids (Table 1), the use of supercritical solvents should accelerate the diffusion process. Therefore the total extraction rate may be faster. However, as we discussed in Chapter II and III, supercritical fluids usually have less solvation power than liquids. At pressures of 170-200 atm, the density of CO$_2$ is even higher than that of methanol. But the experimental data showed that CO$_2$ was still a much weaker solvent than methanol (5). This weaker solvent strength could slow down the desorption kinetics when the solute-matrix bond is broken. Especially when large and polar solutes are involved, the limiting step in SFE could be surface kinetics rather than diffusion in the solvent. In addition, when water exists near the matrix surface, CO$_2$ may not be able to break the water-matrix bonds. The penetration of the solute through the water film could also become a slow step in the extraction process.

In this chapter, based on the studies of enhanced-fluidity solvents in Chapter II and III, initial studies comparing enhanced-fluidity extraction and SFE were undertaken.

**THEORETICAL CONSIDERATIONS**

The extraction mechanisms of chemical compounds from adsorbing materials are extremely complicated. The overall extraction proceeds by more than one step. The
removal of the analytes from the surface of the matrix is usually the first. If a general first-order kinetic model is applied to this step, the rate constant, \( k \), could be described as:

\[
k = k_0 e^{-\frac{E_a}{RT}}
\]  

[1]

where \( k_0 \) is the preexponential factor, \( E_a \) is the activation energy of desorption, \( R \) is the universal gas constant, and \( T \) is temperature. Usually thermal energy can overcome the energy barriers associated with weakly adsorbed species. To achieve fast desorption of those strongly adsorbed species, the activation energy must be reduced by selective interaction of the solvent with the matrix-solute complex in order to break the bond.

\( \text{CO}_2 \) is a weaker solvent compared to other supercritical fluids (nitrous oxide and difluorodichloromethane) and most of the commonly used organic solvents, such as methanol. In supercritical fluid chromatography (SFC), many of studies have shown that \( \text{CO}_2 \) cannot desorb large or polar compounds from common stationary phases. Methanol is used as one of the modifiers to lower the activation energy of the desorption from the stationary phases. In extractions, the matrices are much more complicated than stationary phases in SFC. The matrix may be fly ash, river sediment, and soils, or relatively simple ones, such as sand, and Florisil. There are many different types of active sites and surface structures in these matrices. Therefore, the chemical and physical interactions between analyte molecules and the matrix are expected to be much stronger than those in SFC. Extraction recovery improvements are often observed in SFE when organic modifiers are added to \( \text{CO}_2 \). One hypothesis of the cause for this
enhanced recovery suggests that the interaction between the modifier and the matrix is the predominant interaction (6). If CO₂ itself is too weak for strong solute-matrix interactions, the desorption kinetics will be the limiting step.

The second step in the extraction is to overcome any other barriers near the surface. Minerals are widely distributed in most of the environmental matrices and often form a major or even the sole constituent of the material fraction. When present, the high internal surface area of these minerals can be the major water-holding reserve. In soil, for example, these minerals consist of mica-like silicate unit layers about 0.95 nm thick which are interleaved with one or more sheets of water molecules that incorporate the exchangeable cations necessary to balance the negative charge on the silicate layers. These water molecules interact with both the cations and the oxygens on the silicate surface. The water molecules on the soil surfaces are sufficiently static to allow their position to be identified by X-ray diffraction (7). Further away from the silicate surface, there are grossly expanded phases in which the interlayer water must be much more liquid-like. Studies (8) showed that the thin water film on the surface may cause slow solute desorption. Carbon dioxide has a solvent strength which is usually compared to liquid hexane. The solubility of water in CO₂ is extremely low under normal supercritical fluid extraction conditions (< 0.01% v/v). It is expected that CO₂ cannot efficiently break any water layers on the matrix surface. Since the active sites which hold water can also interact strongly with other polar molecules, polar co-solvents need to be considered to improve the extraction.
The last step of the extraction process is the mass transport of solvated solute molecules to the bulk of the fluid. The primary driving force within small pores of the matrix is molecular diffusion. The total time this step takes, however, depends on both solute diffusion and the porosity of the matrix. Clearly, this is the step where supercritical fluids have big advantages over liquids. If the solubility of the solute in CO₂ is high enough, CO₂ should take shorter time to complete the diffusion step than methanol does. There are different approaches to understand solubility or interactions between solvent and solute. Solubility parameter theory is one of them (9). The solubility parameter is defined as:

\[ \delta = \left( \frac{\Delta E_v}{V} \right)^{0.5} \]  

where \( \Delta E_v \) is the energy of vaporization, \( V \) is the molar volume. This theory has been successful to predict solubilities of liquid molecules. Because it doesn’t include interactions between solute and solvent, it could be very wrong especially when solvation of solids, often larger and polar molecules, are considered. For example, phenanthrene and anthracene have solubility parameters 9.8 and 9.9, respectively. But their solubilities in benzene are as different as 0.21 and 0.008 mole fraction, respectively (10). The Kamlet-Taft system, however, is based on experimental data describing different molecular level interactions. This system was discussed and used in Chapter II and III. Experimentally measured Kamlet-Taft parameters for supercritical fluids and liquids, including CO₂ and methanol are available in literature (11,12). Although methanol is a stronger solvent than CO₂, chromatographic experiments in SFC (13) and solubility
studies (14) showed supercritical CO$_2$ was strong enough to carry small PAHs. In other words, the advantages of SFE over liquid extractions may be mainly caused by faster solute diffusivity in supercritical fluids. If large and polar molecules are considered, however, CO$_2$ needs to be modified.

To optimize the extraction, the limiting step needs to be defined. Because of the complexity of the matrix and the extraction fluids, any of the three steps above could be the rate-determine step. A very porous matrix may require longer time for desorbed analytes to diffuse out of the pours. Therefore, diffusion would be the limiting step. If solute molecules interact strongly to the active sites on the surface, the kinetic process could be the slowest. The existence of water can also slow down the extraction process. Supercritical fluid extraction with CO$_2$ and liquid extraction with solvents such as methanol are the two extremes. If the solute solubility is high enough, SFE with CO$_2$ accelerates the diffusion step dramatically while methanol favors the first steps more. In this chapter, an initial study was undertaken to compare extractions with methanol/CO$_2$ and supercritical CO$_2$ with a simple matrix, sea sand.

**EXPERIMENTAL SECTION**

**Extraction System**

A diagram of the experimental set-up is given in Figure 31. All extractions were performed using an ISCO (Lincoln, NE) SFX™ supercritical fluid extractor which has a wide range of operational temperature and pressure. There are two extraction vessels available and the volume of the vessel can be 0.5, 2.5, or 10 mL. In order to have
Figure 31. Diagram of the extraction system.
better control of the extraction process, only one vessel was used at a time and the vessel volume was maintained at 2.5 mL throughout the entire experiment. Two ISCO 260D syringe pumps with necessary valves and connecting tubing were used to supply either neat CO₂ or mixtures of CO₂ and methanol to the extractor. The pumps were maintained at room temperature. The collection of the extracted materials was performed by inserting the outlet restrictor into a 10 cm x 15 mm i.d. glass vial containing 5 mL methylene chloride. The collection restrictor was a piece of 50 μm i.d. fused silica tubing (Polymicro Technologies). Although no heat was applied to the collection cell, the restrictor was put through a piece of coiled stainless steel tube which was heated to 150 °C to prevent plugging of the restrictor.

Materials

Ultra high purity SFE grade CO₂ was purchased from Scott Specialty Gases (Plumsteadville, PA). The maximum concentrations for oxygen and water in CO₂ were stated less than 2 and 0.5 ppm, respectively. Methanol (99.9%) was obtained from Mallinckrodt, Paris, KT. Methylene chloride (99.99%) was purchased from Aldrich. A standard test mixture solution which included PAH Fluoranthene, Chrysene, Dibenzo(a,h)anthracene, Benzo(ghi)perylene, and Indeno(1,2,3-cd)pyrene was obtained from Supelco, Bellfonte, PA. p-terphenyl was obtained from Aldrich Chemical (Milwaukee, WI). All chemicals were used without further purification. A marine sediment (SRM 1941) was purchased from National Institute of Standards & Technology (NIST). SRM 1941 was prepared from the marine sediment collected in the Chesapeake
Bay at the mouth of the Baltimore (MD) Harbor. The sea sand (100-270 mesh) was obtained from Jenneile Chemical Co. (Cincinnati, OH).

**Sample Preparation**

70 g of purchased sea sand were cleaned by Soxhlet extraction with 200 mL of methylene chloride for 30 hours. The washing was continued with methylene chloride until there was no gas chromatographic difference between the pure methylene chloride solvent and the methylene chloride used in Soxhlet extraction. The sand was dried by use of a stream of nitrogen for hours. A methylene chloride solution which consisted of 70 $\mu$g of each test PAH was spiked into the sand. The spiked sea sand was dried by a stream of nitrogen slowly within four hours. The dried sample was kept in an amber glass bottle and mixed daily for more than two weeks. The SRM 1941 from NIST was stored under 30°C in dark as recommended (15).

**Extraction and Gas Chromatographic Procedures**

Approximately one gram of spiked sea sand was weighted and then placed in the extraction vessel. Each extraction started one minute after the extraction vessel was filled with the fluid. 5 mL of methylene chloride was used as collection solvent spiked with 10 $\mu$g of the internal standards ($p$-terphenyl). The flow rate was controlled at about 0.3 mL/min by varying the length of the restrictor. The extraction was stopped immediately when a specified volume of fluid in the pump had passed through the extraction vessel. If methanol was involved in the extraction, the extract was slowly dried by a stream of nitrogen. 1 mL of methylene chloride was then added to the residues and evaporated down to 0.1 mL. If no methanol was used, the methylene
chloride extract solution was evaporated down to 0.1 mL directly by use of the nitrogen stream. About 1 μL of the concentrated methylene chloride solution was injected in a GC system.

The GC system, Hewlett Packard HP5890 series II, was equipped with a 30 m x 250 μm i.d. (0.25 μm film thickness) fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID). Helium was used as carrier gas at flow rate of 1.2 mL/min. The initial oven temperature was 120 °C for 2 min, programmed to 300 °C at 10 °C/min and held for 12 min. Identification of all peaks was done by use of a Hewlett Packard GC-MS system.

**RESULTS AND DISCUSSION**

**Spiked Sea Sand Sample**

Sea sand was used as the matrix to start the study. Sea sand is a more homogenous matrix than other matrices, such as soil and river sediment. Sea sand consists of silica dioxide as well as metal ions and metal oxides. Metal ions and metal oxides are either attached to the surface or in the silica-oxygen lattice (16). Spiked sand is commonly used in extraction studies (17,18) because sand usually has less contaminants and it is easy to clean. However, the spiked solute adsorption equilibrium must be reached before any extraction. Studies (3,4) showed that more than 24 hours is necessary for most of the organic compounds to equilibrate in matrices, such as soil. Hawthorne et al. (19) showed that the extracted native naphthalene in an urban air particulate was ca. 5 times lower than the extracted spiked D₈-naphthalene which had
been aged 15 hours. In the study reported in this chapter, the spiked sea sand was kept in dark for more than two weeks (450 hours) and mixed daily before extractions. To be certain that the PAHs were uniformly distributed within the sea sand particles at the beginning of the extraction, random sampling was also done. 5% standard error for 4 extractions and double GC injections was observed.

**Existence of Water in the Spiked Sea Sand**

When supercritical CO₂ was used as the solvent, maintaining control of the flow rate was difficult. Since there is a large volume CO₂ expansion across the restrictor, a dramatic temperature drop occurs. Within 10 min of extraction, the temperature of the collection cell can go as low as -50 °C (20), which is lower than the freezing point of CO₂ (-44 °C). If small quantities water are extracted and then move through the restrictor, ice formation in the restrictor can also occur. Restrictor plugging occurred even when the restrictor was heated to 200 °C. Heating the very end of the restrictor with a heat gun could release the flow if a total plug occurred during the extraction. In this study, the volume of CO₂ (238 atm and 25 °C) was used instead of time as an indicator of extent of extraction because the restrictor was easily plugged either partially or totally. It was difficult to control the flow rate therefore the extraction time. The average flow rate was ca. 0.3 mL/min.

The restrictor plugging with CO₂ was often caused by a small amount of water being carried out of the extractor. After the sea sand used in this study was Soxhlet extracted and cleaned at the boiling point of methylene chloride, no heating process was provided. Keay and Wild (21) studied water adsorption on different kinds of matrices.
They reported that each $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ in the matrix could attach 11 or more water molecules. None of these water molecules were desorbed until high temperatures were reached. Even $\text{Na}^+$ had 5 water molecules. It took temperatures higher than 500 °C to completely get the water out of the matrices. Russell and Farmer (22) found that all metal ions in different matrices could be saturated by water after 18 hours by exposure to room humidity. In other words, water from the air might have been adsorbed during the two week storage time of the spiked sea sand.

**Extractions with Supercritical CO$_2$**

Neat CO$_2$ was used under supercritical conditions to extract PAHs from the spiked sea sand. The experiments were done at three different temperatures, 40, 50 and 60°C. The pressure of CO$_2$ was kept at 248, 204, and 146 atm, respectively so that the density of CO$_2$ was constant at 0.8 g/cm$^3$. Therefore, the solvent strength of CO$_2$ was constant. Based on the studies on diffusion in supercritical fluids by Sassiat et al. (23), solute diffusivities in CO$_2$ do not change in this temperature range at constant density. Figure 32-35 show the recoveries of all PAHs when different amounts of CO$_2$ were used to extract. Because dibenzo(a,h)anthracene and benzo(ghi)perylene could not very well separated by GC, the total recoveries of these two PAHs were plotted. As discussed in the theory section of this chapter, increasing temperature should speed up the desorption step. Since no accurate data are readily available on the activation energy, a typical activation energy value, 20 kJ/mol, in reversed-phase liquid chromatographic systems (24) was used as an estimation of activation energy. The rate constant $k$ then would increase by a factor of 1.5 from 40 to 60°C. Because the diffusivities of the analytes
Figure 32. SFE recoveries of fluoranthene at different temperatures with CO$_2$. (●) 40 °C; (▲) 50 °C; (+) 60 °C.
Figure 33. SFE recoveries of chrysene at different temperatures with CO₂. (●) 40 °C; (▲) 50 °C; (+) 60 °C.
Figure 34. SFE recoveries of dibenzo(a,h)anthracene and benzo(ghi)perylene at different temperatures with CO$_2$. (*) 40 °C; (▲) 50 °C; (+) 60 °C.
Figure 35. SFE recoveries of indeno(1,2,3-cd)pyrene at different temperatures with CO$_2$. (●) 40 °C; (▲) 50 °C; (+) 60 °C.
were maintained unchanged by holding the densities of CO₂, the rate of the diffusion step of the extraction was constant at different temperatures. In other words, the overall extraction rate should have increased with temperature if the desorption step was the limiting step. However, no significant recovery change was observed.

To explain the experimental results, two possibilities are proposed. The first possibility is that the actual activation energy of the desorption is higher than that in chromatographic systems. The temperature variation was not enough to affect the extraction rate. In reversed-phased liquid chromatographic systems, strong solvents, such as methanol and acetonitrile, are used as mobile phases while nonpolar stationary phases, such as C₁₈, are used. With CO₂ as a extraction solvent, the solute molecules may be adsorbed more strongly in the sea sand with higher desorption activation energies. Hawthorne et al. (19) studied SFE extraction recoveries of PCBs from a river sediment with CO₂. It was found that raising the extraction temperature from 50 - 200°C resulted in quantitative recoveries even though the density of CO₂ was reduced by half. In the temperature range from 40 - 60 °C, CO₂ might be able to desorb those molecules which were adsorbed to the weak active sites but too weak to those which interacted with strong adsorption sites on the sand surface.

The second explanation is that one of the last two steps, removal of the desorbed solutes away from the matrix surface and the solute diffusion step, controlled the overall extraction speed. Since the existence of water was observed, the water film could become resistance to the desorbed solute molecules to diffuse away from the matrix, especially those nonpolar PAHs. By raising the extraction temperature as shown by
Hawthorne *et al.* (19), better extraction recovery was achieved. This improvement could be also caused by water dehydration in the matrix. Since analyte diffusivities were maintained constant in this experiment, it seemed that the extraction rate was limited by solute diffusion. In the next discussion, the data in methanol/CO$_2$ mixtures will show that diffusion step did not affect extraction recoveries.

**Extraction with Methanol/CO$_2$ Mixtures**

Extractions with methanol/CO$_2$ mixtures were undertaken at 238 atm and 40, 50, and 60 °C, respectively. At each mole fraction of CO$_2$, 4 mL of solvent at 238 atm and 25 °C were delivered through the extraction cell. Figure 36-39 shows the recoveries of the analytes. The interesting result was that all PAHs had better extraction recoveries with methanol/CO$_2$ mixtures. For example, the recovery of Indeno(1,2,3-cd)pyrene at 60 °C increased from 31% with neat CO$_2$ to 86% with mole fraction of CO$_2$ at 0.6. When pure methanol was used, the recoveries were slightly lower than those with 0.6 mole fraction of CO$_2$. As observed in the extractions with neat CO$_2$, temperature change from 40 to 60 °C did little to the measured recoveries.

Sassiat *et al.* experimentally measured diffusion coefficient of benzene in methanol/CO$_2$ mixtures at 50 °C (23). It showed that the diffusion coefficient of benzene decreased continuously from neat CO$_2$ to methanol (see figures in Chapter II and III). If this trend holds for the PAHs used in this study, the diffusion step of the extraction should be slower with methanol/CO$_2$ mixtures than that with neat CO$_2$. Since the extraction recoveries of the PAHs decreased with mole fraction of CO$_2$, the desorption kinetics and/or the removal of the desorbed solute away from the sand surface must be
Figure 36. Extraction recoveries of fluoranthene at different temperatures with methanol/CO$_2$ mixtures. (●) 40 °C; (▲) 50 °C; (+) 60 °C.
Figure 37. Extraction recoveries of chrysene at different temperatures with methanol/CO$_2$ mixtures. (•) 40 °C; (▲) 50 °C; (+) 60°C.
Figure 38. Extraction recoveries of dibenzo(a,h)anthracene and benzo(ghi)perylene at different temperatures with methanol/CO$_2$ mixtures. (●) 40 °C; (▲) 50 °C; (+) 60 °C.
Figure 39. Extraction recoveries of indeno(1,2,3-cd)pyrene at different temperatures with methanol/CO₂ mixtures. (•) 40 °C; (▲) 50°C; (+) 60 °C.
improved. Methanol is often used as modifier in supercritical fluid chromatography to increase the solvent strength of CO$_2$ as well as to compete for the strong adsorption sites on the surface of the stationary phase. It is believed that methanol could interact with adsorption sites more strongly and has lower desorption activation energy than supercritical CO$_2$. With mixtures of methanol and CO$_2$, the desorption kinetics were likely accelerated. The existence of methanol around the sand surface also helped to break the water film because methanol interacts strongly with both acidic and basic active sites. The properties of methanol and methanol/CO$_2$ mixtures were fully discussed in Chapter II and III.

Another difference between neat CO$_2$ and methanol in the extraction process was matrix flocculation characteristics of suspensions. Fernandez and Quigley studied different soil samples in water, alcohol, and xylene (25). Because of the decreased dielectric constant from water ($\epsilon = 80$), alcohols ($\epsilon = 20-30$), to xylene ($\epsilon = 2$), the thickness of the double layers also decreased in the same order. Larger soil clusters were easily seen in xylene under scanning electron photomicrographs. Soil swelling studies with water, acetone and carbon tetrachloride were also reported by Green et al. (26). Water gave the largest matrix swelling percentage and carbon tetrachloride ($\epsilon = 2.24$) was as least 10 times lower. Acetone ($\epsilon = 20.7$) gave intermediate results. It was suggested that the percentage swelling of the matrices depended upon the transfer of immersion fluid into the pores of the matrix. Since CO$_2$ also has a small dielectric constant ($\epsilon = 1.6$), it is expected that the polar sand matrix may not be "wetted" well.
in \( \text{CO}_2 \). The formation of clusters and less solvent in pores would decrease the total surface area where \( \text{CO}_2 \) could interact with analyte molecules.

An unexpected result was the high extraction recoveries with pure methanol. It is usually believed that liquids cannot achieve high extraction recoveries within short period of time because of low solute diffusivities. This assumption is valid only when the speed of the extraction is limited by the diffusion step. Since the porosity, surface area, and surface structure of the matrix varies with different samples, the limiting step of the extraction process can be any of the three steps. The specific sea sand used in this study may have low surface area and large pore size. If solute diffusion is not the rate-determine step, methanol is expected to have higher extraction recoveries.

Almost no restrictor plugging was observed in the extractions with methanol/CO\(_2\) mixtures. Water is almost immiscible in neat \( \text{CO}_2 \) under the operational conditions, but a 0.5 \( \text{CO}_2 \) mole fraction methanol/CO\(_2\) mixture can dissolve as much water as 10-15%. In addition, the smaller compressibility of methanol makes the temperature drop smaller. The low frozen point (-96°C) of methanol also makes water stay in liquid state.

**Extraction of River Sediment with Methanol/CO\(_2\) Mixtures**

The solvent strength of supercritical \( \text{CO}_2 \) was reported to be comparable to those of hexane based on solubility parameters (27), but polar modifiers such as methanol improved both solvent strength of \( \text{CO}_2 \) and extraction selectivity. When polar analytes instead of nonpolar PAHs are considered, neat \( \text{CO}_2 \) itself cannot achieve good results. For example, Dobbs *et al.* (28) reported that the addition of 0.035 mole fraction of methanol in \( \text{CO}_2 \) increased the solubility of 2-aminobenzoic acid by 620%. For benzoic
acid, the solubility enhancement was significantly greater with methanol than with acetone or \( n \)-octane. To demonstrate the difference between \( \text{CO}_2 \) and methanol/\( \text{CO}_2 \) mixtures in extracting strength, 1 g of the river sediment purchased from NIST was extracted with and a at 238 atm and 60 °C. 8 mL of \( \text{CO}_2 \) and 4 mL of 0.5 \( \text{CO}_2 \) mole fraction methanol/\( \text{CO}_2 \) mixture were allowed to pass through the extraction vessel. Figure 40 and 41 show the chromatograms. 10 \( \mu \text{g} \) \( p \)-terphenyl, as indicated with arrows, was added into the collection cell as internal standard. Few compounds were extracted with neat \( \text{CO}_2 \). The large peaks from 15 to 35 min were long chain (20-30) hydrocarbons. When 0.5 \( \text{CO}_2 \) mole fraction methanol solution was used, however, the chromatogram shows many more peaks some of which had higher concentrations than the internal standard.

**Summary**

The brief initial experiments show that enhanced-fluidity fluids can be more powerful in term of extracting large and polar compounds. Since extraction matrices are extremely complicated, the extraction limiting step needs to be defined each time. When the solvent strength of supercritical \( \text{CO}_2 \) is strong enough, the extraction speed is improved only if the solute diffusion step is the controlling step. Methanol/\( \text{CO}_2 \) mixtures are able to maintain the solvent strength of liquid methanol while the solute diffusivity can be incased. Clearly, more experiments need to be done.
Figure 40. GC chromatogram of the SFE extract of river sediment with CO₂.
Figure 41. GC chromatogram of the extract of river sediment with 0.5 CO$_2$ mole fraction methanol/CO$_2$ mixture.
Future Work of Enhanced-Fluidity Solvents

(A) High temperature extraction studies would be interesting. In fact, raising temperature should favor all three steps in the extraction process. High extraction recoveries are expected. But the extraction cost will also increase in the larger scale.

(B) Almost all of the real samples involved in extractions contain water. The percentage of water in a sample could be 80% or higher. Extraction recoveries vary dramatically with percent water. Because of the poor miscibility of CO₂ and water, drying agents have to be used previous of the extraction. It is possible that these drying agents may make the matrix even more complicated. More strong adsorption sites would also be introduced to the system. Methanol/CO₂ mixtures, as described in Chapter III, can be miscible with water. A 50% methanol/CO₂ mixture dissolves as much as 10-15% water under the common extraction conditions. Polar methanol molecules can also compete with water for the matrix surface. As a result, the water film on the surface may be easily moved. Restrictor plugging will not be a technical problem with large amount of methanol is present.

(C) Different environmental samples, such as suburban dust, fly ash, and river sediment, should be studied with enhanced-fluidity solvents. Different samples have different matrix structures and porosities, therefore, extraction limiting step may be different. By varying the composition of the enhanced-fluidity mixtures, i.e., the percentage of the compressed gas in liquids, one should be able to maximize the extraction rate. In other words, a larger percentage of the liquid ought to be used if the desorption kinetics or the water film resistance is controlling the extraction rate, while
more compressed gas, even pure supercritical fluids should be used when the diffusion step is the limiting step.

**LITERATURE CITED**


CHAPTER V

RETENTION OF C₆₀ AND C₇₀ FULLERENES ON REVERSED-PHASE HPLC STATIONARY PHASES

INTRODUCTION

The development of a bulk method to produce the third allotrope of carbon, buckminster fullerenes (1) has caused the development of completely new areas of chemistry and materials science. The surge of new fullerene-related discoveries continues (2). Optical isomers of C₇₆, C₇₈, C₈₂, C₈₄ were theoretically predicted (3,4) and recently experimentally separated and identified (5). Numerous compounds with one or more metal atoms inside the fullerene cage have been produced (6).

As discussed in Chapter I, to facilitate these new developments, scientists use various chromatographic techniques to separate and purify the fullerenes. Among the high efficiency separations, reversed-phase HPLC columns have been used. The mobile phases used along with those reversed-phase columns, however, are nonpolar such as hexane and pentane. To understand the mechanisms of fullerene separations, a comparison of the separation of C₆₀ and C₇₀ fullerenes on five different columns with four different types of polysiloxane-based stationary phases are reported in this chapter. Commonly used solvents, such as hexane or mixtures of hexane and methylene chloride are compared to more polar mobile phases, such as, methanol and diethyl ether mixtures.
Also the specific type of molecular level interactions involved in the separation of these fullerenes was evaluated. A linear solvation energy relationship (LSER) which correlates Kamlet - Taft solvent parameters with retention was used to identify these interactions.

EXPERIMENTAL

Columns

The columns included an end-capped monomeric octadecyl polysiloxane, ODS-Hypersil (Shandon Scientific), \textit{C18}; an end-capped phenyl dimethylpolysiloxane, phenyl-2 Hypersil (Shandon Scientific), \textit{Phen}; a experimental non end-capped diphenyl methylpolysiloxane, diphenyl Hypersil, \textit{DP-K}. All the columns contained 5 \(\mu\)m particles with 120 \(\AA\) pore size, and dimensions of 250 x 4.6 mm as supplied by Keystone Scientific, Bellefonte, PA. In addition two end-capped diphenylmethylpolysiloxane, Supelcosil column (Supelco, Bellefonte, PA), with 5 \(\mu\)m particles, 100 \(\AA\) pore size were studied. One of these columns was analytical scale with 250 x 4.6 mm dimensions, \textit{DP-S}; and the other was semiprep scale with dimensions of 250 x 21.2 mm. A polymeric ODS, Vydac 201TP (The Separations Group, Hesperia, CA) with 5 \(\mu\)m particle size, 300 \(\AA\) pore size, \textit{PI8}, was also studied. A prep scale column was home-packed with alumina (activity 1, neutral, 80 mesh) purchased from Alltech (Alltech Associates, Inc., Deerfield, Illinois). A tap-fill dry-packing method was used. After the 6.5 cm i. d. empty glass column was filled with hexane, about 165 grams of alumina were slowly added from a funnel. The packing was consolidated by tapping firmly on the side of the
column 2-3 time a second. To avoid any air bubbles in the packing bed, the column was kept wet all the time. The length of the column was 40 cm.

**Instrumentation**

The chromatographic system included an ISCO LC-2600 syringe pump (ISCO, Lincoln NE), a Valco W-series high pressure injection valve (Valco Instruments, Houston, TX) with a 20 µl injection volume and a Millipore 991 Photodiode Array Detector (Millipore, Waters Chromatography Division, Milford, MA.) All separations were done at room temperature.

** Fullerene Production and Purification**

The raw fullerene soot was prepared by the arc welding technique which was first developed by Smalley and coworkers (7). A small brass tube (10 cm i.d., 15 cm long) was fitted with electrically insulated brass end flanges that had Cajon ultratorr fittings for the manipulation of gas and the insertion of electrodes. Two copper rods (0.25 inch i.d., 20 cm long), which were directed through centrally located fittings, served as high current electrical feedthroughs and graphite rod holders. This arrangement permitted external manipulation of the spacing between the graphite rods to be arc welded. Graphite rods of 0.156 inch i.d. were evaporated by arc welding at 75 A. The whole apparatus was submerged in cooling water, and the tube had a replaceable window which extended out of the water so that the operator could watch the arc welding and keep the graphite rods almost touching. This operation was performed in an atmosphere of 150 torr of helium. A one gram portion of soot could be generated in about 1 hour (8). Raw soot was extracted with benzene. An example extraction process is: 500 mg of raw soot
was placed in a round bottom flask which was connected to a water-cooled condenser. 100 mL boiling benzene for 3 hrs was used to extract the fullerenes from the raw soot. Typical extraction yields for the fullerenes were 8 % (wt/wt) fullerenes in soot. The ratio $C_{60}$ to $C_{70}$ in the extract was between 4:1 and 5:1.

A home-packed alumina prep scale column was used to purify $C_{60}$ and $C_{70}$. This system was first recommended by Diederich et al. (9). The benzene extract was added into about 10 grams of alumina. After benzene was evaporated by a stream of nitrogen, the uniform fullerene-adsorbed alumina was put on the top of the column packing bed and the elution began immediately. A mixture of 95% hexane and 5% toluene was first used to elute $C_{60}$, then the percentage of toluene was increased to 10%, 20%, and finally 50% for $C_{70}$. Although the full procedure took longer than hours, the benzene extract from approximately 0.5 grams of the original soot, or 20 mg $C_{60}$ and 5 mg $C_{70}$ could be separated. $C_{70}$ was about 80% pure. An unknown portion of $C_{60}$ and $C_{70}$ was believed lost to the column bed permanently. Benzene solutions of these pre-purified $C_{60}$ and $C_{70}$ were used for the experiments described later.

**Materials**

All chemicals used in this study were used as delivered without further purification. Diethyl ether ($> 99.99\%$) and benzene ($> 99.99\%$) were purchased from J. T. Baker, Inc., Phillipsburgh, NJ. Methanol (99.9% purity) was obtained from Mallinckrodt, Paris, KT. The dyes used to determine the Kamlet-Taft solvent parameters were 4-nitroaniline ($99\%$), 2-nitroanisole ($99\%$) were obtained from Aldrich Chemical Co. Inc., and $N,N$-dimethyl-p-nitroaniline was purchased from Eastman Kodak.
A standard test mixture of polynuclear aromatic hydrocarbons purchased from Supelco, Bellfonte, PA. was also used in the study. This test mix included: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(b)fluoranthenes, benzo(k)fluoranthenes, benzo(ghi)perylene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

**Density Measurements**

The molar volume of the methanol/diethyl ether mixtures were determined by measuring the solution densities with a DMA 512 density meter (Anton Paar USA, Inc., Warminister, PA) and a TU16D constant temperature bath (Techne Inc., Princeton, NJ). The density meter was calibrated with distilled water at atmospheric pressure and 27°C.

**Data Analysis**

A NEC 386SX PowerMate-Plus computer was used for data collection and analysis. The multivariate linear regression of the chromatographic data and the solvatocromatic data was obtained using SYSTAT software (SYSTAT, Inc., Evanston, IL). The statistical data provided complies with the "Recommendation for Reporting Results of Correlation Analysis in Chemistry using Regression Analysis 10". R, the coefficient of multiple correlation is reported. An R value approaching 1 indicates excellent correlation. The standard deviation of the regression, s, is reported. The F-test at the 95% confidence level is used to indicate the statistical significance of the model and the student’s t test at the 95% level was used to indicate the significance of the independent variable’s coefficients. In addition to these recommendations, the
adjusted coefficient of multiple determination ($R_a^2$) is also reported. This variable is substantially lower than the coefficient of multiple determination ($R^2$) when too many independent variables are used in a model. A more detailed description of the regression analysis of LSER was previously published (11).

RESULTS AND DISCUSSION

Our initial separations of fullerenes used phenyl polysiloxane columns. We anticipated that the retention and the separation factor might be higher for the phenyl columns than other reversed-phase columns, because of π-π interactions between the phenyl rings on the stationary phase and the condensed rings of the different fullerenes. Hexane and pentane were initially used as the mobile phases. The fullerenes could be separated with these mobile phases, but the efficiency was low. Figure 42 is a sample chromatogram using a phenyl column, Phen, as stationary phase and pentane as mobile phase. Under these conditions the efficiency was low and separation factor was $\alpha = 1.33$. Mixed mobile phases which contained a low percentage of cosolvent, such as ethanol, methanol, tetrahydrofuran and diethyl ether, improved the efficiency substantially. Figure 43 demonstrates the increased efficiency found when diethyl ether/pentane mixtures were used as the mobile phase with the Phen column. In addition, the retention of the fullerenes was practically unaffected by the addition of substantial quantities of diethyl ether to the pentane (see Figure 44) and the selectivity decreased slightly.

Neat diethyl ether also worked well as a mobile phase for the separation of fullerenes. All five columns were tested with diethyl ether as a mobile phase. Short
Figure 42. Separation of $C_{60}$ and $C_{70}$ on the phenyl polysiloxane column (Phen). Mobile phase: pentane; flow rate: 1 mL/min; detection wavelength: 330 nm.
Figure 43. Dependence of column efficiency (number of theoretical plates) on the percentage of diethyl ether in pentane for fullerene separations on the Phen column. (○) $C_{60}$; (+) $C_{70}$.
Figure 44. Variation of capacity factors with percentage of diethyl ether in pentane using the phenyl polysiloxane stationary phase (Phen). (+) benzene; (○) C<sub>60</sub>; (△) C<sub>70</sub>.
retention times and improved efficiency (2000-4000 plates) were achieved. Figure 45 is a sample chromatogram of a high molecular weight fraction of the fullerene extract. This fullerene fraction was obtained by first extracting the soot with hot trichlorobenzene. 20 μL of the supernatant were injected onto and separated by the monomer ODS column. As described later, the ODS column was used because it provided the highest efficiency with diethyl ether. The high molecular weight fraction of the fullerene chromatogram (portion of the chromatogram with retention time > 4.2 minutes) was collected for 15 injections. The resulting 250 mL sample was desolvated with a stream of N₂. The residue was then dissolved in 100 μL benzene. 20 μL of this yellow solution were injected onto and separated by the same column and the chromatogram in Figure 45 was obtained. The identity of C₆₀, C₇₀, C₇₆, and C₈₄ chromatographic peaks were determined from published UV/Vis spectra. Mass spectrometry was used to confirm the identification of the fullerene extract. Figure 46 shows the Fourier transform ion cyclotron resonance mass spectrum of the concentrated high molecular weight fraction before the final separation shown in Figure 45. (The relative abundance of the ions in the mass spectrum may not be indicative of the relative proportions of fullerenes because the relative ionization efficiencies of fullerenes are unknown.)

Because diethyl ether was a good mobile phase for fullerene separations more polar cosolvents with diethyl ether were also considered. 20% mixtures of alcohol/diethyl ether were considered. Because fullerene solubility in polar solvents was expected to be low, hexanol/diethyl ether was first considered followed by more polar alcohols such as ethanol and methanol. Of the solvents considered, methanol provided
Figure 45. Chromatogram of high molecular weight fullerenes. Mobile phase: diethyl ether; flow rate: 1 mL/min; column: monomeric C18; detection wavelength: 330 nm.
Figure 46. Laser desorption, negative ion FT-ICR mass spectrum of the sample used in Figure 45.
the greatest control of mobile phase solvent strength and therefore the retention and selectivity. The efficiency of C\textsubscript{60} on the C\textsubscript{18} column increased from 1400 to 2600 theoretical plates when the proportion of methanol in the mobile phase was increased from 0 - 20% and was invariant for mobile phase compositions that ranged from 20-70% methanol. Only for compositions > 70% methanol did the efficiency lower and tailing begin due to lowered solubility in the mobile phase. Figure 47 and 48 illustrate how the retention of C\textsubscript{60} and C\textsubscript{70} is affected by the addition of increasing amounts of methanol to diethyl ether. The ln k' increased with the percentage methanol added to the mobile phase for both C\textsubscript{60} and C\textsubscript{70} on all of the stationary phases studied. Cox \textit{et al.} (12) reported that retention of C\textsubscript{60} and C\textsubscript{70} closely resembled the retention of planar molecules with similar molecular "footprints". They showed that under their chromatographic conditions the retention of C\textsubscript{60} was similar to that of triphenylene and the retention of C\textsubscript{70} was intermediate between that of benzo[a]pyrene and coronene. However, with the 0-50% methanol/diethyl ether mobile phases, all of the polynuclear aromatic hydrocarbon standards (listed in the experimental section), were unretained on all five columns.

Figure 49 shows the variation of the separation factor for the methanol/diethyl ether solvent system on all five columns. Surprisingly, the separation factors were higher when ODS stationary phases were used as opposed to phenyl or diphenyl columns. The separation factors obtained with the methanol/diethyl ether ODS system were significantly higher than those previously reported by Jinno \textit{et al.} (13) for a C\textsubscript{60} and C\textsubscript{70} separation using monomeric and polymeric ODS with n-hexane as the mobile phase. In
Figure 47. Variation of capacity factors of $C_{60}$ with percent methanol in diethyl ether mobile phase on different columns. (◊) polymeric ODS, P18; (●) monomeric ODS, C18; (△) diphenyl, DP-S; (○) diphenyl, DP-K; (+) phenyl, Phen.
Figure 48. Variation of capacity factors of C$_{70}$ with percent methanol in diethyl ether mobile phase on different columns. Column identifications are given in Figure 47.
Figure 49. Variation of separation factors with percent methanol in diethyl ether mobile phase on different columns. Symbols are the same as in Figure 47.
addition, Figures 47-49 show the polymeric phase, Vydac 201 TP, was more retentive and more selective than monomeric ODS for the separation of the fullerenes. Exactly opposite trends were found in the previous study (13) when n-hexane was used as the mobile phase. In the previous study, the same polymeric ODS was practically nonretentive toward C\textsubscript{60} and C\textsubscript{70}, while the monomer ODS provided capacity factors of 0.58 and 0.92 for C\textsubscript{60} and C\textsubscript{70}, respectively and a separation factor of 1.65. The observed differences in fullerene retention and selectivity between this work and that of Jinno et al. is primarily caused by marked difference in the observed behavior of the polymeric ODS stationary phase. The monomeric ODS columns used in both studies performed similarly. The distinctly different behavior of the polymeric ODS is quite interesting. The different retention characteristics of the polymeric stationary phase were most likely caused by the different mobile phases use in the two studies. The observed differences cannot be explained by variation in mobile phase shielding of surface silanols. Hexane would afford minimum shielding of surface silanols compared to methanol/diethyl ether; however when hexane was used as the mobile phase lesser fullerene retention was observed. Perhaps the two mobile phases affect the surface structure of the polymeric ODS phase differently. Further investigations are needed to completely understand this phenomenon.

Using the data in Figures 47 and 48, a comparison of the different columns was made. Table 5 shows the comparison of the number of theoretical plates required to achieve baseline separation (resolution R\textsubscript{s} = 1.5) for the five chromatographic columns when 20% methanol/diethyl ether is used as the mobile phase. Due to the higher
Table 5. Number of plates required to achieve resolution of 1.5 with a 20% methanol/diethyl ether mixture.

<table>
<thead>
<tr>
<th>column</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C18$</td>
<td>351</td>
</tr>
<tr>
<td>Phen</td>
<td>2142</td>
</tr>
<tr>
<td>$DP-K$</td>
<td>4405</td>
</tr>
<tr>
<td>$DP-S$</td>
<td>1936</td>
</tr>
<tr>
<td>P18</td>
<td>179</td>
</tr>
</tbody>
</table>
observed separation factors, the polymeric and monomeric ODS columns require the least theoretical plates to achieve baseline separation. The Phen and DP-S stationary phases require approximately 10 times the number of theoretical plates as P18 and, due to the low separation factor, DP-K requires approximately double the number of theoretical plates as DP-S. However, under the experimental conditions described in Figures 47-49 and for the 20% methanol/diethyl ether mobile phase composition, the measured efficiency of C_{60} and C_{70} was approximately 3000 for the C18 column and 1500-1800 for the PI8, DP-K, DP-S, and Phen columns. These chromatographic conditions were scaled-up to semiprep scale using the diphenyl column described in the experimental section. This column was used because it was readily available in our laboratory. The extract used in the analytical-scale separations was first doubly concentrated. We were able to inject 100 μL of this concentrated extract directly onto the semiprep scale column and obtain resolution of 0.8-1.0 for flow velocities of 15 mL/min. From the previous discussion it is obvious that the diphenyl polysiloxane columns are the poorest choice for fullerene separations under these chromatographic conditions. Therefore, much better performance should be expected when semiprep polymeric or monomeric ODS columns are used under these chromatographic conditions.

To better understand the retention mechanism involved in the separation of fullerenes with the methanol/diethyl ether mixtures on polysiloxane-based stationary phases, a linear solvent free energy relationship (LSER) between retention (ln k') and measured solvent strength parameters was developed. LSER's assume that attractive interactions can be correctly categorized by a nonspecific interaction term that measures
the effect of dipolarity and specific terms that describe the effect of hydrogen bond donor and acceptor interactions on solvent properties. In addition, the aforementioned independent variables must be orthogonal and the assumption is made that there is a linear free energy relationship between the each attractive term and the specified solvent effect. Finally, to complete the description of the solvent effect, an endothermic term that describes the formation of a cavity in the solvent to accommodate the solute must also be included in this model (14). Kamlet and Taft with coworkers described the adsorption of gases and liquids on solids using LSERs (15,16). In addition, Carr and coworkers demonstrated that LSERs can be quite useful in describing the partitioning of solutes in HPLC (17). Equation 1 is a generalized LSER that describes the transfer of a solute from mobile phase to stationary phase in HPLC.

\[ \ln k' = A + B\Omega + C\pi^* + D\alpha + E\beta \]  \hfill [1]

Kamlet-Taft solvatochromic parameters (\(\pi^*\), \(\alpha\), \(\beta\)) were used to describe the exoergetic interactions. The \(\pi^*\) measures the ability of the medium to stabilize charged or dipolar solutes by virtue of dipolar or induced dipolar interactions. The \(\alpha\) parameter measures hydrogen bond donation capability and \(\beta\) measures the ability of solvent to accept hydrogen bonds. The most common method of modeling the cavity formation term, \(\Omega\), is to use the cohesive energy density which is the square of the Hildebrand solubility parameter (14). We chose to use (molar volume)\(^{-1}\) to represent the variation in cohesive energy density (and therefore the cavity formation term) of this mixed
solvent. This is a reasonable approximation when the attractive portion of the interaction potential controls the cohesive energy density of the solvent (18).

The $\pi^*$, $\alpha$, $\beta$ parameters of the solvent mixture were experimentally determined by measuring the variation of the UV/Vis spectrum of solvatochromic dyes which are listed in the experimental section. More detail on the measurement of the solvent strength parameters was previously published (19). The molar volume of the mixtures was determined from experimentally measured solution density. The cavity formation parameter used in the model was generated by dividing all the (molar volume)$^{-1}$ values by that of methanol. This was necessary to keep the cavity formation parameter in the same range as the other solvent strength parameters. Figures 50 and 51 illustrates the variation in the solvent strength and cavity formation parameters as a function of percent methanol in the mixture. The hydrogen bond donator strength, $\alpha$, and the hydrogen bond acceptor strength, $\beta$, of the mixture increased substantially over the composition range of 0 - 20 % methanol, then with increasing proportions of methanol the lewis acidity and basicity varied minimally. As more diethyl ether is added methanol-methanol hydrogen bonds must break to maintain the polarity of the mixture at close to a constant value. The cavity formation term varies linearly with added methanol.

The relative values of the coefficients $B$, $C$, $D$, and $E$ describe the relative importance of cavity formation, dipolarity, hydrogen bond accepting or donating properties of the solvent to the retention of the fullerenes. When the capacity data shown in Figures 50 and 51 were fit to solvent strength parameters, models that included the $\alpha$ and $\beta$ parameters were inadequate. They did not pass the F-test even at the 75%
Figure 50. Measured solvent strength parameters of methanol/diethyl ether mixtures. (○) \( \pi^* \); (♦) \( \Omega \).
Figure 51. Measured solvent strength parameters of methanol/diethyl ether mixtures. (+) $\alpha$; (◊) $\beta$. 
confidence level. Only models which included exclusively the cavity formation term and the dipolarity term fit the data well. These models were valid at the 97% confidence level using the F-test. For all columns, the regression coefficients for the cavity formation parameter, $B$, and dipolarity, $C$, were positive and negative, respectively. Tables 6 and 7 list the regression coefficients for all five columns. Figures 52 and 53 show the relative magnitude of the regression coefficients. This indicates that increased energy necessary to form a cavity in the solvent caused increased retention and increasing solvent dipolarity decreased retention. The signs of these coefficients are as expected from the endothermic and exothermic nature of the respective terms. However, the relative magnitude of these regression coefficients is interesting. For all five stationary phases the cavity formation regression coefficient was the larger of the two. However, for the monomeric and polymeric phases the cavity formation coefficient predominates. This suggests that solvophobic forces control the fullerene retention with ODS and these mobile phase mixtures.

Since the involvement of the mobile phase in retention is described by the cavity term and solvent strength parameters, the intercept term, $A$, of the model should represent the interaction between a solute molecule and the stationary phase. In other words, $A$ could be treated as a measure of "pure" retention of a solute on a specific stationary phase when no mobile phase is applied, therefore all four other terms would be zero. By comparing the relative size of the $A$ term for different columns, the retention of the fullerenes on the stationary phases is expected to decrease in the following order: diphenyl, $DP-S >$ diphenyl, $DP-K >$ phenyl, $Phen >$ ODS, $C18 >$
Table 6. Model Describing Retention of C60 on Different Columns (Model \( \ln k' = A + B\Omega + C\pi \)).

<table>
<thead>
<tr>
<th>column</th>
<th>( n^a )</th>
<th>( N^b )</th>
<th>coefficient (( t ) test)(^c )</th>
<th>statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>C18</td>
<td>9</td>
<td>6</td>
<td>-3.472(48.0)</td>
<td>12.072(39.7)</td>
</tr>
<tr>
<td>Phen</td>
<td>10</td>
<td>7</td>
<td>-2.815(29.2)</td>
<td>5.019(13.8)</td>
</tr>
<tr>
<td>DP-K</td>
<td>10</td>
<td>7</td>
<td>-2.374(16.0)</td>
<td>4.799(8.5)</td>
</tr>
<tr>
<td>DP-S</td>
<td>10</td>
<td>7</td>
<td>-1.802(5.8)</td>
<td>7.683(6.5)</td>
</tr>
<tr>
<td>PI8</td>
<td>8</td>
<td>5</td>
<td>-4.238(17.6)</td>
<td>12.651(9.8)</td>
</tr>
</tbody>
</table>

\(^a\text{Number of data point.}\) \(^b\text{The degrees of freedom.}\) \(^c\text{Number in parenthesis is student’s} \( t \text{ test value for the coefficient.}\)
Table 7. Model Describing Retention of C<sub>70</sub> on Different Columns (Model ln k' = A + BΩ + Cτ*).

<table>
<thead>
<tr>
<th>column</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>coefficient (t test)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>CI8</td>
<td>7</td>
<td>4</td>
<td>-3.235(21.2)</td>
<td>13.464(12.8)</td>
</tr>
<tr>
<td>Phen</td>
<td>10</td>
<td>7</td>
<td>-2.488(18.7)</td>
<td>6.766(13.4)</td>
</tr>
<tr>
<td>DP-K</td>
<td>10</td>
<td>7</td>
<td>-1.950(11.6)</td>
<td>6.192(9.7)</td>
</tr>
<tr>
<td>DP-S</td>
<td>10</td>
<td>7</td>
<td>-1.897(6.8)</td>
<td>8.842(8.4)</td>
</tr>
<tr>
<td>P18</td>
<td>7</td>
<td>4</td>
<td>-3.682(16.9)</td>
<td>14.072(9.4)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of data point.  <sup>b</sup> The degrees of freedom.  <sup>c</sup> Number in parenthesis is student's t test value for the coefficient.
Figure 52. Comparison of the relative values of model coefficients of C$_{60}$ for different columns. A: model intercept; B: the coefficient for the cavity formation term; and C: the coefficient for the dipolarity term.
Figure 53. Comparison of relative values of model coefficients of $C_{70}$ for different columns. Symbols are the same as in Figure 52.
ODS, PI8. This is exactly the order of retention that Jinno (13) found for fullerenes with n-hexane as a mobile phase. We had also originally anticipated that the fullerenes would be more retentive on phenyl columns. However, with the diethyl ether/methanol mobile phases the reverse retention order was observed for phenyl and ODS phases. As mentioned above, coefficients $B$ and $C$ describe different interactions between the solvent and the solute. However, for a chromatographic separation these parameters would be describing solvent-solute interaction in the bulk fluid and near the surface of stationary phase. Different $B$ and $C$ values from different columns may indicate that the solute and the solvent interactions near the surface were different. In both Tables 6 and 7, CI8 and PI8 had similar cavity formation and dipolarity coefficients; DP-K and Phen, both from the same vendor, also had similar $B$ and $C$ values. Therefore, similar surfaces gave similar solvent-solute interaction indicators, $B$ and $C$.

**CONCLUSIONS**

This chapter demonstrates that the fullerenes are soluble enough to use solvents as polar as methanol in their separation. A nonaqueous reversed-phase method of separating $C_{60}$ and $C_{70}$ is described. A linear solvation energy relationship (LSER) provided significant evidence to support the proposed retention mechanism. With the methanol/diethyl ether mobile phase, monomeric and polymeric ODS provided the highest selectivity.
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