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EVALUATION OF ENHANCED-FLUIDITY MOBILE PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

DISSERTATION

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

By

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****

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1995

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CHAPTER I

THE MOBILE PHASE IN CHROMATOGRAPHY

INTRODUCTION

Elution chromatography is a separation method where the analytes separated are distributed between a mobile phase and a stationary phase. The distribution or partitioning of analytes between the mobile and stationary phase is described by equation 1:

\[ K = \frac{C_s}{C_m} \]  

(1)

where \( K \) is the partition coefficient; \( C_s \) is the concentration of analyte in the stationary phase; and \( C_m \) is the concentration of analyte in the mobile phase. To separate analytes in a chromatography experiment the analytes must have different partition coefficients. Therefore, the differential partitioning of analytes between the stationary and the mobile phase is the basis for separation in elution chromatography.

The stationary phase can be a solid, a solid with ligands chemically-attached to the surface, or a film of liquid coated on a solid support. Figure 1 is a generic phase
diagram of a pure substance. Solid, liquid, gas and supercritical fluid states are
delineated along with the major variants of chromatography that employ these states as
mobile phases. The transition between a solid-liquid and liquid-gas is abrupt and is
delineated by a solid line. The supercritical state is defined as a fluid above its critical
temperature ($T_c$) and critical pressure ($P_c$) and is unique in that the gas-supercritical
and liquid-supercritical transitions are continuous. As is shown in Figure 1, the mobile
phase can be a gas, liquid or supercritical fluid. These mobile phase distinctions
describe the three major classifications of elution chromatography: gas chromatography
(GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC). The
use of different mobile phases and the manipulation of their physical parameters
provide chromatographic advantages and disadvantages that are particular to the state
of the mobile phase employed.

**Efficiency and Time of Analysis**

The number of theoretical plates, $N$, for a given chromatographic band is a
measure of the quality or efficiency of a chromatographic column. $N$ is typically
calculated assuming the chromatographic band is a Gaussian distribution. With this
assumption $N$ is calculated using equation 2:

$$N = \left( \frac{t_R}{\sigma_t} \right)^2$$ (2)
Figure 1. Phase diagram delineating gas, solid, liquid, and supercritical fluid states.
where $t_R$ is the elution time at the center of the chromatographic band and $\sigma^2$ is the variance of the band in units of time. Plate height, $H$, is also a measure of chromatographic efficiency and is inversely related to the number of theoretical plates:

$$H = \frac{L}{N}$$

(3)

where $L$ is the length of the chromatographic column. While both plate number and plate height are used to express chromatographic efficiency, plate height is more useful in elution chromatography because it relates column efficiency to mobile phase velocity.

The van Deemter equation is often used to describe the relationship between plate height, $H$, and linear velocity of the mobile phase, $u^1$

$$H = A + \frac{B}{u} + Cu$$

(4)

$A$ and $B$ are coefficients that describe band dispersion due to multiple flow paths and longitudinal diffusion, respectively. $C$ describes the band dispersion due to the combined resistance to mass transfer in the mobile and stationary phases. In packed columns, $A = 2\lambda d_p$ and $B = \gamma D_m$ where $\lambda$ and $\gamma$ are column packing constants. For well packed columns $\lambda$ and $\gamma$ have values of 0.5 and 0.6, respectively. $d_p$ is the particle diameter and $D_m$ is the diffusion coefficient of the solute in the mobile phase.

$$C = f(k')d_p^2/D_m$$ where $k'$ is the capacity factor, a measure of solute retention.

$$C = f(k')d_p^2/D_m$$ only if it is assumed that the resistance to mass transfer in the mobile
and stationary phases is primarily attributed to diffusion in the mobile and stagnant mobile phases, and if the film thickness of the stationary phase is significantly less than the particle diameter. In practice these assumptions are valid.\(^1\)

Taking the derivative of the van Deemter equation with respect to \(u\), setting the derivative equal to zero, and substituting in the definitions of \(B\) and \(C\) an expression for the optimum velocity, \(u_{\text{opt}}\), is obtained:

\[
D_m = \frac{D_m}{d_p} \left( \frac{2\gamma}{f(k^*)} \right)^{1/2}
\]

The optimum velocity is the mobile phase velocity at which a minimum value of \(H\) and a maximum value of \(N\) is produced. The minimum value of \(H\) can be calculated by substituting the value for the optimum linear velocity into equation 4 to obtain:

\[
H_{\text{min}} = 2\lambda d_p + 2d_p \sqrt{2\gamma f(k^*)}
\]

Equation 6 predicts that for packed columns the minimum value for \(H\) depends predominantly on column parameters such as particle diameter and packing quality, and that the minimum plate height can be achieved regardless of mobile phase characteristics. Equation 5 shows that the optimum velocity is a function of column parameters, but is also directly proportional to the diffusion coefficient of the solute through the mobile phase. This relationship predicts that rapid solute diffusion results in an increase in optimum velocity and is reflected chromatographically as an increase
in efficiency per unit time or decreased analysis time. Therefore, low viscosity fluids that allow rapid solute diffusion and fast analysis times are preferred mobile phases in elution chromatography.

**GAS CHROMATOGRAPHY**

From an efficiency and time of analysis point of view, GC is superior to supercritical fluid chromatography (SFC) and high performance liquid chromatography (HPLC). The high diffusion rates of analytes and the low viscosities of gases are the primary reasons behind the high efficiencies and fast analysis times gas chromatography affords. Typical diffusivities and viscosities for gases, supercritical fluids and liquids are shown in Table 1. Diffusion of analytes through gases are typically 4 and 2-3 orders of magnitude higher than in liquids and supercritical fluids, respectively. In addition, the viscosities of gases are 2 and 1-2 orders of magnitude less than in liquids and supercritical fluids, respectively.\(^2\)

In GC the mobile phase is inert and does not contribute to partitioning. Partitioning depends only on analyte - stationary phase interactions and the vapor pressure of the analyte. Gas chromatography is limited to the separation compounds with relatively high vapor pressures or volatile compounds. It is estimated that only 20% of all chemical compounds are volatile enough to be separated by GC.\(^3\) The remaining 80% includes compounds of low volatility or thermally labile compounds.
Table 1. Physical properties of gases, supercritical fluids, and liquids.²

<table>
<thead>
<tr>
<th></th>
<th>Gas</th>
<th>Supercritical Fluid</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (g cm⁻¹ s⁻¹)</td>
<td>10⁻¹</td>
<td>10⁻⁴ - 10⁻³</td>
<td>10⁻²</td>
</tr>
<tr>
<td>Diffusion Coefficients (cm²/s)</td>
<td>10⁻¹</td>
<td>10⁻⁴ - 10⁻³</td>
<td>10⁻⁵</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>10⁻³</td>
<td>0.3 - 0.8</td>
<td>1</td>
</tr>
</tbody>
</table>
Many compounds of special interest, such as biologically related compounds, fall into these categories.

**SUPERCritical FLUID CHROMATOGRAPHY**

Supercritical fluid chromatography (SFC) is a viable separation technique complimentary to gas chromatography and high performance liquid chromatography.

From a speed of analysis point of view (SFC) is superior to HPLC, but inferior to GC. The diffusivities and viscosities of typical supercritical fluids are intermediate between gases and liquids and are the physical properties that determine the intermediate position of SFC between GC and HPLC in terms of chromatographic speed of analysis.

In SFC, partitioning is a function of several variables including: temperature, pressure, mobile phase density, mobile phase composition, and stationary phase composition.

There are more variables available in optimizing a separation in SFC than in GC. Supercritical fluid mobile phases used in SFC have several advantages compared to the gas mobile phases employed in GC. Supercritical fluid mobile phases have solvation power that facilitates the separation of non-volatile and high molecular weight compounds that cannot be separated by GC. In addition, supercritical fluids with a low critical temperatures can be used to separate thermally-labile compounds. Attractive properties for supercritical fluid mobile phases are low critical temperature, low critical pressure, and low chemical reactivity. In addition, low toxicity, low flammability, high
Table 2. Critical temperatures and pressures for supercritical fluids.\textsuperscript{4}

<table>
<thead>
<tr>
<th>Fluid</th>
<th>$T_c$ (°C)</th>
<th>$P_c$ (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>31.0</td>
<td>72.9</td>
</tr>
<tr>
<td>Nitrous oxide</td>
<td>36.5</td>
<td>71.5</td>
</tr>
<tr>
<td>Sulfur hexafluoride</td>
<td>45.6</td>
<td>37.1</td>
</tr>
<tr>
<td>Xenon</td>
<td>16.6</td>
<td>57.7</td>
</tr>
<tr>
<td>Butane</td>
<td>152.1</td>
<td>37.5</td>
</tr>
<tr>
<td>Pentane</td>
<td>196.6</td>
<td>33.3</td>
</tr>
<tr>
<td>Dichlorodifluoromethane</td>
<td>111.9</td>
<td>40.9</td>
</tr>
<tr>
<td>Fluoroform</td>
<td>26.2</td>
<td>48.0</td>
</tr>
<tr>
<td>Ammonia</td>
<td>132.4</td>
<td>112.1</td>
</tr>
<tr>
<td>Methanol</td>
<td>239.5</td>
<td>79.9</td>
</tr>
<tr>
<td>Ethanol</td>
<td>240.8</td>
<td>60.6</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>263.7</td>
<td>51.0</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>272.4</td>
<td>47.7</td>
</tr>
<tr>
<td>Water</td>
<td>374.2</td>
<td>218.4</td>
</tr>
</tbody>
</table>
purity, cost and compatibility with commonly used detectors are important. Table 2 lists critical temperatures and pressures of several fluids. Carbon dioxide has many of the properties that are attractive for a supercritical mobile phase, and is the most commonly used mobile phase in supercritical fluid chromatography.

SFC is ultimately limited in its ability to separate high molecular weight and/or polar compounds. The primary reason behind this limitation is the limited ability of carbon dioxide to dissolve polar compounds. As was mentioned previously, low critical temperatures and pressures are preferred in the selection of a supercritical fluid mobile phase. Unfortunately, this requirement eliminates the use of many of the more polar fluids such as methanol, ethanol, and water. Supercritical NH₃ has been investigated and found to solubilize polar analytes that are insoluble in supercritical carbon dioxide. However, supercritical NH₃ has a relatively high critical temperature (132.4°C) and is reactive with stationary phases and column support materials and is therefore of limited use in chromatography.

The most common approach to increasing the polarity of supercritical fluid mobile phases is addition of a miscible cosolvent to the primary fluid. These miscible components are called modifiers or entrainers and are generally used in concentrations of less than 10 mol %. Modifiers have been shown to increase the solubility of several polar compounds in modified carbon dioxide. When using modifiers in carbon dioxide based mobile phases, caution must be taken to insure the fluid mixture is single phase. Poor efficiency and irreproducible chromatography result if a multiple phase
mobile phase exists. The use of modifiers in supercritical fluid chromatography has expanded the range of SFC to larger and more polar compounds and increased the number of compounds that can be separated in SFC. However, even with the use of modifiers, SFC has found limited success in the separation of large and/or polar compounds when compared to HPLC. The reasons behind these limitations remain the limited polarity and solvent strength of the mobile phases employed in supercritical fluid chromatography.

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

High performance liquid chromatography (HPLC) is presently the chromatographic method of choice for the separation of nonvolatile compounds. Conventional liquids have stronger solvating power than supercritical fluids. Large compounds, such as proteins and high molecular weight polymers, require liquid mobile phases for separation. In conventional HPLC, partitioning is a function of mobile phase composition and stationary phase composition. The solvent strength of the mobile phase in HPLC is varied by changing the mobile phase composition. The large number and the wide polarity range of liquids and liquid mixtures used in HPLC provides the separation of larger and more polar compounds than is possible by SFC and GC. However, HPLC typically has larger pressure drops across the chromatographic column, longer analysis times and lower efficiencies than GC and SFC. The large pressure drops, long analysis times and low efficiencies of HPLC in comparison to GC
and SFC are results of the high viscosities and low diffusivities of commonly used liquids in comparison to those of gases and supercritical fluids.

Numerous studies were successful in decreasing the viscosity and increasing diffusion in liquid mobile phases in HPLC by elevating the column temperature.\textsuperscript{23,24,25,26} In some of these cases, the advantages of lower pressure drops, shorter analysis times and increased efficiency were realized.\textsuperscript{24,25,26} However, to achieve significant increases in diffusion in common liquid mobile phases, temperatures in excess of 100 °C are often required. The use of extreme temperatures increases the frequency of undesirable reactions at the surface of the column and degradation of thermally-labile solutes and stationary phases.

**ENHANCED FLUIDITY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

Enhanced fluidity or low viscosity liquid mixtures have been demonstrated as mobile phases in HPLC.\textsuperscript{27,28,29} An enhanced-fluidity liquid mixture is defined as an associated liquid, such as an alcohol, to which high proportions of a low viscosity liquid, such as CO\textsubscript{2}, are added. Enhanced-fluidity high performance liquid chromatography was first investigated in liquid - solid chromatography with a methanol/CO\textsubscript{2} eluent and a porous glassy carbon (PGC) as the stationary phase. Chromatographic advantages, such as increased optimum linear velocity, lower column pressure drops, and decreased analysis times were obtained without significantly
lowering the mobile phase solvent strength in these methanol/CO₂ mobile phase mixtures.²⁷,²⁸

Initial studies on the use of enhanced fluidity liquids in reversed phase HPLC have also been performed. When CO₂ was added to a methanol/water mobile phase, lower plate height and decreased analysis times were achieved. It was also shown that solvent strength was maintained with the addition of CO₂ to the methanol/water mobile phase because of the ability of CO₂ to readily break hydrogen bonds in these mixtures.²⁸,²⁹

The results of this previous work demonstrated column pressure drops and analysis times that were superior to commonly-used HPLC but inferior to SFC. These studies indicated the intermediate chromatographic performance of enhanced fluidity HPLC to commonly used HPLC and SFC was due to a decrease in mobile phase viscosity and an increase in solute diffusion in enhanced-fluidity liquids relative to commonly employed HPLC mobile phases. In addition, the solvent strength of the enhanced fluidity mobile phases was similar to that of common HPLC mobile phases.

**GOALS OF RESEARCH**

The goal of this research is to provide common HPLC mobile phases with some of the positive attributes of SFC, such as low viscosities, rapid solute diffusion, high efficiencies, fast analysis times, and lower column pressure drops while maintaining solvent strength similar to common liquids and liquid mixtures. The studies described
in this dissertation build upon previous work in the characterization of enhanced-fluidity liquids as mobile phases in HPLC. Research fundamental to the use and understanding of enhanced fluidity mobile phases in reversed phase HPLC is reported along with an initial study using enhanced-fluidity liquids as mobile phases in normal phase HPLC.

Similar to the use of modifiers in SFC and mobile phase mixtures in HPLC, the mobile phase in enhanced fluidity HPLC must be in a single liquid phase. Maintaining an enhanced-fluidity liquid in the single phase imposes constraints on the composition, pressure, and temperature of enhanced fluidity mixtures. While phase diagrams delineating the single liquid phase region for nonpolar/CO₂ mixtures are often available in the literature, phase information on polar solvent/CO₂ systems, such as methanol/H₂O/CO₂ and acetonitrile/CO₂, is limited. In Chapter II comprehensive phase diagram studies on methanol/CO₂, methanol/H₂O/CO₂, acetonitrile/CO₂, and acetonitrile/H₂O/CO₂ mixtures are performed to determine the single liquid phase regions. These studies will provide valuable information about the single phase conditions useful for reversed-phase enhanced-fluidity HPLC.

In Chapter III diffusion coefficients for benzene, anthracene, and phenol in several methanol/H₂O/CO₂ mixtures at ambient and elevated temperatures are determined and compared to the diffusion coefficients of the same solutes in a methanol/H₂O mixture over the same temperature range. This information is
fundamental to evaluating the performance of these liquid mixtures as mobile phases in HPLC.

Reversed-phase HPLC separations using mixtures similar to those studied in Chapter III are then characterized in Chapter IV. A direct comparison of the improvements in chromatographic performance in HPLC due to elevating the column temperature and/or by the use of enhanced fluidity mixtures as mobile phases is obtained.

In Chapter V the variation in retention with the addition of CO$_2$ to a methanol/H$_2$O mobile phase is compared to the variation in retention with a methanol/H$_2$O mobile phase where the mole fraction of methanol is varied. This comparison is made for three different solute sets of interest: polycyclic aromatic hydrocarbons, probucol and related compounds, and a vitamin test mixture.

Finally, an initial study of enhanced-fluidity mobile phases in normal-phase HPLC with a hexane/CO$_2$ mobile phase is described in Chapter VI.
LIST OF REFERENCES


CHAPTER II

PHASE DIAGRAM STUDIES OF METHANOL-H₂O-CO₂ AND
ACETONITRILE-H₂O-CO₂ MIXTURES

INTRODUCTION

Methanol, acetonitrile and H₂O are the most commonly-used eluents in
reversed-phase HPLC. The viscosities of acetonitrile, methanol, and H₂O are 0.35 cP,
0.56 cP, and 0.89 cP, respectively at 25°C and ambient pressure.¹ H₂O is often added
to a methanol or acetonitrile mobile phase in reversed phase HPLC to increase the
polarity of the mobile phase and the selectivity of the separation. There are, however,
disadvantages to the use of methanol/H₂O and acetonitrile/H₂O mixtures as mobile
phases in HPLC. The viscosities of methanol/H₂O and acetonitrile/H₂O mixtures are
greater than that of either of its components. For example, at 0.46 mole fraction
methanol/H₂O and at 0.24 mole fraction acetonitrile/H₂O, the mixture viscosities reach
maximum values of 1.62 cP and 1.01 cP, respectively.¹ As discussed in Chapter I,
some have used elevated temperatures to decrease the viscosity of liquid mobile phases.
At 60°C the viscosities of the methanol/H₂O and acetonitrile/H₂O mixtures described
above decrease to 0.81 cP and 0.53 cP. The effects of mobile phase viscosity in HPLC will be discussed in detail in Chapters III and IV.

Supercritical fluids have markedly lower viscosities than liquid solvents commonly-used in HPLC. Supercritical CO₂ (T_c = 31.06°C, P_c = 72.86) is the most commonly used mobile phase in supercritical fluid chromatography. The viscosity of supercritical CO₂ at 40°C and 136 atm is 0.065 cP; while the viscosity of liquid CO₂ at 25°C and 136 atm is 0.082 cP. These viscosities are approximately an order of magnitude less than the viscosities of the liquids used in reversed phase HPLC.

Previous work in this research group demonstrated that large proportions of liquid CO₂ in methanol and in a methanol/H₂O mobile phase decreased the overall viscosity of these mobile phases. Elevating the temperature also decreases the viscosity of liquids. We proposed that similar additions of liquid CO₂ to acetonitrile, methanol/H₂O and acetonitrile/H₂O mixtures would also lower the overall viscosity. By elevating the temperature of these low viscosity or enhanced fluidity mixtures, the mixture viscosity should decrease further.

Consistent with mobile phase mixtures in conventional HPLC and in the use of modifiers in SFC, the mobile phase in enhanced fluidity HPLC must be in a single liquid phase. Because, the addition of CO₂ and temperature elevation are the primary methods of decreasing the viscosity of the mobile phase, it is important to know the compositions, temperatures, and the pressures required to maintain a single liquid phase in these mixtures.
Several studies on the binary phase behavior of methanol/CO\(_2\)\(^{6,7,8,9,10,11,12,13,14,15,16}\) and H\(_2\)O/CO\(_2\)\(^{17,18,19,20,21,22,23,24}\) have been reported. While methanol/CO\(_2\) and H\(_2\)O/CO\(_2\) phase information is available, limited phase information exists in the literature on methanol/H\(_2\)O/CO\(_2\) mixtures. Francis reported a ternary phase diagram for the methanol/H\(_2\)O/CO\(_2\) system at approximately 21-26°C and 65 atm.\(^{25}\) Yoon et al. reported ternary phase diagrams for the methanol/H\(_2\)O/CO\(_2\) system at 40 °C and at pressures of 69.1, 98.7, and 118.5 atm. They also report some additional three phase data at temperatures of 32, 35, and 38 °C in the pressure range of 71.1 - 80.0 atm.\(^{26}\) Chang and Rousseau published vapor-liquid equilibrium data for methanol/H\(_2\)O/CO\(_2\) mixtures at - 30°C, -15°C, 0°C and 25°C where the H\(_2\)O to methanol ratio was constant at 0.2 and the mole fraction of CO\(_2\) is varied from 0.00-0.65.\(^{6}\) Page determined six methanol/H\(_2\)O/CO\(_2\) isopleths over the temperature range of 25-100°C where the H\(_2\)O to methanol mole ratio varied from 0.08-0.30 and mole fraction of CO\(_2\) varied from 0.54-0.92.\(^{27}\) Except for the few isopleths reported by Page, little methanol/H\(_2\)O/CO\(_2\) phase information exists at temperatures other than 25 °C and 40 °C. Unfortunately, previously published information is not extensive enough to insure or accurately predict a single liquid phase for most methanol/H\(_2\)O/CO\(_2\) mixtures of interest in enhanced fluidity HPLC.

Almost no information on acetonitrile/CO\(_2\) and acetonitrile/H\(_2\)O/CO\(_2\) mixtures has been published. Page reported interpolated data from a single acetonitrile/CO\(_2\) isotherm at 35°C\(^{27}\) and Ziegler et. al. estimated the vapor-liquid critical loci for
acetonitrile/CO₂ using a peak-shape method. To our knowledge, no information on acetonitrile/H₂O/CO₂ has been reported at this time.

In this chapter the single liquid phase regions for methanol/CO₂, methanol/H₂O/CO₂, acetonitrile/CO₂ and acetonitrile/H₂O/CO₂ applicable to enhanced fluidity HPLC are determined. Liquid/liquid, vapor/liquid or vapor/supercritical fluid phase boundaries are determined by visual observation using a high pressure, variable volume view cell.

**EXPERIMENTAL**

**View Cell**

Figure 2 is a diagram of the experimental setup. A custom designed, high pressure, variable volume view cell was purchased from Temco, Inc. (Tulsa, OK). A diagram of the view cell is shown in Figure 3. The 0-35 ml cell is rated to 585 atm at a maximum temperature of 176 °C. The stainless steel cylindrical sample chamber (3.89 cm i.d. x 2.95 cm) is enclosed on one end by a threaded brass cap containing a stainless steel volume displacer and an attached pneumatic assembly that drives the volume displacer allowing the cell volume to be varied. The pneumatic portion and sample volume are isolated from one another by two sets of high pressure seals on either side of a region at atmospheric pressure. A borosilicate glass window (1" diameter x 0.86" thick) is mounted within the pneumatic assembly. The other end of the sample chamber consists of a larger window (1.5" diameter x 0.75" cm thick) for visual observation of
Figure 2. System for determining phase transitions.
Figure 3. Diagram of high pressure variable volume view cell.
the cell contents. Buna-N, ethylene propylene, viton, and teflon encapsulated silicon o-rings were all tried and found unacceptable as seals in contact with the cell contents. The fluids and conditions used caused extraction of the Buna-N, ethylene propylene, and viton o-rings forming a yellow solution and contaminating the cell contents. The teflon portion of the teflon encapsulated silicon o-rings cracked under pressure causing seals to fail at elevated pressures and allowing the silicon portion to extract into the cell contents forming an orange solution. In addition, the graphite filled teflon spacers that came standard with the cell extracted and formed a yellow-green solution when immersed in methanol. Teflon o-rings, sizes 120 and 325, obtained from McMaster-Carr Supply Co. (Chicago, IL), were found acceptable as high pressure seals and did not extract into the cell contents. The teflon o-rings were positioned around each window and around the volume displacer. Teflon spacers made in-house were installed on either side of the o-rings. All materials in contact with the cell contents were stainless steel, borosilicate glass, or teflon.

The pneumatics assembly, that was isolated from the cell contents, was sealed with Buna-N o-rings of sizes 216 and 223 (McMaster-Carr). The volume displacer was moved pneumatically by applying or releasing pressure via an ISCO model 260D syringe pump (Lincoln, NE) filled with H₂O. The pump was operated in the constant flow and refill modes at rates of ± 0.02 to ± 2 ml/min.
Temperature Measurement and Control

The view cell is equipped with dual 1/8" NPT pipe fittings near the viewing window of the sample chamber. An Omegaclad type J thermocouple, enclosed in a 1/16" stainless steel sheath, from Omega Engineering, Inc. (Stamford, CT) was installed through one NPT fitting via a Parker A-Lok thermocouple connector (Forberg Scientific of Ohio, Inc., Columbus, OH). The thermocouple extended into the center of the sample portion of the view cell and monitored the temperature of the cell contents directly. A CN9000 series temperature controller (Omega Engineering, Inc.) was used to monitor and control the cell temperature. The temperature controller reads and controls to ± 0.1 °C with a maximum temperature of 200 °C. The exterior of the stainless steel cell was surrounded by three mica band heaters (2- 4.5" i.d. x 1.5" wide, 240 V and 400 W each, and 1- 4.5" i.d. x 2.0" wide, 240 V and 500 W), obtained from Watlow (St. Louis, MO). These heaters were wired in parallel to a 220 V Powerstat power supply (Hughes-Peters, Columbus, OH). The Powerstat was set at 20 percent full scale such that 45 volts were delivered to the band heaters to heat the cell. The entire view cell was wrapped in an insulating jacket made from Flexweave 1000 tape (1/8" thick x 4" wide), obtained from The Carborundum Co. (Niagara Falls, NY), to minimize heat loss and temperature fluctuations.

Prior to installation, the thermocouple was calibrated in a Techne TU-16D immersion circulator H₂O bath (Techne, Inc., Princeton, NJ) relative to the Techne Tempunit® thermoregulator and a Fisher thermometer (-20-110 °C, Pittsburgh, PA)
from 30 to 70 °C. Thermometer temperatures were estimated to 0.1 °C and used for the calibration curve. The correlation coefficient for the calibration curve was 0.99999.

**Pressure Measurement**

To the other sample chamber port, a three-way valve (Scientific Systems, Inc., State College, PA) was attached with 1/16" stainless steel tubing. A Setra model 204 pressure transducer (Setra Systems, Inc., Acton, MA) was connected to the open side of the valve. The transducer that is functional from 0 to 5000 psi (340.2 atm), was factory calibrated before use. Output was read from a Fluke 77 multimeter (John Fluke Mfg Co., Inc., Everett, WA) operated on the volt DC setting and readable to ± 1 psi (0.1 atm) from 0 to 3200 psi (217.8 atm) and to ± 10 psi (0.7 atm) above 3200 psi.

**Sample Mixing**

The thickness of the stainless steel cell and the motion of the volume displacer made the use of a magnetic stir bar for mixing the cell contents very difficult. The cell was modified in-house to replace the small rear window with a mixer assembly (see Figure 3). A long cylindrical stainless steel chamber, open to the cell contents, was sealed with the Teflon o-rings and the spacers previously used with the window. The chamber protruded beyond the cell dimensions by approximately 9 cm. A stainless steel circular paddle was mounted on a stainless steel shaft (21.5 cm long x 1 cm wide x 0.3 cm thick). A 5/16" x 24/16" Teflon coated octagonal spinbar® (VWR Scientific, West Chester, PA) was fitted near the end of the shaft. The end of the stainless steel shaft was inserted into the mixer chamber until the paddle touched the plunger so that
the end of the shaft fitted with the spin bar was near the closed end of the mixer chamber. Six ceramic ring magnets (1.079" o.d. x 0.597" i.d. x 0.250" thick, McMaster-Carr) were used on the exterior of the mixer chamber to manually to push and pull the mixer through the entire sample volume manually. The stainless steel paddle (3.5 cm diameter x 0.15 cm thick) contains 24 small holes (0.5 cm diameter) so that the fluid was effectively circulated throughout the cylindrical cell. The mixer assembly adds 12 ml to the overall cell volume and requires no permanent cell modification. Furthermore, visual observation of the cell contents was still possible through the large window at the other end of the view cell.

**Mixture Preparation**

HPLC grade methanol and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ) and specified at 100.0% purity with a H₂O content of < 0.01% and 0.002% for methanol and acetonitrile, respectively. H₂O was O.S.U. distilled and was delivered to a NANOpure II system (SYBRON|Barnstead Boston, MA). Extraction/chromatography grade carbon dioxide without a helium pad was obtained from Air Products and Chemicals (Allentown, PA) specified at > 99.9999 % purity and was used as received. Impurities specified were H₂O (< 250 ppb), Total hydrocarbons, C₁ - C₂₀ (<50 ppb), and total halocarbons (< 1.0 ppb).

Prior to preparing a mixture in the view cell, the sample chamber of the view cell was charged with CO₂ to approximately 21 atm through the three-way valve and
checked for leaks. The contents were then emptied so that only CO₂ remained inside the cell at atmospheric pressure.

All mixtures were prepared on a mole fraction basis calculated from the weight of material added. Methanol/H₂O stock solutions were prepared by adding methanol and H₂O in varying proportions to a 500 glass bottle. The bottle was weighed to ±0.05 g before and after the methanol and H₂O were added. The number of moles of methanol and H₂O were calculated from the mass of each solvent added to the container. The methanol/H₂O mixtures are expressed as the ratio, R, of moles of H₂O to moles of methanol. Stock solutions and experiments were performed for methanol/H₂O mixtures with H₂O/methanol mole ratios of 0.2490 ± 0.0005, 0.3318 ± 0.0007, 0.4298 ± 0.0006, and 0.5633 ± 0.0006 the corresponding H₂O/methanol volume ratios are 10/90, 13/87, 16/84 and 20/80.

Each time a phase diagram experiment was performed, an aliquot of methanol or the methanol/H₂O mix was taken from the stock solution and degassed by sonication. A weighed quantity of methanol or methanol/H₂O mix was added through a three way valve open to the view cell (see Figure 2) from a 10 or 30 ml glass syringe equipped with the appropriate valve fitting and ferrule. The syringe was weighed to ± 0.0001 g before and after methanol or methanol/H₂O mix transfer. Between experiments the stock solution was sealed and refrigerated.

The number of moles of the methanol/H₂O mixture introduced into the view cell was calculated using the following equality:
where $M$ is the total number of moles in the methanol/H$_2$O in the stock solution, $G$ is the total mass of the stock solution in grams, $g$ is the mass of the mixture added to the view cell in grams, and $m$ is the number of moles of the mixture introduced into the view cell.

The number of moles of H$_2$O, $m_{\text{water}}$, was calculated using the following equation:

$$m_{\text{water}} = m \left( \frac{R}{1 + R} \right)$$

where $R$ is the ratio of moles of H$_2$O to methanol. The number of moles of methanol was calculated by subtracting the moles of H$_2$O from the total moles of mixture added to the cell.

CO$_2$ was maintained in an ISCO model 260D syringe pump operated in constant pressure mode at 122.5 atm. A 40 ml stainless steel vessel with an operating pressure of 122.5 atm, was equipped with a two-way valve and was used as a transfer vessel (Whitey Co., Highland Heights, OH). A known volume of CO$_2$ was added to the empty vessel. After weighing to ± 0.01 g, the vessel was connected to the three-way valve of the view cell. The valves were opened, allowing the cell to fill with CO$_2$. 
until equal pressures were reached. Because the cell and transfer vessel are of approximately equal volumes, no more than half of the vessel contents could be transferred at ambient temperatures. Successful transfers of more than half the vessel contents were facilitated by gently heating the 40 ml stainless steel vessel with a heat gun. The vessel was then reweighed in order to calculate the number of moles of CO₂ delivered into the mixture. The Ideal Gas Law (PV = nRT) was used to approximate the amount of residual CO₂ remaining in the cell prior to loading the cell with methanol, methanol/H₂O mixtures, or CO₂. This correction factor was added to the total moles delivered from the stainless steel vessel.

The mixer assembly, shown in Figure 3, was used for all phase diagram measurements in this study. After transferring the methanol, or methanol/H₂O and CO₂, the cell was pressurized, with mixing, until only a one-phase liquid remained. The temperature controller was then set to the desired temperature. Pressurizing the cell well above the single liquid phase transition temperature insured that phase separation did not take place during temperature stabilization. Upon temperature stabilization, phase transitions were determined by depressurizing the cell at a rate not exceeding 6 atm/min. Depressurizing the cell at rates exceeding 6 atm/min made visual observation of the phase transition and recording accurate temperature and pressure readings difficult. At least four measurements were recorded at each temperature for all reported observations. Below the critical point of the mixture, phase transitions were noted by a bubble point at the top of the cell. Near and above the critical point, color
changes called critical opalescence, and pools of liquid at the bottom of the cell (dew point) were observed as the liquid separated from the fluid. Efforts were not made to locate the critical point exactly but visual observation of the dew point indicated when the critical temperature was surpassed. This basic procedure was used to determine the single liquid phase for all mixtures in this study.

The procedure described above was the same procedure used to prepare acetonitrile/H₂O stock solutions, calculate acetonitrile/H₂O mole fractions introduced into the view cell, and prepare acetonitrile/CO₂ and acetonitrile/H₂O/CO₂ mixtures in the view cell. Stock solutions and experiments were performed for acetonitrile/H₂O mixtures with H₂O/acetonitrile mole ratios of 0.3206 ± 0.0006, and 0.7316 ± 0.0008 the corresponding volume ratios are 10/90 and 20/80. One deviation from the methanol and methanol/H₂O procedure was that more than half of the 40 ml stainless steel vessel contents could be transferred when acetonitrile and acetonitrile/H₂O mixtures were used. This indicates that acetonitrile and acetonitrile/H₂O mixtures have a negative volume of mixing with CO₂. In addition, the teflon seals appeared to contract during acetonitrile/CO₂ and acetonitrile/H₂O/CO₂ experiments and failed more frequently than during methanol/CO₂ and methanol/H₂O/CO₂ experiments.
RESULTS AND DISCUSSION

CO₂

Initially, the vapor/liquid equilibrium line for CO₂ was followed from 23-32 °C to check the accuracy of the pressure and temperature measurements. This experiment could have been performed by filling the cell approximately half full of liquid CO₂, and simply monitoring the change in vapor pressure with temperature. However, we chose to determine the vapor/liquid line by visual observation of the bubble point because we anticipated using this method to determine the single phase region in binary and ternary fluid mixtures. This experiment was performed without the mixer assembly. The mixer assembly was used with binary and ternary fluid mixtures to prevent composition gradients. A volume of CO₂ was transferred from the syringe pump directly to the view cell. Pressure was applied by decreasing the cell volume via the pneumatically driven volume displacer until only liquid CO₂ filled the cell volume. After temperature equilibration, the cell was slowly depressurized until the first bubble of vapor (bubble point) appeared at the top of the cell. This process was repeated a minimum of four times at each temperature. The standard deviation in pressure at each temperature was < 0.14 atm. Figure 4 shows the experimental results from this study and literature values. The agreement between our experimental results and a line drawn through the literature values²⁸ was within a relative error of 0.9 %. The critical point of CO₂ was observed with a color change from clear to opaque, yellow, and dark brown at 31.0-31.1 °C and 72.6 atm compared to the IUPAC value of 31.06 °C and 72.86 atm.²⁸
Figure 4. Vapor/Liquid equilibrium line for carbon dioxide, this study (▲), IUPAC values (●). 28
**Methanol - CO₂**

Eight compositions of methanol/CO₂ mixtures were studied over the 0.00 - 1.00 mole fraction CO₂ range with nine experimentally determined isotherms at 25 °C followed by 10°C increments from 30-100°C. Table 3 lists the experimental pressure, temperature, and composition (P-T-x) values. All Tables listing P-T-x values and the associated standard deviation, SD, in phase transition pressure are at the end of this chapter. Figure 5 shows the experimentally determined isotherms. Good agreement was observed between our experimental data and others in the literature. Figure 6 shows a comparison of the data from this study with literature values from several others for the 25°C, 50°C, and 100°C isotherms. Table 3 shows methanol/CO₂ compositions with the temperature range in which phase transitions changed from bubble point (bp) to dew point (dp). Figure 7 is a three dimensional phase diagram showing the single phase region for methanol/CO₂ mixtures between 25 - 100°C. Each intersection on the gridded surface represents one P-T-x measurement. The region above the surface is either a single liquid or supercritical fluid phase. Below the gridded surface a two phase liquid-vapor region was present.

**Acetonitrile - CO₂**

Ten compositions of acetonitrile/CO₂ were studied over the 0-100 mole percent CO₂ range with six experimentally determined isotherms at 25, 35, 50, 70, 80, and 100°C. Table 4 lists the experimental P-T-x values with the associated standard deviation (SD) in the phase transition pressure. Figure 8 shows the experimentally
Figure 5. Methanol/CO₂ vapor-liquid equilibrium isotherms at 25 °C (➕), 30 °C (□), 40 °C (▲), 50 °C (φ), 60 °C (●), 70 °C (▼), 80 °C (▼), 90 °C (Ο), and 100 °C (♦).
Figure 6. Methanol/CO\textsubscript{2} vapor-liquid equilibrium isotherm comparison of experimental results of this study at 25°C (▲), 50°C (●), and 100°C (▼) with results of references 6, 7, 11 at 25°C (▲), 7, 10 at 50°C (●), and 7, 13, 10, 15 at 100°C (▼).
Figure 7. Phase equilibrium diagram of methanol/CO$_2$ as a function of P-T-x from 0-100°C. The region above the surface is single phase.
Figure 8. Acetonitrile/CO₂ vapor-liquid equilibrium isotherms at 25 °C (+), 35 °C (O), 50 °C (▲), 70 °C (▼), 80 °C (♦), and 100 °C (□).
determined isotherms. This is first time a comprehensive acetonitrile/CO₂ phase
diagram has been reported. Page et al. attempted to map the acetonitrile/CO₂ phase
diagram for use in SFC, but were not successful because acetonitrile was determined to
be reactive to the Buna-N, Viton, and ethylene propylene seals used in their system.²⁷
Good agreement was observed between our experimental data and the limited data
available.²⁷ Figure 9 shows a comparison of this study with literature data at 35°C.
Table 2 shows acetonitrile/CO₂ compositions with the temperature and pressure range
in which phase transitions changed from bubble to dew point. Figure 10 is a three
dimensional phase diagram showing the single phase region for acetonitrile/CO₂
mixtures between 25 - 100°C. Each intersection on the gridded surface represents one
P-T-x measurement. The region above the surface is either a single liquid or
supercritical phase, while below the surface is two phase (liquid-vapor).

**Type I Phase Behavior**

Scott and van Konynenburg have divided binary phase diagrams into five basic
types.²⁹ Type I phase behavior is illustrated in Figure 11 where a P-T-x diagram is
projected onto a pressure - temperature (P-T) surface. In Type I mixtures, the two
components of the mixture are chemically similar and are generally miscible as liquids
(an exception will be discussed below). The critical mixture curve in Type I mixtures is
continuous between the critical points of component a and component b. A single
supercritical fluid phase occurs above the critical mixture curve. Lines A-E describe
different shapes of the the critical mixture curve in Type I mixtures and will be
Figure 9. Acetonitrile/CO₂ vapor-liquid equilibrium isotherm comparison of experimental results of this study at 35°C (▲) with reference 27 (➕).
Figure 10. Phase equilibrium diagram of acetonitrile/CO₂ as a function of P-T-x from 0-100°C. The region above the surface is single phase.
Figure 11. P-T diagram of Type I binary phase behavior.
discussed below. Below the critical mixture curve and bounded by the vapor-liquid critical lines of the pure components on each side, single vapor phase, two phase vapor-liquid, and single liquid phase regions are possible. Type I mixtures generally do not exhibit liquid/liquid separation at low temperatures.\textsuperscript{30,31,32}

A critical mixture curve forming a straight line connecting the critical points of component a and b (Figure 11, Line A) indicates that components a and b exhibit ideal phase behavior and small departures from Raoult's law. Components exhibiting this variation of Type I phase behavior have very similar physical and critical properties. CO\textsubscript{2} / N\textsubscript{2}O mixtures display this variation of Type I phase behavior.\textsuperscript{30,31,32} The critical properties of CO\textsubscript{2} (T\textsubscript{c} = 31.0°C, P\textsubscript{c} = 72.9 atm, V\textsubscript{c} = 94 ml/mol) are very comparable to the critical properties of N\textsubscript{2}O (T\textsubscript{c} = 36.5°C, P\textsubscript{c} = 71.5 atm, V\textsubscript{c} = 97 ml/mol).\textsuperscript{33}

Critical mixture curves exhibiting pressure mimima between components a and b (Figure 11, Line B) exhibit phase behavior representative of positive deviations from Raoult's law. Mixtures of polar and nonpolar components often display this phase behavior. Benzene/methanol mixtures exhibit this variation of Type I Phase behavior.\textsuperscript{31,32}

If the binary critical mixture curve exhibits a temperature maximum greater than either of the components, gas-gas immiscibility occurs (Figure 11, Line C). In this variation of Type I phase behavior, components a and b can be at temperatures greater than either of their critical temperatures and thus can exist as mutually immiscible
supercritical fluids. Hydrogen chloride/ethyl ether mixtures display this variation of Type I phase behavior.3¹,3²

If the binary critical mixture curve exhibits a temperature minimum less than either of the components, liquid-liquid immiscibility can occur (Figure 11, Line D). This is the only variation of Type I phase behavior where liquid-liquid immiscibility occurs. This variation of Type I phase behavior is distinguished from Type II phase behavior by the absence of a liquid-liquid-vapor line. Ethane/CO₂ mixtures are examples of this variation of Type I phase behavior.³⁰,³¹,³²

The most common variation of Type I phase behavior is when the critical mixture curve exhibits a maxima in pressure at a temperature in between the critical temperatures of the pure components a and b (Figure 11, Line E). This type of phase behavior is manifest when components a and b have similar critical pressures but differences in critical temperatures or volumes. In this variation of Type I phase behavior the components are mutually miscible in each other as liquids.³⁰,³²

According to this classification scheme methanol/CO₂ displays the most common variation of Type I phase behavior.³⁰,³² Methanol and CO₂ are mutually miscible in each other as liquids (see Figures 5 and 7). Brunner experimentally determined the critical mixture of curve methanol/CO₂ by visually observing the disappearance of the two phases in equilibrium with minute changes in temperature or composition.⁸ Zeigler et al. have also estimated the critical mixture curve of methanol/CO₂ using a peak shape method with SFC chromatographic equipment.¹⁶ In
both these studies the critical mixture curve was found to be continuous between the
critical points of CO₂ and methanol with a maxima in pressure at a temperature
intermediate of the critical temperatures of CO₂ and methanol. The critical
temperature, critical pressure, and critical volume of methanol is 239.4°C, 79.7 atm,
and 118 ml/mol, respectively. The critical pressure and critical volume of CO₂
(Pc = 72.9 atm, Vc = 94 ml/mol) are very similar, however, there is a difference in the
critical temperature of methanol (Tc = 239.4°C) and that of CO₂ (Tc = 31.0°C).

In this study, bubble point (bp) to dew point (dp) transitions were observed
below 100°C for three different methanol/CO₂ mixtures of 0.7100, 0.8208, and 0.9117
mole fraction CO₂. A transition of bubble point to dew point with changes in either the
temperature, pressure or composition indicates the critical mixture curve has been
transversed. The P-T-x conditions where transitions from bubble point to dew point
occurs are listed in Table 3 and are consistent with previously documented critical
temperatures and pressures for these mixtures.

Acetonitrile/CO₂ can also be classified as displaying Type I phase behavior of
the most common kind. Acetonitrile and CO₂ are mutually miscible in each other as
liquids (see Figures 8 and 10). The single liquid phase region of acetonitrile/CO₂ is
formed at lower pressures than methanol/CO₂ mixtures at similar compositions. Zeigler
et al. also estimated the critical mixture curve of acetonitrile/CO₂ and found the curve
to be continuous between the critical points of CO₂ and acetonitrile with a maxima in
pressure at a temperature in intermediate of the critical temperatures of CO₂ and
acetonitrile. The critical temperature, critical pressure, and critical volume of acetonitrile is 274.7°C, 47.7 atm, and 173 ml/mol, respectively. The critical pressure of CO₂ (P_c = 72.9 atm) is comparable to that of acetonitrile. However, the critical temperatures are significantly different for acetonitrile (T_c = 274.7°C) and CO₂ (T_c = 31.0°C), and moderate differences in the critical volumes exist between acetonitrile (V_c = 173 ml/mol) and CO₂ (V_c = 94 ml/mol).

In this study, bubble point (bp) to dew point (dp) transitions were observed below 100°C for one acetonitrile/CO₂ mixture; 0.9037 mole fraction CO₂ in acetonitrile. The P-T-x condition where the transition from bubble point to dew point is listed in Table 4 and is consistent with previously documented critical temperatures and pressures for these mixtures. The critical temperatures reported by Zeigler et al. are higher for acetonitrile/CO₂ mixtures than for methanol/CO₂ mixtures. The higher critical temperatures for acetonitrile/CO₂ mixtures in comparison to methanol/CO₂ mixtures noted by Ziegler et al. and in this study may be attributed to the relatively large differences in critical temperatures and volumes between acetonitrile and CO₂ compared to the differences critical temperatures and volumes between methanol and CO₂. Studies have also shown that there is significant association between methanol and CO₂ in liquid mixtures. This may also contribute lower critical temperatures for methanol/CO₂ mixtures than acetonitrile/CO₂ mixtures.
**Methanol - H₂O - CO₂**

Nine isotherms at 25°C followed by 10°C increments from 30 - 100°C were determined for methanol/H₂O/CO₂ mixtures with H₂O/methanol mole ratios (R) of 0.2490, 0.3318, 0.4298, and 0.5633. Tables 5-8 lists the experimental P-T-x values.

For R = 0.2490 eleven compositions of methanol/H₂O/CO₂ mixtures were studied over the 0.00 - 0.87 mole fraction CO₂ range, at R = 0.3318 three compositions of methanol/H₂O/CO₂ were studied over the 0.40 - 0.55 mole fraction CO₂ range, at R = 0.4298 five compositions of methanol/H₂O/CO₂ were studied over the 0.00 - 0.44 mole fraction CO₂ range, and at R = 0.5633 four compositions of methanol/H₂O/CO₂ were studied over the 0.00 - 0.33 mole fraction range. Figures 12, 13, 14, and 15 show the isotherms for methanol/H₂O/CO₂ mixtures with R = 0.2490, 0.3318, 0.4298, and 0.5633, respectively. Transitions from liquid to the supercritical fluid state were not observed in these mixtures because the critical temperatures of the mixtures exceeded the temperature limits of this study.

Triangular or ternary phase diagrams are often used to describe the phase behavior of three component mixtures. Triangular diagrams are generated with isothermal, isobaric systems where the liquid and vapor phases are sampled and their compositions determined. With the variable volume view cell system used in this study the pressure was varied to observe phase transitions, thus only extrapolated data from isotherms determined in this study can be used to construct triangular diagrams. Figures 16 and 17 are triangular diagrams comparing methanol/H₂O/CO₂ equilibrium
Figure 12. Methanol/H$_2$O/CO$_2$ phase equilibrium isotherms with $R = 0.2490$ at 25 °C (+), 30 °C (□), 40 °C (▲), 50 °C (○), 60 °C (●), 70 °C (▼), 80 °C (▽), 90 °C (○), and 100 °C (◇).
Figure 13. Methanol/H₂O/CO₂ phase equilibrium isotherms with \( R = 0.3318 \) at 25 °C (†), 30 °C (☐), 40 °C (▲), 50 °C (◇), 60 °C (●), 70 °C (▽), 80 °C (▼), 90 °C (○), and 100 °C (♦).
Figure 14. Methanol/H$_2$O/CO$_2$ phase equilibrium isotherms with $R = 0.4298$ at 25 °C ($\oplus$), 30 °C (□), 40 °C (▲), 50 °C (◇), 60 °C (○), 70 °C (▽), 80 °C (▼), 90 °C (◆), and 100 °C (◆).
Figure 15. Methanol/H$_2$O/CO$_2$ phase equilibrium isotherms with $R = 0.5633$ at 25 °C (⊕), 30 °C (□), 40 °C (▲), 50 °C (○), 60 °C (●), 70 °C (▼), 80 °C (▽), 90 °C (○), and 100 °C (◆).
Figure 16. Triangular phase diagrams comparing methanol/H$_2$O/CO$_2$ equilibrium data of reference 26 (▲) with extrapolated single liquid phase equilibrium data from this study (●) at 69.1 atm and 40°C.
Figure 17. Triangular phase diagrams comparing methanol/H₂O/CO₂ equilibrium data of reference 26 (▲) with extrapolated single liquid phase equilibrium data from this study (●) at 118.5 atm and 40°C.
data of Yoon et al. with extrapolated single liquid phase equilibrium data from this study at 69.1 atm and at 118.5 atm and 40°C. There is good agreement between the extrapolated data from this work and that of Yoon et al. These triangular diagrams illustrate the miscibility of methanol with H₂O and methanol with CO₂, they also show that H₂O and CO₂ are not readily miscible. Comparison of Figure 16 where the pressure is 69.1 atm with Figure 17 where the pressure is 118.5 atm show that the single phase region for the methanol/H₂O/CO₂ system increases with pressure under isothermal conditions.

Figure 18, 19, 20, and 21 are three dimensional phase diagrams showing the single liquid phase region for methanol/H₂O/CO₂ mixtures between 25 - 100°C with mole ratios of 0.2490, 0.3318, 0.4298, and 0.5633, respectively. Each intersection on the gridded surface represents one P-T-x measurement. The region above an to the left of the surface is a single liquid phase. Below the gridded surface a two phase liquid-vapor region is present. The 0.00 - 0.40 mole fraction CO₂ range was not studied at R = 0.3318, the pressures necessary to maintain a single liquid phase at the low CO₂ mole fraction range are predicted to be bracketed by the pressures required to maintain a single liquid phase in the methanol/H₂O/CO₂ system with H₂O/methanol mole ratios (R) of 0.2490 and 0.4298. Similar to the triangular phase diagrams, Figures 18 - 21 show the pressure required to maintain a single liquid phase increases as the mole fraction of H₂O and/or the mole fraction of CO₂ in methanol/H₂O/CO₂ mixtures...
Figure 18. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.2490 as a function of P-T-x from 0-100°C. The region above and to the left of the surface is single phase.
Figure 19. Phase equilibrium diagram of methanol/H$_2$O/CO$_2$ at $R = 0.3318$ as a function of P-T-x from 0-100°C. The region above and to the left of the surface is single phase.
Figure 20. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.4298 as a function of P-T-x from 0-100°C. The region above and to the left of the surface is single phase.
Figure 21. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.5633 as a function of P-T-x from 0-100°C. The region above and to the left of the surface is single phase.
increases. This is again attributed to the immiscibility of H₂O and CO₂ in each other except in relatively low concentrations.

The low H₂O, low CO₂ mole fraction portions of the methanol/H₂O/CO₂ ternary system mimic the Type I binary phase behavior discussed previously. However, when the mole fraction of CO₂ or H₂O is increased such that the pressure required to obtain a single liquid phase exceeds the vapor pressure for CO₂, the contents in the cell go from liquid-vapor or liquid-liquid-vapor to liquid-liquid, these mixtures exhibit an upper critical solution temperature (UCST) line. The upper critical solution temperature (UCST) is the temperature at which the liquids critically merge to form a single liquid phase as the temperature is raised. The UCST line drops from high temperatures to a temperature below the critical temperatures of all of the components in the mixture and terminates at an upper critical end point (UCEP) where it is met by a liquid-liquid-vapor (LLV) line. Figure 22 illustrates the upper critical solution temperature line, the upper critical end point and the liquid-liquid-vapor line on a pressure temperature (P-T) surface for methanol/H₂O/CO₂ mixtures. For methanol/H₂O/CO₂ mixtures in this study, at temperatures below the UCST line (to the left of the UCST line in Figure 22) the mixtures exhibit liquid-liquid separation. At temperatures above the UCST line (to the right of the UCST line in Figure 22) a single liquid phase can be reached. However, further increases in temperature at isobaric conditions may cause vapor-liquid separation in some methanol/H₂O/CO₂ mixtures. Figure 23 shows experimentally determined methanol/H₂O/CO₂ isopleths from this study with those of
Figure 22. Illustration of the upper critical solution temperature (UCST) line in methanol/H$_2$O/CO$_2$ mixtures.
Figure 23. Isopleth comparison of experimental results of this study at methanol/H₂O/CO₂ compositions of 0.3416/0.1133/0.5451 (♦), and 0.2148/0.0535/0.7317 (●), with reference 27 at methanol/H₂O/CO₂ compositions of 0.275/0.078/0.657 (▲), and 0.210/0.062/0.728 (➕).
Page et al.\textsuperscript{27} Figure 23 shows that with a 0.210/0.062/0.728 mole fraction methanol/H\textsubscript{2}O/CO\textsubscript{2} mixture at 220 atm temperatures of above approximately 60°C must be reached to obtain a single liquid phase. With this methanol/H\textsubscript{2}O/CO\textsubscript{2} mixture a single liquid phase persists past 100°C. Figure 23 also shows that with a 0.3416/0.1133/0.5451 mole fraction methanol/H\textsubscript{2}O/CO\textsubscript{2} mixture at 170 atm temperatures of above approximately 30°C must be reached to obtain a single liquid phase. However, further increasing the temperature above approximately 61°C at the same pressure results in two phase vapor-liquid separation.

Experimentally determined methanol/H\textsubscript{2}O/CO\textsubscript{2} isotherms at 25°C from this study with work reported in the literature are shown in Figure 24.\textsuperscript{6,36} From these data it appears that there is an inflection point at \( \approx 63.5 \) atm (vapor pressure of CO\textsubscript{2} at 25°C) in these graphs. The onset of the UCST line in methanol/H\textsubscript{2}O/CO\textsubscript{2} mixtures occurs at this point. After this point, the pressure required to obtain a single liquid phase increases dramatically with added proportions of CO\textsubscript{2} at 25°C. Figure 24 along with Figures 18-21 show that the onset of the UCST line in methanol/H\textsubscript{2}O/CO\textsubscript{2} mixtures occurs at lower mole fractions of CO\textsubscript{2} as the mole fraction of H\textsubscript{2}O (R value) in the mixture increases.

\textbf{Acetonitrile - H\textsubscript{2}O - CO\textsubscript{2}}

Five compositions of acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} with a H\textsubscript{2}O/acetonitrile mole ratio, R, of 0.3206 were studied over the 0.00 - 0.28 mole fraction CO\textsubscript{2} range. Six isotherms at 25, 35, 50, 70, 80, and 100°C were determined. Table 9 lists the experimental P-T-x
Figure 24. Phase equilibrium diagram of methanol/H$_2$O/CO$_2$ as a function of P-x at 25°C. The region above and to the left of the points are single phase. This study at $R = 0.0000$ (.), $R = 0.2490$ (▼), $R = 0.3318$ (♦), $R = 0.4298$ (▼), and $R = 0.5633$ (〇). Reference 6 at $R = 0.2000$ (〇). Reference 36 at $R = 0.1200$ (▲) and $R = 0.1810$ (●).
values. Figure 25 shows the experimentally determined isotherms.

Acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} mixtures with R values of 0.7316 were also studied and are listed in Table 10, however, a single liquid phase was never obtained with this mixture at 25°C and pressures under 300 atm.

The single phase region of acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} mixtures with a H\textsubscript{2}O/acetonitrile ratio of 0.3206 between 25 - 100°C is shown in Figure 26. Acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} mixtures can also display a UCST line. The onset of the UCST line occurs at lower mole fractions of CO\textsubscript{2} and lower H\textsubscript{2}O content for acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} mixtures than in the methanol/H\textsubscript{2}O/CO\textsubscript{2} mixtures. For example, the UCST line exhibited by the 0.588/0.195/0.217 mole fraction acetonitrile/H\textsubscript{2}O/CO\textsubscript{2} mixture is illustrated in Figures 25 and 26 by the relatively high pressure, 120.9 atm, necessary to obtain a single liquid phase at 25.1 °C and the moderate pressures, 24.3 atm and 54.6 atm, necessary to maintain a single liquid phase at 35.4 °C and 99.2 °C, respectively.

**Impact of Phase Behavior in Enhanced-Fluidity HPLC and SFC**

Mixtures displaying the most common variation of Type 1 phase behavior are the most desirable for enhanced-fluidity HPLC and SFC. If methanol/CO\textsubscript{2} mixtures are prepared (for example in a syringe pump) at 25°C a minimum pressure of ca. 70 atm should be maintained to insure a single liquid phase at this temperature with all possible methanol/CO\textsubscript{2} compositions (see Figures 5 and 7). Because liquid/liquid separation does not occur in these Type 1 phase mixtures with a decrease in temperature, no
Figure 25. Acetonitrile/H$_2$O/CO$_2$ phase equilibrium isotherms with $R = 0.3206$ at 25 °C (♦), 35 °C (○), 50 °C (▲), 70 °C (▽), 80 °C (●), and 100 °C (□).
Figure 26. Phase equilibrium diagram of acetonitrile/H₂O/CO₂ at R = 0.3206 as a function of P-T-x from 0-100°C. The region above and to the left of the surface is single phase.
special precautions need to be taken to insure a single liquid phase if the temperature decreases as long as the pressure of the system remains at 70 atm. However, the pressure required to maintain a single liquid phase does increase with an increase in temperature (see Figures 5 and 7). For example, at 50°C, 80°C, and 100°C pressures of ca. 100 atm, 145 atm, and 160 atm should be maintained to insure a single phase at all methanol/CO₂ compositions.

If acetonitrile/CO₂ mixtures are prepared (for example, in a syringe pump) at 25°C a minimum pressure of approximately 65 atm should be maintained to insure a single liquid phase at this temperature with all possible acetonitrile/CO₂ compositions. At 50°C, 80°C, and 100°C pressures of ca. 95 atm, 130 atm, and 145 atm should be maintained to insure a single phase at all acetonitrile/CO₂ compositions (see Figures 8 and 10).

Because liquid/liquid separation may occur as the temperature decreases in many of the methanol/H₂O/CO₂ mixtures, it is important to maintain the temperature of the syringe pump within prescribed limits to insure a single liquid phase. In addition, at elevated temperatures higher pressures may be necessary to maintain a single liquid phase with many of the methanol/H₂O/CO₂ mixtures. The phase behavior of methanol/H₂O/CO₂ mixtures is an excellent example of why experimental P-T-x information is important when using enhanced fluidity liquids as mobile phases in HPLC.
Figure 24 illustrates the methanol/H$_2$O/CO$_2$ mixtures that are useable as mobile phases for enhanced-fluidity HPLC if the mixtures are prepared and maintained at 25°C (for example in a syringe pump). For H$_2$O/methanol mole ratio (R value) of 0.2490 at 204 atm and 25°C, 0.00 - 0.73 mole fraction of CO$_2$ can be added to the mixture; while at the same pressure, single liquid phase mixtures with methanol/H$_2$O/CO$_2$ mixtures having R values of 0.3318, 0.4298 and 0.5633 can be obtained with 0.00 - 0.54, 0.00 - 0.38 and 0.00 - 0.27 mole fractions of CO$_2$, respectively.

The onset of the UCST line at low mole fractions of CO$_2$ and H$_2$O in acetonitrile/H$_2$O/CO$_2$ mixtures limits the usefulness of these mixtures for enhanced-fluidity HPLC. A single liquid phase for acetonitrile/H$_2$O/CO$_2$ mixtures with a H$_2$O/acetonitrile mole ratio (R value) of 0.3206 at 121 atm and 25°C, can only be obtained if the mole fraction of CO$_2$ is limited to 0.00 - 0.21.
Table 3. Experimental P-T-x vapor-liquid equilibrium data for the methanol(1) - CO₂(2) system.

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Table 3 (continued). Experimental P-T-x vapor-liquid equilibrium data for the methanol - carbon dioxide system.

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<th>SD</th>
<th>(x_1)</th>
<th>(x_2)</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
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Table 4. Experimental P-T-x vapor-liquid equilibrium data for the acetonitrile(1) - CO$_2$(2) system.

<table>
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<tr>
<th>$x_1$</th>
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<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
<th>$x_1$</th>
<th>$x_2$</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
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Table 5. Experimental P-T-x single phase transition data for the methanol(1) - H₂O(2) - CO₂(3) system at x₂/x₁=0.2490.

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<thead>
<tr>
<th>Temp. Press. (°C)</th>
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<th>Temp. Press. (°C)</th>
<th>SD (atm)</th>
</tr>
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<td>x₁ = 0.669, x₂ = 0.167, x₃ = 0.164</td>
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<td>99.6</td>
</tr>
<tr>
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Table 5 (Continued). Experimental P-T-x single phase transition data for the methanol(1) - H₂O(2) - CO₂(3) system at x₂/x₁=0.2490.

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<th>SD</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
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Table 6. Experimental P-T-x single phase transition data for the methanol(1) - H$_2$O(2) - CO$_2$(3) system at $x_2/x_1=0.3318$

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</thead>
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<td>(°C) (atm)</td>
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</tbody>
</table>

$x_1 = 0.4480, x_2 = 0.1486, x_3 = 0.4034$

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>(°C) (atm)</td>
<td>(°C) (atm)</td>
</tr>
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</table>

$x_1 = 0.4090, x_2 = 0.1357, x_3 = 0.4553$

$x_1 = 0.3416, x_2 = 0.1133, x_3 = 0.5451$

<table>
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<tr>
<td>(°C) (atm)</td>
<td>(°C) (atm)</td>
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Table 7. Experimental P-T-x single phase transition data for the methanol(1) - H₂O(2) - CO₂(3) system at x₂/x₁=0.4298

<table>
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<th>SD</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
</tr>
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<td>99.1</td>
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x₁ = 0.5061, x₂ = 0.2175, x₃ = 0.2763

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<th>SD</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
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<td>99.4</td>
<td>178.0</td>
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x₁ = 0.3963, x₂ = 0.1703, x₃ = 0.4334

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<th>SD</th>
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<td>59.5</td>
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<td>185.0</td>
<td>0.3</td>
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<td>187.3</td>
<td>0.1</td>
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<td>0.2</td>
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<td>191.5</td>
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<td>0.05</td>
<td>89.3</td>
<td>196.0</td>
<td>0.05</td>
</tr>
<tr>
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<td>200.2</td>
<td>0.1</td>
<td>99.2</td>
<td>200.2</td>
<td>0.1</td>
</tr>
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Table 8. Experimental P-T-x single phase transition data for the methanol(1)-H₂O(2)-CO₂(3) system at x₂/x₁ = 0.5633.

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<th>Temp. Press. SD</th>
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<td>(atm)</td>
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<td>x₁ = 0.5759, x₂ = 0.3244, x₃ = 0.0998</td>
<td>x₁ = 0.504, x₂ = 0.284, x₃ = 0.212</td>
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<td>30.9</td>
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<tr>
<td>88.9</td>
<td>63.5</td>
</tr>
<tr>
<td>99.7</td>
<td>68.0</td>
</tr>
</tbody>
</table>

x₁ = 0.4651, x₂ = 0.2620, x₃ = 0.2729 | x₁ = 0.4292, x₂ = 0.2418, x₃ = 0.3290 |
| 25.1            | 115.8           | 0.8             | 25.0            | > 306           |
| 30.3            | 111.4           | 0.4             |                  |                 |
| 40.0            | 113.4           | 0.2             |                  |                 |
| 49.8            | 121.3           | 0.2             |                  |                 |
| 59.5            | 131.0           | 0.2             |                  |                 |
| 69.5            | 141.3           | 0.2             |                  |                 |
| 79.4            | 150.2           | 0.4             |                  |                 |
| 89.2            | 157.6           | 0.3             |                  |                 |
| 99.2            | 164.0           | 0.5             |                  |                 |
Table 9. Experimental P-T-x single phase transition data for the acetonitrile(1) - H₂O(2) - CO₂(3) system at x₂/x₁=0.3206.

<table>
<thead>
<tr>
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<th>Temp. Press. SD</th>
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</thead>
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<td>('C) (atm)</td>
<td>('C) (atm)</td>
</tr>
<tr>
<td>x₁ = 0.672, x₂ = 0.223, x₃ = 0.105</td>
<td>x₁ = 0.595, x₂ = 0.197, x₃ = 0.208</td>
</tr>
<tr>
<td>25.2 10.3 0.05</td>
<td>25.4 19.7 0.05</td>
</tr>
<tr>
<td>35.6 12.8 0.1</td>
<td>35.5 23.4 0.2</td>
</tr>
<tr>
<td>49.6 17.0 0.2</td>
<td>49.9 30.2 0.05</td>
</tr>
<tr>
<td>69.9 21.9 0.1</td>
<td>69.7 39.3 0.1</td>
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<tr>
<td>79.5 24.5 0.3</td>
<td>79.4 44.0 0.1</td>
</tr>
<tr>
<td>99.2 30.0 0.5</td>
<td>99.0 53.2 0.2</td>
</tr>
</tbody>
</table>

x₁ = 0.588, x₂ = 0.195, x₃ = 0.217
25.1 120.9 7.9
35.4 24.3 0.05
50.1 30.7 0.05
69.9 40.4 0.2
79.3 45.2 0.1
99.2 54.6 0.6

x₁ = 0.547, x₂ = 0.181, x₃ = 0.272
25.0 >306.0

x₁ = 0.542, x₂ = 0.180, x₃ = 0.278
25.0 >306.0
Table 10. Experimental P-T-x single phase transition data for the acetonitrile$_1$-water$_2$-carbon dioxide$_3$ system at $x_2/x_1=0.7316$.

<table>
<thead>
<tr>
<th>Temp. Press. SD</th>
<th>Temp. Press. SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(°C)$</td>
<td>$(atm)$</td>
</tr>
<tr>
<td>$x_1 = 0.5218$, $x_2 = 0.3817$, $x_3 = 0.0965$</td>
<td>$x_1 = 0.466$, $x_2 = 0.341$, $x_3 = 0.193$</td>
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<td>25.0</td>
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<tr>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>&gt;204.0</td>
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LIST OF REFERENCES


29. van Konynenburg, P. H.; Scott, R. L. Phil. Trans. R. Soc. 1980, 298, 495.
34. Souvignet, I.; Olesik, S. V.; unpublished data.
35. McHugh, M. A.; Krukonis, V. J., Supercritical Fluid Extraction Principles and Practice; Butterworths Scientific: Massachusetts, 1986.
CHAPTER III

DIFFUSION OF ANALYTES IN ENHANCED-FLUIDITY LIQUIDS

INTRODUCTION

To evaluate enhanced-fluidity high performance liquid chromatography (HPLC) and compare it to commonly performed HPLC, an understanding of the diffusion process within the single phase region of these unique solvents is important. Almost no information exists on the diffusion of solutes in enhanced-fluidity liquids. Only the diffusion coefficients of benzene in methanol/CO₂ mixtures over the composition range of 0.00 to 1.00 mole fraction CO₂ in methanol at 40°C and 148 atm have been reported.¹

In this chapter experimental diffusion coefficients of benzene, anthracene, and phenol in methanol, and a methanol/H₂O mixture at ambient and elevated temperatures are compared to diffusion coefficients for the same solutes in a series of methanol/H₂O/CO₂ mixtures at ambient and elevated temperatures.

In addition a method for estimating the single liquid phase region of methanol/H₂O/CO₂ mixtures at elevated temperatures is described herein. In this method, standard chromatographic equipment readily available in our laboratory was
used. However, this method is limited in that information about the single liquid phase region of methanol/H₂O/CO₂ at room temperature must be known a priori.

**EXPERIMENTAL**

**System for Diffusion Coefficient and Phase Diagram Measurements**

Figure 27 shows the instrumentation used to measure the diffusion coefficients and phase diagram. The system consisted of an ISCO LC-2600 syringe pump (ISCO, Lincoln, NE), a Valco W-series high pressure injection valve with an injection volume of 60-nL (Valco Instruments, Houston, TX), a fused silica (Polymicro Technologies, Phoenix, AZ) capillary tube 19.90-m long x 320-μm i.d. and a Spectra-Physics UV2000 UV/vis absorbance detector equipped with a capillary flow cell (model 9550-0155). The flow cell for detection was created by removing the polyimide coating from a 5-mm length of the 320-μm i.d. tubing and centering it in the capillary flow cell. The detector wavelength was 204 nm for benzene, 252 nm for anthracene, and 211 nm for phenol. A Carlo Erba Fractovap 4160 gas chromatographic oven was used. The mobile phase was preheated for the elevated temperature experiments by placing a two meter length of 1/16-in stainless steel tubing inside the oven, after the syringe pump, and prior to the injector. This was added as a precaution to prevent band broadening caused by the thermal gradients that might otherwise occur between the injector and the column. A Setra 204 series pressure transducer (Setra Systems Inc. Acton, MA) was placed in-line after the
Figure 27. System for measuring diffusion coefficients.
detector and before the flow restrictor. The outlet pressure of the open tube was 
monitored because the system pressure must be maintained above a minimum pressure 
to prevent the methanol/H₂O/CO₂ mixture from separating into two phases (liquid-gas). 
The measured pressure drop across the open tube in these experiments was less than 
one atm under all conditions and its effect is negligible in these diffusion coefficient 
measurements. All experiments in this study were performed under conditions in which 
the methanol/H₂O/CO₂ mixture was a single liquid phase. The flow control for the 
chromatographic system was maintained by an appropriate length of 100, 50, or 20-μm 
i.d. fused silica capillary tubing (Polymicro Technologies, Phoenix, AZ). The inlet 
pressure of the capillary tubing was maintained at 136 atm throughout the diffusion 
coefficient and phase diagram studies.

When using a coiled tube to determine diffusion coefficients, secondary flow 
contributions can cause incorrect measurements. To eliminate possible errors due to 
secondary flow contributions, the aspect ratio (ratio of the coil diameter to tube i.d.) 
must be as large as possible. The aspect ratio in this experiment was 625 with linear 
velocities of 1.8-3.8 cm/s. Secondary flow does not contribute to the flow pattern 
under these conditions.

Materials

The analytes used for the diffusion coefficient experiments were benzene, 
anthracene and phenol. The concentrations of analytes were 5.55 x 10⁻³ M, 1.78 x 10⁻⁴ 
M and 7.18 x 10⁻³ M for benzene, anthracene and phenol in methanol, respectively.
Using the 60 nl injection loop, these concentrations correspond to 0.026 µg benzene, 0.002 µg anthracene and 0.041 µg phenol injected. Supercritical fluid grade CO₂ from Scott Specialty Gases (Plumsteadville, PA) was used in the diffusion coefficient and phase diagram measurements. The methanol was HPLC-grade obtained from J.T Baker (Phillipsburg, NJ).

Methanol/H₂O/CO₂ mixtures were prepared using two ISCO LC-2600 high pressure syringe pumps. A known volume of a methanol/H₂O mixture at a composition of 0.70/0.30 mole fraction was placed in one pump. Liquid CO₂ at 136 atm and ambient temperature was held in another pump. Using the known density of CO₂ at these conditions the appropriate volume was calculated and then delivered to the pump holding the methanol/H₂O mixture to make a given methanol/H₂O/CO₂ mixture. The mixture was then pressurized to 204 atm and allowed to equilibrate at ambient temperature for at least 12 hours to ensure complete mixing of the solution. In the diffusion coefficient experiments methanol, a methanol/H₂O mixture of 0.70/0.30 mole fraction methanol/H₂O and four enhanced-fluidity mixtures, 0.56/0.24/0.20, 0.52/0.23/0.25, 0.49/0.21/0.30, and 0.42/0.18/0.40 mole fraction methanol/H₂O/CO₂ were studied.

Data Analysis

Data were collected on an IBM-AT compatible computer using a data collection program written in our lab with ASYST 2.1 (Macmillan Software Company, New York, NY). The data were collected at a variable sampling rate that was
controlled by the final analysis time of the separation. Data were collected at a sampling rate of 6-12 points/s for the diffusion experiments. The data were analyzed using PeakFit 3.0 (PeakFit Analysis Software, Jandel Scientific, San Rafael, CA). Theoretical plates were determined by fitting a Gaussian peak to the experimental data. Gaussian fits to the experimental data had $R^2 \geq 0.999$

RESULTS AND DISCUSSION

Phase Diagram Measurement using an Open Tube

Prior to obtaining the variable volume view cell described in Chapter II, the single liquid phase region of a methanol/H$_2$O/CO$_2$ mixture was estimated at 136 atm using this system. The methanol/H$_2$O mole ratio was held constant at 0.70/0.30; the temperature and mole fraction CO$_2$ were then varied. The mixture in the pump was always in the single liquid phase at ambient temperature and 136 atm for compositions containing 0 - 0.40 mole fraction of CO$_2$. This was verified by the standard method of determining phase behavior which is visual observation with a fixed volume view cell and was also supported by previously published work. The pump delivered the mixture to an open tube (19.90-m of 320-μm i.d. fused silica tubing) that was inside the gas chromatographic oven. The UV/vis detector with its capillary detection cell was attached to the open tube and outside the oven. The point of detection was 5.2 cm from the heated region of the oven.
The single phase region was estimated dynamically, with the mixture flowing through the open tube. Under these conditions, the oven temperature was elevated in 10 °C increments. After each temperature increase, the system was allowed to equilibrate until the detector baseline stabilized. The temperature was increased stepwise in this way until the phase boundary was reached. The phase boundary was apparent because the detected base line immediately became very noisy. The oven temperature was then decreased by 10 °C until a stable baseline was again obtained. The temperature was then increased 2 °C at a time until the lowest temperature where the noise was observed was identified. This method was useful in estimating the phase boundary. However, it cannot be used to determine whether the non-single phase conditions correspond to two or multidimensional phase conditions. More comprehensive and quantitative phase diagrams were described in Chapter II.

Figure 28 shows a comparison of the phase boundary points obtained by this method with the phase boundary extrapolated from experimental data from Chapter II. There is inconsistency between the phase transition points measured using the open tube technique and the variable volume view cell in Chapter II. In particular, the single phase transition point measured by the open tube technique at 0.30 mole fraction CO₂ and 60 °C is well within the single phase region determined in Chapter II. Phase boundary measurements by the open tube method are susceptible to many sources of error such as local heating of the capillary tube with elevation in oven temperature and an approximately 15 minute time delay for the volume of the capillary tube to
Figure 28. The single liquid phase boundary of methanol/H$_2$O/CO$_2$ at 136 atm where the methanol/H$_2$O is at a constant 0.70/0.30 mole ratio and the amount of CO$_2$ is varied. Estimated using the open tube technique (●). Extrapolated from data in Chapter II (▼).
completely pass by the detector when the temperature of the tube is decreased. Also
the accuracy and precision is not as high when preparing mobile phase mixtures on a
CO₂ density basis as with on the weight measurements used in Chapter II.

In summary, the dynamic open tube method for estimating single liquid phase
boundaries should only be used when phase information does not exist and when
instrumentation to more accurately determine phase boundaries is not available. Phase
information obtained from the open tube method should be used conservatively.

**Diffusion Coefficients**

The chromatographic band broadening method⁵ was used to determine
experimental diffusion coefficients, Dₘ, of solutes in the various mobile phase mixtures.
This technique was previously used in this laboratory to determine solute diffusion
coefficients in binary and ternary supercritical mixtures.⁶ In this technique, analyte is
introduced as a sharp pulse into a capillary tube, and carried through the length of the
tube by the desired mobile phase, then eluted as a Gaussian peak. If the flow pattern of
the mobile phase inside the tube is laminar, the dispersion or broadening of the peak is
attributed to molecular diffusion along the axis of the tube and radial diffusion across
the parabolic flow profile. The peak variance is then related to the diffusion coefficient
of the solute by the following expression:

\[ \sigma^2 = \frac{2 D_m L}{u} + \frac{d^2 uL}{96 D_m} \] (1)
where $\sigma^2$ is the peak variance, cm$^2$; $D_m$, is the diffusion coefficient of the analyte in the mobile phase, cm$^2$/s; $L$ is the length of the tube, cm; $u$ is the average linear velocity, cm/s; and $d$ is the internal diameter of the tube, cm. This relationship correctly describes the band dispersion in an open tube as long as:

$$\sigma < t; \quad \frac{Ld}{t D_m} > 100; \quad tD_m/d^2 > 0.2$$

where $t$ is the elution time for the center of mass of the peak.$^7,^8$ These conditions were readily met by this study.

The first term on the right side of equation 1 represents dispersion due to diffusion along the axis of the tube and the second term represents dispersion due to radial diffusion across the parabolic flow profile. In practice the first term is negligible if the average linear velocity is greater than $140 \, D_m/d$.$^1$ This condition was met in these experiments. Chromatographic plate height, $H$, by definition is

$$H = \frac{\sigma^2}{L}$$

and the average linear velocity, $u$, is equal to $L/t$. Therefore the diffusion coefficient of the solute can be determined using equation 4.$^1$

$$D_m = \frac{d^2L}{96tH}$$
All of the terms on the right side of the equation 4 were determined experimentally to provide experimental $D_m$ values. The variance of the bands was determined by fitting Gaussian distributions to the eluted peaks using PeakFit software. Each reported diffusion coefficient is the average of 5 - 10 independent measurements. Figure 29 shows data for benzene at four different mobile phase conditions. Experimentally measured diffusion coefficients for benzene, anthracene, and phenol with the accompanying 95% confidence limits are listed in Tables 11, 12, and 13, respectively.

Many studies have shown that over a wide range of pressure and temperature, liquid viscosity and solute diffusion coefficients are inversely related by the relationship

$$D_m \propto \eta^q$$

For example, Figure 30 shows the inverse relationship of diffusion coefficient and viscosity for methanol at elevated pressures. As the temperature is elevated the experimentally measured diffusion coefficient of benzene through methanol at 136 atm increases. In contrast, experimentally determined methanol viscosities at 98.7 atm reported in the literature decrease with temperature.\textsuperscript{10,11}

Figure 31 shows the variation in the experimentally measured diffusion coefficients, $D_m$, for benzene as a function of temperature (26 °C to 120 °C) in a series of enhanced-fluidity mixtures where the mole ratio of methanol/H\textsubscript{2}O was maintained at 0.70/0.30 and the amount of added CO\textsubscript{2} was varied from 0 to 0.40 mole fraction at
Figure 29. Data and associated plate counts for diffusion coefficient measurements of benzene at four different mobile phase conditions: (A) 0.70/0.30 methanol/H_2O at 26°C, (B) 0.70/0.30 methanol/H_2O at 60°C, (C) 0.49/0.21/0.30 methanol/H_2O/CO_2 at 26°C, and (D) 0.49/0.21/0.30 methanol/H_2O/CO_2 at 58°C.
Table 11. Experimental diffusion coefficients for benzene in methanol and methanol/H₂O/CO₂ mixtures at 136 atm where the methanol/H₂O mole ratio is constant at 0.70/0.30 and the mole fraction of CO₂ in the mixture is varied.

\[ D_m \times 10^{-5} \text{ cm}^2/\text{s} \]

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<th>0.40</th>
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<td>26</td>
<td>1.49 (±0.03)</td>
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<td>2.78 (±0.07)</td>
<td>3.03 (±0.06)</td>
<td>3.68 (±0.06)</td>
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</tr>
<tr>
<td>27</td>
<td>2.56 (±0.05)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>32</td>
<td>2.90 (±0.05)</td>
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<td></td>
<td>3.44 (±0.05)</td>
<td>4.42 (±0.14)</td>
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</tr>
<tr>
<td>40</td>
<td>3.16 (±0.06)</td>
<td>1.99 (±0.02)</td>
<td>3.35 (±0.07)</td>
<td>3.74 (±0.15)</td>
<td>4.01 (±0.08)</td>
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<tr>
<td>50</td>
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<td>6.29 (±0.07)</td>
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<tr>
<td>58</td>
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<td>12.62 (±0.44)</td>
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<tr>
<td>60</td>
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<tr>
<td>80</td>
<td>5.18 (±0.13)</td>
<td>3.88 (±0.10)</td>
<td>5.85 (±0.21)</td>
<td>9.48 (±0.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
<td></td>
<td>17.77 (±1.60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>6.53 (±0.05)</td>
<td>5.10 (±0.08)</td>
<td>7.90 (±0.07)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>8.08 (±0.05)</td>
<td>6.54 (±0.11)</td>
<td>9.84 (±0.43)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis are 95% confidence intervals.
Table 12. Experimental diffusion coefficients for anthracene in methanol/H₂O/CO₂ mixtures at 136 atm where the methanol/H₂O mole ratio is constant at 0.70/0/30 and the mole fraction of CO₂ the mixture is varied.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>0.00</th>
<th>0.20</th>
<th>0.25</th>
<th>0.30</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>1.01 (±0.03)</td>
<td></td>
<td></td>
<td>2.02 (±0.08)</td>
<td>2.27 (±0.04)</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.36 (±0.01)</td>
<td>2.32 (±0.10)</td>
<td>2.48 (±0.04)</td>
<td>2.64 (±0.05)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>4.04 (±0.12)</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td>7.00 (±0.30)</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.95 (±0.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>2.70 (±0.03)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3.63 (±0.07)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>4.66 (±0.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis are 95% confidence intervals.
Table 13. Experimental diffusion coefficients for phenol in methanol/H$_2$O/CO$_2$ mixtures at 136 atm where the methanol/H$_2$O mole ratio is constant at 0.70/0.30 and the mole fraction of CO$_2$ in the mixture is varied.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Mole Fraction CO$_2$ in the H$_2$O/Methanol Mixture</th>
<th>$D_m \times 10^{-5}$ cm$^2$/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.30</td>
</tr>
<tr>
<td>26</td>
<td>1.00 (±0.02)</td>
<td>1.74 (±0.03)</td>
</tr>
<tr>
<td>40</td>
<td>1.36 (±0.01)</td>
<td>3.00 (±0.03)</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>7.62 (±0.29)</td>
</tr>
<tr>
<td>60</td>
<td>1.97 (±0.03)</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>2.78 (±0.01)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3.69 (±0.03)</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>4.85 (±0.11)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis are 95% confidence intervals.
Figure 30. Variation in diffusion coefficients of benzene (⊕) in methanol at 136 atm and variation in viscosity (△) of methanol at 98.7 atm with temperature.10,11
Figure 31. Variation in diffusion coefficients of benzene in (+) 0.70/0.30 mole fraction methanol/H2O, (▲) 0.56/0.24/0.20 mole fraction methanol/H2O/CO2, (●) 0.52/0.23/0.25 mole fraction methanol/H2O/CO2, (♦) 0.49/0.21/0.30 mole fraction methanol/H2O/CO2, and (■) 0.42/0.18/0.40 mole fraction methanol/H2O/CO2 with temperature at 136 atm.
136 atm. The error bars in the figure indicate 95% confidence intervals. For data points without error bars, the 95% confidence interval is within the size of the data point. The magnitude of the increase in diffusion coefficient with temperature is greatest for the mixtures with the highest proportion of CO₂. Figure 32 shows the variation in the diffusion coefficients, D_m, for anthracene over the same temperature range for two mixtures, the 0.70/0.30 mole fraction methanol/H₂O mobile phase and the 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂. Figure 32 shows that the diffusion coefficients of anthracene are similarly affected by the enhanced fluidity solvents. Temperatures in excess of 120 °C must be applied to achieve an approximately five-fold increase in the diffusion coefficients of benzene and anthracene with the methanol/H₂O mobile phase. However, elevating the temperature dramatically increases the diffusion coefficients of benzene and anthracene for the enhanced-fluidity mixtures with increasing proportions of CO₂. At 58 °C with the 0.49/0.21/0.30 methanol/H₂O/CO₂ mixed mobile phase, the diffusion coefficients increased almost nine-fold and seven-fold for benzene and anthracene, respectively, relative to their diffusion coefficients at 26 °C with a methanol/H₂O mobile phase. In addition, the diffusion coefficients of benzene and anthracene are approximately two times greater with the 0.49/0.21/0.30 methanol/H₂O/CO₂ mixture at room temperature than the methanol/H₂O mobile phase at the same temperature. Temperatures in excess of 60 °C must be applied to the methanol/H₂O mobile phase to achieve the same increase in diffusion coefficient as can be obtained by using the enhanced-fluidity mixture at room
Figure 32. Variation in diffusion coefficients of anthracene in (●) 0.70/0.30 mole fraction methanol/H$_2$O, and (♦) 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$ with temperature at 136 atm.
temperature. Similar variation in the diffusion coefficients of phenol with temperature was observed with these mixtures, these diffusion coefficients are listed in Table 13.

For a large number of liquid mixtures, including hydrogen-bonded systems, the variation of diffusion coefficient with temperature is often described by Eyring rate theory (equations 6 and 7):

\[ \ln D_m = A + B/T \]  
\[ B = -E_d/R \]

where \( A \) is pre-exponential, \( E_d \) is activation energy of diffusion, \( R \) is the Boltzmann constant, and \( T \) is absolute temperature.\(^{12,13,14}\) Figure 33 shows a linear fit of variation of \( \ln D_m \) with \( 1/T \) for benzene in the 0.70/0.30 mole fraction methanol/H\(_2\)O mixture and for the 0.49/0.21/0.30 mole fraction methanol/H\(_2\)O/CO\(_2\) mixture. Figure 34 also shows a linear fit for the variation of \( \ln D_m \) with \( 1/T \) for anthracene in the same mobile phase mixtures. While the Eyring rate theory correctly describes the variation in diffusion coefficients for benzene and anthracene in the 0.70/0.30 mole fraction methanol/H\(_2\)O mixture, equations 6 and 7 clearly do not describe well the temperature dependence of the diffusion coefficient for benzene or for anthracene in the methanol/H\(_2\)O/CO\(_2\) enhanced-fluidity mixtures.
Figure 33. Plot of ln $D_m$ versus $1/T$ for benzene with (✦) 0.70/0.30 mole fraction methanol/H$_2$O and (♦) 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$. 
Figure 34. Plot of In $D_m$ versus $1/T$ for anthracene with (+) 0.70/0.30 mole fraction methanol/H$_2$O and (♦) 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$. 
CONCLUSIONS

From the data presented, it is clear that the mobile-phase viscosity decreases with increasing temperature and the extent of this decrease is greatest for the enhanced-fluidity mixtures containing the highest proportions of CO₂. The addition of a low viscosity liquid, such as CO₂, to a methanol/H₂O mixture increases the diffusion coefficients of benzene, anthracene, and phenol approximately 2-fold at room temperature. Temperature elevation results in significantly higher solute diffusion with enhanced-fluidity liquids compared to solvents commonly used for high performance liquid chromatography. Although, the Eyring Rate Theory describes well the temperature dependence of diffusion coefficients for most liquids, it does not correctly predict the temperature dependence of diffusion coefficients in enhanced-fluidity liquids.
LIST OF REFERENCES


CHAPTER IV

REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
USING ENHANCED-FLUIDITY LIQUIDS AS MOBILE PHASES

INTRODUCTION

In this chapter HPLC separations using methanol/H_2O and methanol/H_2O/CO_2 mobile phase mixtures similar to those studied in Chapter III are characterized. A direct comparison of improvements in chromatographic performance in HPLC due to increased column temperature and/or the use of enhanced-fluidity mixtures is obtained.

In addition we demonstrate that the low column pressure drop caused by the use of an enhanced-fluidity mobile phase allows ready serial coupling of columns for the generation of a large number of theoretical plates. These coupled columns were applied to the separation of a complex coal tar standard.

EXPERIMENTAL

Chromatographic System

Figure 35 shows the chromatographic system. The chromatographic system consisted of an ISCO LC-2600 syringe pump (ISCO, Lincoln, NE), a Valco W-series high pressure injection valve with an injection volume of 200-nL (Valco Instruments,
Figure 35. Enhanced-fluidity HPLC system.
Houston, TX), a BDS Hypersil C18, 150-mm x 1-mm column packed with 5-μm
diameter particles (Keystone Scientific, Bellefonte, PA) and a Spectra-Physics UV2000
UV/vis absorbance detector equipped with a capillary flow cell (model 9550-0155).
The oven was from a Carlo Erba Fractovap 4160 gas chromatograph. The mobile
phase was preheated for the elevated temperature experiments by placing a two meter
length of 1/16-in stainless steel tubing inside the oven, after the syringe pump, and prior
to the injector. This was added as a precaution to prevent chromatographic band
broadening caused by the thermal gradients that would otherwise occur between the
injector and the column. However, over the temperature range studied (25 - 60 °C) it
was later determined that the presence of the preheater tube did not affect the band
width of the chromatographic peaks. A 1/16-in o.d. x 0.004-in i.d. x 10-cm polished
stainless steel tube (Alltech Assoc., Deerfield, IL) was used to connect the injector to
the column to minimize extracolumn band broadening. The flow cell for detection was
created by removing the polyimide coating from a 5-mm section of 100-μm i.d. fused
silica tubing (Polymicro Technologies, Phoenix, AZ) and centering it in the capillary
flow cell. The detector wavelength was 254 nm. An Omega model PX931-5KSV
pressure transducer (Omega Engineering, Stamford, CT) was placed in-line after the
detector and before a post-detection restrictor. The outlet pressure of the column was
monitored because the column pressure must be maintained above a minimum pressure
to prevent the methanol/H₂O/CO₂ mixture from separating into two phases (liquid-gas).
All experiments in this study were performed under conditions in which the
methanol/H₂O/CO₂ mixture was a single liquid phase. The flow control for the chromatographic system was maintained by an appropriate length of 20, 15, or 10-µm i.d. fused silica tubing (Polymicro Technologies, Phoenix, AZ). The column inlet pressure was maintained at 204 atm throughout the chromatographic experiments except when using the subsequently defined RT mobile phase with 4 columns in series, under these conditions a column inlet pressure of 320 atm was required to obtain the appropriate mobile phase linear velocity.

**Materials**

SFC/SFE grade CO₂ supplied by Air Products (Allentown, PA) was used in all the chromatographic separations. The methanol was HPLC-grade obtained from J.T Baker (Phillipsburg, NJ).

Methanol/H₂O/CO₂ mixtures were prepared using two ISCO LC-2600 high pressure syringe pumps. A known volume of a methanol/H₂O mixture at a composition of 0.70/0.30 mole fraction was placed in one pump. Liquid CO₂ at 136 atm and ambient temperature was held in another pump. Using the known density of CO₂ at these conditions the appropriate volume was calculated and then delivered to the pump holding the methanol/H₂O mixture to make a given methanol/H₂O/CO₂ mixture. The mixture was then pressurized to 204 atm and allowed to equilibrate at ambient temperature for at least 12 hours to ensure complete mixing of the solution. All the experiments described in this chapter were performed using a methanol/H₂O mixture of
0.70/0.30 mole fraction methanol/H₂O or a methanol/H₂O/CO₂ mixture of 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂.

The primary chromatographic test mixture used in this study was a methanol solution containing 0.71 μl/ml benzene, 0.079 mg/ml naphthalene, 0.021 mg/ml anthracene, 0.082 mg/ml pyrene, 0.036 mg/ml benz[a]anthracene, 0.071 mg/ml benzo[e]pyrene, 0.039 mg/ml benzo[a]pyrene, and 0.046 mg/ml benzo[ghi]perylene. A Supelco test mix of 16 polyaromatic hydrocarbon compounds was also separated and illustrated in the text (catalog # 4-8905). This test mix was received in a methylene chloride/benzene solvent. This solvent was evaporated to dryness and replaced with methanol. The final concentration of each individual component of this test mix was 0.030 mg/ml. SRM 1597, a complex mixture of PAH isolated from coal tar, was obtained from The National Institute of Standards and Technology (Gaithersburg, MD).

Data Analysis

Data were collected on an IBM-AT compatible computer using a data collection program written in our lab with ASYST 2.1 (Macmillan Software Company, New York, NY). The data were collected at a variable sampling rate that was controlled by the final analysis time of the separation. The sampling rate was always fast enough so that each chromatographic band had no less than 40 data points across the entire band. The chromatograms were analyzed using PeakFit 3.0 (Peakfit Analysis Software, Jandel Scientific, San Rafael, CA). Theoretical plates were determined by
fitting a Gaussian peak to the experimental data. Plate height versus linear velocity curves were fit to the van Deemter equation using the nonlinear least square subroutines of SYSTAT 5.0 (SYSTAT, Inc., Evanston, IL).

RESULTS AND DISCUSSION

Injection profile

All chromatograms using the enhanced-fluidity liquids have a noticeable vacancy peak that appears prior to the elution of benzene. Vacancy peaks often appear in chromatograms when mixed mobile phases are used with an injection solvent that is not the same composition as the eluent. Several different solvents were examined as possible injection solvents. The list of solvents examined includes methanol, water, methanol/H₂O combinations, acetone, acetonitrile, carbon disulfide, and methylene chloride. All these solvents left a large injection profile. Methanol was chosen as the injection solvent because it had the smallest injection profile, it is the main component in the mobile phases employed and all test compounds were readily soluble in it. The validity of using methanol in this study as the injection solvent was verified by comparing chromatograms when methanol and the methanol/H₂O/CO₂ mobile phase were used as the injection solvent.

The sample was injected with the pressurized mobile phase by splitting mobile phase from the pump with a 3-way valve placed in line before the injection valve. The majority of the mobile phase was delivered through the tubing, injector, column,
detector, restrictor in that order as usual. The remaining mobile phase moved from the three way valve to a 1.2 ml extraction cell containing the sample solutes. 1.2 ml of the chromatographic test mix was delivered to the extraction cell with a glass pipet and the sample solvent (methanol) was evaporated with a stream of nitrogen. This process was repeated once. The total volume of the test mix delivered to the cell and evaporated was ≈ 2.4 ml. The cell containing the sample solutes was then put in line between the 3-way valve and the injector. The methanol/H2O/CO2 mobile phase then solvated and pressurized the contents of the extraction cell. The cell and sample loop were maintained at the head pressure of the system by a valve positioned after the injector. A restrictor directly after the valve was created with a length of 5 μm id fused silica. By opening the post injector valve, sample was allowed to flow from the extraction cell and fill the sample loop. Using this system injection of the 16 component standard was performed in triplicate. This was compared to triplicate injections of the 16 component standard using a conventional syringe guide and waste line respectively.

Figure 36 shows chromatograms using methanol as the injection solvent and using the 0.49/0.21/0.30 mole fraction methanol/H2O/CO2 mobile phase as the injection solvent. This comparison shows no difference in terms of efficiency (plate height) or selectivity (k') for the separation of the 16 component PAH test mix. However, the phase inequilibrium introduced by using methanol as the injection solvent is shown by the injection profile. A large positive deflection followed by a large negative deflection in the signal was observed when using methanol as the injection
Figure 36. Chromatograms with the EF mobile phase at 204 atm using (A) methanol and (B) 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ mixture (EF mixture) as the injection solvent. (s) solvent; (1) benzene; (2) naphthalene; (3) acenaphthalene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluorene; (8) pyrene; (9) benz[a]anthracene; (10) chrysene; (11) benzo[b]fluoranthene; (12) benzo[k]fluoranthene; (13) benzo[a]pyrene; (14) dibenzo[a,h]anthracene; (15) indeno[1,2,3-cd]pyrene; (16) benzo[ghi]perylene.
solvent. Only a small negative deflection in the baseline when using the mobile phase as the sample solvent is observed. The small negative deflection is attributed to methanol that was not completely evaporated under the nitrogen stream. In addition, some of the more volatile low molecular weight components (benzene, naphthalene, acenaphthene and fluoranthene) were lost when the methanol was evaporated under the nitrogen stream. Because there was no observable effect on the chromatographic performance when using methanol as the injection solvent, besides the observed vacancy peak, methanol was used throughout this study.

Chromatograms

From the results of the Chapter III, two mixture compositions at two temperatures were chosen for further characterization by chromatographic analysis. These include the methanol/H$_2$O mixture at 26 °C and at an elevated temperature of 60 °C; and the 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$ mixture at 26 °C and 60 °C. Hereafter in the text the 0.70/0.30 mole fraction methanol/H$_2$O mixture at 26 °C is designated as the RT (room temperature) mixture. The same mixture at 60 °C is designated as the ET (elevated temperature). Also, the 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$ mixture at 26 °C is designated as EF (enhanced-fluidity) and this mixture at 60 °C is designated as the ET-EF (elevated temperature - enhanced fluidity) mixture. The 60 °C temperature was chosen because raising the temperature to achieve optimum separation may be a reasonable choice. However if the temperature is increased too much the stationary phase and the column often degrade faster than at
room temperature. The 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ enhanced-fluidity mixture was chosen because it showed the greatest variation in diffusion coefficients with temperature change. For later reference, Table 14 shows the benzene and anthracene diffusion coefficients from Chapter III for mixture compositions that are similar to those that were further characterized through chromatography. The inlet pressure of the chromatographic column was maintained at 204 atm instead of 136 atm (pressure where diffusion coefficients was measured). This was done to insure that even with the pressure drop across the column, the mobile phase would remain in a single liquid phase.

Figure 37 shows chromatograms of the eight component PAH test mixture using the RT, ET, EF and ET-EF mobile phases, respectively. The average number of theoretical plates for peaks 3-7 for each chromatogram is also shown. These chromatograms were collected at approximately the same linear velocities (ca. 0.115-0.120 cm/s). Under all conditions baseline resolution was achieved for all components. Efficiency, or the number of theoretical plates for the separations increases in the order of room temperature (RT) < elevated temperature (ET) < enhanced fluid (EF) < elevated temperature - enhanced fluidity (ET-EF). Retention and selectivity is highest with the methanol/H₂O mobile phase at room temperature (RT) and lowest for the enhanced-fluidity mobile phase at elevated temperature (ET-EF). This is not surprising since increasing the temperature and/or the addition of a nonpolar component, CO₂, to
Table 14. Variation in diffusion coefficients of benzene and anthracene at 136 atm with mobile phase conditions.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mobile Phase Composition (Mole Fraction)</th>
<th>Temperature (°C)</th>
<th>$D_m \times 10^{-5}$ (cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>0.70 MeOH / 0.30 H$_2$O</td>
<td>26</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>0.70 MeOH / 0.30 H$_2$O</td>
<td>60</td>
<td>2.85</td>
</tr>
<tr>
<td></td>
<td>0.49 MeOH / 0.21 H$_2$O / 0.30 CO$_2$</td>
<td>26</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>0.49 MeOH / 0.21 H$_2$O / 0.30 CO$_2$</td>
<td>58</td>
<td>12.62</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.70 MeOH / 0.30 H$_2$O</td>
<td>26</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>0.70 MeOH / 0.30 H$_2$O</td>
<td>60</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>0.49 MeOH / 0.21 H$_2$O / 0.30 CO$_2$</td>
<td>26</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>0.49 MeOH / 0.21 H$_2$O / 0.30 CO$_2$</td>
<td>58</td>
<td>7.00</td>
</tr>
</tbody>
</table>
Figure 37. Chromatograms at 204 atm for different mobile phase conditions: RT; ET; EF; and ET-EF. (s) Solvent; (1) benzene; (2) naphthalene; (3) anthracene; (4) pyrene; (5) benzo[a]anthracene; (6) benzo[e]pyrene; (7) benzo[a]pyrene; (8) benzo[ghi]perylene.
the mobile phase in reversed-phase chromatography increases the strength of the mobile phase.

It is interesting to note that the retention and selectivity are very similar between the chromatogram with a methanol/H₂O mobile phase at elevated temperature (ET) and the chromatogram using the enhanced-fluidity mobile phase at room temperature (EF). It is also interesting that the measured diffusion coefficients, average number of theoretical plates, and pressure drop across the chromatographic column (which will be discussed later in this chapter) are similar between these two conditions. This shows that to some extent similar results can be obtained by elevating the temperature of the mobile phase and by using enhanced-fluidity mixtures as the mobile phase.

Plate Height

Both the van Deemter and the Knox equations describe well the variation of plate height, \( H \), with linear velocity, \( u \), in HPLC.²³ Although the Knox equation fit our data equally well, for simplicity, we chose to characterize our results in terms of the van Deemter equation (equation 1).

\[
H = Au + \frac{B}{u} + \sum C_i u
\]  

(1)

\( A \) is a constant which represents the band dispersion due to multiple flow paths. \( B \) describes the band dispersion contribution due to longitudinal diffusion; and the \( C \)
terms are measures of band dispersion caused by the resistance to mass transfer in the mobile, stagnant mobile, and stationary phases.

For packed columns the constants in the van Deemter equation can be more specifically described by equation 2:

\[
H = 2\lambda d_p + \frac{2\gamma D_m}{u} + \frac{f_1(k')}{D_m} d^2_p u + \frac{f_2(k')}{D_s} d^2_f u
\]  

(2)

\(\lambda, \gamma\) are packing constants which for well packed columns have values of 0.5 and 0.68, respectively; \(d_p\) is the particle diameter; \(f_1(k')\) and \(f_2(k')\) are functions of \(k'\); \(d_f\) is film thickness of stationary phase; \(D_s\) is the diffusion coefficient of the solute in the stationary phase; and the other symbols were previously defined. Because the \(C_i\) coefficient attributed to the stationary phase nonequilibrium is due primarily to diffusion in the stagnant mobile phase within the pores of the particles, \(D_s\) can be written as:

\[
D_s = \beta D_m
\]  

(3)

where \(\beta\) is a constant < 1. Equation 2 is then rewritten as:

\[
H = 2\lambda d_p + \frac{2\gamma D_m}{u} + \frac{bu}{D_m}
\]  

(4)
where
\[ b = f_1(k) d_p^2 + f_2(k) \frac{d_f}{\beta} \]  
(5)

By differentiating \( H \) with respect to \( u \), and setting the derivative equal to zero, an expression for the optimum linear velocity, \( u_{\text{opt}} \) is obtained:

\[ u_{\text{opt}} = D_m \sqrt{\frac{2\gamma}{b}} \]  
(6)

If Purnell's equation for \( f_j \) is substituted into equation 5 and \( d_f \ll d_p \) then equation 6 can be rewritten as:

\[ u_{\text{opt}} = D_m \sqrt{\frac{48 \gamma (1+k^2)}{(1+6k' + 11k'^2) d_p^2}} \]  
(7)

Finally, when this equation for \( u_{\text{opt}} \) is substituted into equation 1, an expression for \( H_{\text{min}} \) is obtained:

\[ H_{\text{min}} = 2\lambda d_p + 2 \sqrt{2\gamma \frac{(1+6k' + 11k'^2) d_p^2}{24(1+k')^2}} \]  
(8)
From equations 7 and 8, the $u_{opt}$ is expected to increase as $D_m$ increases and as $k'$ decreases; while for $k' = 0$ and $k' = \infty$, $H_{min}$ will be $1.45d_p$ and $2.48d_p$, respectively.

Plate height versus linear velocity curves for the eight test solutes were collected using the mixed mobile phases at the room temperature (RT), elevated temperature (ET), enhanced-fluidity (EF), and elevated temperature-enhanced fluidity (ET-EF) conditions. The experimental curves covered the linear velocity range of 0 - 0.4 cm/s. These experimental data were fit to the van Deemter equation using nonlinear least squares. The resultant curves had $R^2$ values $\geq 0.999$. Figures 38-41 show the experimental data for pyrene, benz[a]anthracene, benzo[e]pyrene and benzo[a]pyrene and the resultant van Deemter curves (solid lines) for each of the four mobile phase conditions mentioned previously.

From visual observation of the data and from the $H_{min}$ and $u_{opt}$ data obtained from the resultant curves (Table 15), little variation in the minimum plate height, $H_{min}$, was observed among the solvent conditions studied. The observed average $H_{min}$ was 120 $\mu$m which is similar in magnitude to that predicted $H_{min} = 124$ $\mu$m for the conditions when $k' = \infty$. Interestingly, the variation of $k'$ with mobile phase solvent did not affect the $H_{min}$ as predicted by equation 8. However, a shift in the optimum linear velocity to higher values is evident in the order of room temperature < elevated temperature mobile phase < enhanced-fluidity mobile phase < elevated temperature - enhanced fluidity mobile phase.
Figure 38. Variation of plate height with mobile phase linear velocity at 204 atm for different mobile phase conditions for pyrene (△) RT, $k' = 3.77$; (●) ET, $k' = 2.02$; (✚) EF, $k' = 1.82$; (■) ET-EF, $k' = 0.92$. 

Linear Velocity (cm/s)  
H (cm) (E-2)
Figure 39. Variation of plate height with mobile phase linear velocity at 204 atm for different mobile phase conditions for benz[a]anthracene (▲) RT, k' = 5.94; (●) ET, k' = 2.68; (◆) EF, k' = 2.42; (■) ET-EF, k' = 1.07.
Figure 40. Variation of plate height with mobile phase linear velocity at 204 atm for different mobile phase conditions for benzo[e]pyrene (△) RT, $k' = 8.92$; (●) ET, $k' = 3.78$; (+) EF, $k' = 3.85$; (■) ET-EF, $k' = 1.53$. 
Figure 41. Variation of plate height with mobile phase linear velocity at 204 atm for different mobile phase conditions for benzo[a]pyrene (▲) RT, $k'=11.19$; (●) ET, $k'=4.33$; (●) EF, $k'=4.75$; (■) ET-EF, $k'=1.70$. 
Table 15. Variation of capacity factor, minimum plate height, and optimum velocity with mobile phase conditions.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mobile Phase Conditions</th>
<th>$k'$</th>
<th>$H_{\text{min}} \times 10^3$ (cm)</th>
<th>$u_{\text{opt}} \times 10^{-2}$ (cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrene</td>
<td>RT</td>
<td>3.77</td>
<td>1.2</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>2.02</td>
<td>1.2</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>1.82</td>
<td>1.2</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>0.92</td>
<td>1.2</td>
<td>11.5</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>RT</td>
<td>5.94</td>
<td>1.2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>2.68</td>
<td>1.2</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>2.42</td>
<td>1.2</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.07</td>
<td>1.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>RT</td>
<td>8.92</td>
<td>1.3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>3.78</td>
<td>1.2</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>3.85</td>
<td>1.2</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.53</td>
<td>1.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>RT</td>
<td>11.19</td>
<td>1.2</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>4.33</td>
<td>1.2</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>4.75</td>
<td>1.2</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.70</td>
<td>1.1</td>
<td>12.1</td>
</tr>
</tbody>
</table>
Equation 7 was used to understand to what extent the shift in \( u_{opt} \) was caused by variation in \( D_m \) or by variation in \( k' \) for the four mobile phase conditions studied. The RT mobile phase was taken as the reference condition. The approximate value of the diffusion coefficient for pyrene in this mobile phase (\( 1.0 \times 10^{-5} \text{ cm}^2/\text{sec} \)) was determined by substituting the experimental \( u_{opt} \) and \( k' \) into equation 7. The second column in Table 16 shows the \( u_{opt} \) values for pyrene calculated using equation 7 for the case where the diffusion coefficient is held constant at the calculated room temperature (RT) value of \( D_m = 1.0 \times 10^{-5} \text{ cm}^2 \sec^{-1} \) and the capacity factor, \( k' \), varies to the extent experimentally observed for the four mobile mixture conditions. The third column in Table 16 is the experimentally observed \( u_{opt} \) for pyrene for the four mobile phase conditions. By comparing these two columns, clearly the observed decrease in capacity factor alone does not account completely for the observed increase in \( u_{opt} \). From equation 7 the increase in diffusion coefficients (as observed for benzene and anthracene) in the order of RT < ET < EF < ET-EF is the other primary factor controlling the shift in \( u_{opt} \). The fourth column in Table 16 shows the calculated diffusion coefficients for pyrene for the four mobile phase conditions when the experimental \( k' \) and \( u_{opt} \) values are substituted into equation 7. As suggested earlier the trend in these predicted values is the same as observed for the diffusion coefficients of benzene and anthracene.

Table 17 shows the van Deemter constants (\( A, B \) and \( C \)) and their 95% confidence intervals for pyrene, benz[a]anthracene, benzo[e]pyrene, and
Table 16. Comparison for pyrene of the experimental optimum velocity, $u_{opt}$, with the calculated $u_{opt}$ due only to capacity factor variation.

<table>
<thead>
<tr>
<th>Mobile Phase Conditions</th>
<th>Calculated* $u_{opt} \times 10^{-2}$ (cm/s)</th>
<th>Observed $u_{opt} \times 10^{-2}$ (cm/s)</th>
<th>Calculated $D_m \times 10^{-4}$ (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>3.8</td>
<td>3.8</td>
<td>1.0</td>
</tr>
<tr>
<td>ET</td>
<td>4.3</td>
<td>7.0</td>
<td>1.6</td>
</tr>
<tr>
<td>EF</td>
<td>4.4</td>
<td>8.9</td>
<td>2.0</td>
</tr>
<tr>
<td>ET-EF</td>
<td>5.2</td>
<td>11.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* For $D_m = 1.0 \times 10^{-4}$ cm²/s
benzo[a]pyrene at the four studied mobile phase conditions. Because of the fact that most error analysis calculations in nonlinear regression programs underestimate the true error, only trends in the coefficient data that are well beyond the quoted confidence interval will be taken as valid. Variation in the A coefficient among the studied mobile phases is often within the statistical error. The observed B coefficients for the ET, EF and ET-EF conditions were all significantly larger than the RT coefficient and the ET-EF B coefficient was always larger than the ET or EF coefficient. This trend is as expected in that the B coefficient should increase with increasing $D_m$. Also, the measured diffusion coefficients for benzene and anthracene at conditions similar to the ET and EF conditions were very similar which account for the fact that the ET and EF B coefficients were often statistically indistinguishable. For all compounds studied, the C coefficients of the van Deemter curves for the ET, EF, and ET-EF mobile phases are all smaller than those for the RT mobile phase. The decreased C coefficient can be attributed to the combined impact of decreased capacity factors and increased diffusion coefficients under these conditions compared to the room temperature conditions. To obtain a measure of how much impact the change in capacity factors has on the C coefficient relative to the change in the diffusion coefficient, the equation suggested by Purnell for the mobile phase contribution to the C coefficient was used. The C coefficient was calculated by holding the diffusion coefficient for any compound arbitrarily to $1.0 \times 10^{-5}$ cm$^2$/sec. The calculated C coefficients were then compared to the experimentally observed C coefficients. For the
Table 17. Variation in van Deemter equation coefficients with mobile phase conditions.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mobile Phase Conditions</th>
<th>A x 10⁻⁴ (cm)</th>
<th>B x 10⁻⁵ (cm²/s)</th>
<th>C x 10⁻³ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrene</td>
<td>RT</td>
<td>5.8 (±0.8)</td>
<td>1.1 (±0.2)</td>
<td>7.8 (±0.4)</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>4.7 (±1)</td>
<td>2.6 (±0.4)</td>
<td>5.3 (±0.4)</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>3.8 (±0.9)</td>
<td>3.8 (±0.5)</td>
<td>4.8 (±0.3)</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>---</td>
<td>6.8 (±0.2)</td>
<td>5.1 (±0.1)</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>RT</td>
<td>6.3 (±0.8)</td>
<td>1.2 (±0.2)</td>
<td>7.3 (±0.5)</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>4.7 (±0.8)</td>
<td>2.7 (±0.3)</td>
<td>4.9 (±0.3)</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>4.8 (±0.4)</td>
<td>2.9 (±0.2)</td>
<td>4.1 (±0.1)</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.1 (±0.6)</td>
<td>5.9 (±0.3)</td>
<td>4.8 (±0.4)</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>RT</td>
<td>7.1 (±1)</td>
<td>1.0 (±0.3)</td>
<td>7.3 (±0.7)</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>4.8 (±1)</td>
<td>2.6 (±0.4)</td>
<td>5.1 (±0.4)</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>6.3 (±0.9)</td>
<td>2.2 (±0.5)</td>
<td>3.8 (±0.3)</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.6 (±0.7)</td>
<td>5.6 (±0.3)</td>
<td>4.5 (±0.2)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>RT</td>
<td>6.3 (±2)</td>
<td>1.1 (±0.4)</td>
<td>7.3 (±0.9)</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>3.8 (±0.9)</td>
<td>3.2 (±0.3)</td>
<td>5.0 (±0.4)</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>5.4 (±0.8)</td>
<td>2.7 (±0.5)</td>
<td>3.8 (±0.3)</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>5.7 (±0.8)</td>
<td>6.5 (±0.4)</td>
<td>4.5 (±0.3)</td>
</tr>
</tbody>
</table>

Values in parenthesis are 95% confidence intervals.
elevated temperature (ET) conditions of all compounds, the C coefficient decreased relative to the RT condition by an average of 45 % more than that expected from the capacity factor decrease alone. For the EF mixture, the C coefficient decreased for all compounds relative to the RT conditions by 57 % more than that expected from the measured capacity factor decrease alone. However even though the diffusion coefficients are highest for the ET- EF mobile phase, the decrease in the C coefficient was approximately equal to that expected from the measured capacity factor decrease which indicates that another contribution that causes band broadening must have offset the expected lower C coefficient caused by the increased diffusion coefficients. One possible explanation may be that at higher temperatures and higher solvent strength conditions active sites may be more exposed and contribute to the band dispersion.

**Analysis Time**

The time of a separation is described by:

\[ t = \frac{NH (1 + k')}{u} \]  

(9)

where \( t \) is the elution time of the last eluting peak and \( N \) is the efficiency of the column. For a column of specified efficiency, the separation time can be reduced by minimizing the \( H/u \) ratio and the capacity factor. Table 18 shows \( H/u \) for pyrene, benz[a]anthracene, benzo[e]pyrene and benzo[a]pyrene at \( u_{opt} \) and \( u = 0.2 \) cm/sec for all four mobile phase mixtures. For both values of linear velocity, the \( H/u \) ratio and the \( k' \)
Table 18. Data showing variation in analysis time with mobile phase conditions.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mobile Phase Conditions</th>
<th>$k'$</th>
<th>$H_{\min}/u_{eq} \times 10^3$ (s)</th>
<th>$H/u \times 10^3$ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrene</td>
<td>RT</td>
<td>3.77</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>2.02</td>
<td>17</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>1.82</td>
<td>14</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>0.92</td>
<td>10</td>
<td>6.9</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>RT</td>
<td>5.94</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>2.68</td>
<td>16</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>2.42</td>
<td>14</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.07</td>
<td>11</td>
<td>6.7</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>RT</td>
<td>8.92</td>
<td>34</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>3.78</td>
<td>17</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>3.85</td>
<td>16</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.53</td>
<td>10</td>
<td>6.7</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>RT</td>
<td>11.19</td>
<td>31</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>ET</td>
<td>4.33</td>
<td>15</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>EF</td>
<td>4.75</td>
<td>14</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>ET-EF</td>
<td>1.70</td>
<td>9.4</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* $u = 0.20$ cm/s
values decrease substantially in the order of RT > ET > EF > ET-EF which will result in an associated decrease in separation time. By substituting the data in Table 18 into equation 9, at 0.2 cm/sec the separation time is expected to decrease approximately 5-fold between the RT and ET-EF conditions. The chromatograms in Figure 37 were taken at a linear velocity (0.1 cm/sec) which is intermediate between the $u_{opt}$ and $u = 0.2$ cm/sec, and a 5-fold decrease in separation time was observed from 42 minutes to 8 minutes under those conditions.

Figure 42 compares chromatograms for a test mixture for the analysis of polycyclic aromatic compounds on the US EPA target compound list using the RT, ET, EF and ET-EF mobile phases, respectively. The time of analysis decreases substantially by increasing the temperature of the mobile phase (ET) or by the addition of CO$_2$ to the mixture. The time of analysis is also similar for the elevated temperature chromatogram (ET) and the enhanced-fluidity chromatogram (EF). Figure 42 shows that the elevated temperature - enhanced fluidity solvent (ET-EF) provides the fastest analysis time but many of the compounds co-eluted. The ET-EF solvent mixture is thus too strong of a solvent for effective analysis of this PAH mixture.

**Pressure Drop**

Table 19 shows the pressure drop across the chromatographic system and the average number of theoretical plates for peaks 3-7 at the 4 mobile phase conditions defined previously. The data were obtained at approximately the same linear velocity for 1 column and 4 columns in series. The pressure drop across the column decreases
Figure 42. Chromatograms at 204 atm for different mobile phase conditions: (A) RT; (B) ET; (C) EF; and (D) ET-EF. (s) Solvent; (1) benzene; (2) naphthalene; (3) acenaphthylene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) benz[a]anthracene; (10) chrysene; (11) benz[bf]fluoranthene; (12) benzo[k]fluoranthene; (13) benzo[a]pyrene; (14) dibenzo[a,h]anthracene; (15) benzo[ghi]perylene; (16) indeno[1,2,3-cd]pyrene.
Table 19. Variation in column pressure drop and efficiency with mobile phase conditions and column length.

<table>
<thead>
<tr>
<th>One Column</th>
<th>Mobile Phase Conditions</th>
<th>Linear Velocity (cm/s)</th>
<th>δP (atm)</th>
<th>N_{3,7} (plates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>0.196</td>
<td>81.3</td>
<td>7,618</td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>0.204</td>
<td>49.2</td>
<td>8,880</td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>0.207</td>
<td>39.2</td>
<td>9,955</td>
<td></td>
</tr>
<tr>
<td>ET-EF</td>
<td>0.200</td>
<td>24.4</td>
<td>11,068</td>
<td></td>
</tr>
<tr>
<td>Four Columns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>0.189</td>
<td>314.2</td>
<td>30,290</td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>0.199</td>
<td>148.7</td>
<td>40,262</td>
<td></td>
</tr>
<tr>
<td>EF</td>
<td>0.189</td>
<td>144.8</td>
<td>44,124</td>
<td></td>
</tr>
<tr>
<td>ET-EF</td>
<td>0.182</td>
<td>81.9</td>
<td>47,948</td>
<td></td>
</tr>
</tbody>
</table>
in the following order of mobile phase conditions: methanol/H$_2$O at room temperature
> methanol/H$_2$O at elevated temperature > enhanced-fluidity mixture at room
temperature > enhanced-fluidity mixture at elevated temperature. Darcy's law\textsuperscript{7,8} describes the relationship between pressure drop, $\Delta P$, and solvent viscosity, $\eta$, in porous beds, such as chromatographic columns:

\[
\Delta P = \frac{\phi \eta \langle u \rangle L}{d_p^2}
\]

where $\phi$ is the dimensionless flow resistance parameter that typically has values in the range of 500–1000, $L$ is the length of the column, $\langle u \rangle$ is the average linear velocity, and $d_p$ is the particle diameter. This expression and the data in Table 19 show that by elevating the temperature of the methanol/H$_2$O mobile phase from 26 °C to 60 °C the pressure drop across the column due to decreased viscosity of the mobile phase decreases by ca. 40%. The pressure drop and viscosity are also reduced at 26 °C with the enhanced-fluidity mixture by ca. 50% and with enhanced-fluidity mixture at 60 °C by ca. 70% compared to the methanol/H$_2$O mobile phase at 26°C.

In Chapter III the inverse relationship between viscosity and diffusion was described. The trend of decreased pressure drop across the column in the order of room temperature (RT) > elevated temperature (ET) > enhanced fluidity (EF) > enhanced fluidity at elevated temperature conditions (ET-EF) correlates with the experimentally determined diffusion coefficients for benzene and anthracene at similar
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Figure 43. Chromatograms at 204 atm with approximately the same column pressure drop but with (A) the RT mobile phase and 1 column; and (B) the ET-EF mobile phase and 4 columns. (s) solvent; (1) benzene; (2) naphthalene; (3) anthracene; (4) pyrene; (5) benz[a]anthracene; (6) benzo[e]pyrene; (7) benzo[a]pyrene; (8) benzo[ghi]perylene.
the average number of theoretical plates for peaks 3-7 is more than 6 fold greater for the ET-EF separation than for the RT separation. The dramatic increase in theoretical plate number can be attributed to several factors. The 4-fold increase in column length accounts for ca. 64% of the total plate number for the ET-EF separation, leaving a 36% increase in plate number attributable to a combination of a shift in the optimum velocity to higher linear velocities, a decrease in the slope of the mass transfer region of the van Deemter curve, and decreased capacity factors (k'). Efficiency is also somewhat higher for 4 columns than 1 column probably due to decreased relative dead volume.

Figure 44 compares chromatograms of the SRM 1597 complex mixture of PAH isolated from coal tar separated at the same linear velocity with the EF mobile phase on 1 and on 4 columns. These chromatograms were truncated to illustrate the improvement in efficiency when using 4 columns in series compared to one. However, at the same linear velocity, the analysis time is 4 times as long with 4 columns. Figure 45 shows the SRM 1597 separation across 4 columns using the EF mobile phase at longer time. Tentative peak identifications for the chromatographic bands were assigned by injecting the Supelco test mix that contained 16 polyaromatic hydrocarbon compounds immediately after collecting the coal tar chromatogram and superimposing these chromatograms on one another. Perylene was assigned by injecting a perylene in methanol standard. The identifications were further supported by referring to an article
Figure 44. Chromatograms of SRM 1597 coal tar standard using the EF mobile phase at 204 atm with (A) 1 column and (B) 4 columns. (s) solvent; (1) naphthalene; (2) acenaphthalene; (3) fluorene; (4) phenanthrene; (5) anthracene; (6) fluoranthene; (7) pyrene; (8) benz[a]anthracene; (9) chrysene; (10) benzo[b]fluoranthene; (11) benzo[k]fluoranthene; (12) perylene; (13) benzo[a]pyrene.
Figure 45. Chromatograms of SRM 1597 coal tar standard using the EF mobile phase at 204 atm with 4 columns.
(s) solvent; (1) naphthalene; (2) acenaphthalene; (3) fluorene; (4) phenanthrene; (5) anthracene; (6) fluoranthene;
(7) pyrene; (8) benz[a]anthracene; (9) chrysene; (10) benzo[b]fluoranthene; (11) benzo[k]fluoranthene; (12) perylene;
(13) benzo[a]pyrene; (14) dibenz[a,h]anthracene; (15) indeno[123-c,d]pyrene; (16) benzo[g,h,i]perylene.
by Wise et al. and correlating our results with this extensive LC, GC, GC/MS analysis of the same sample.\textsuperscript{9}

CONCLUSIONS

Both the enhanced-fluidity mixtures and elevated column temperatures supplied improvements in HPLC chromatographic performance. Lower viscosity and decreased capacity factors are the prime factors causing the observed improvements. ET and EF conditions provided 1) a shift in $u_{eq}$ to higher linear velocities, 2) decreased C coefficients of the van Deemter curve, 3) decreased separation time and 4) lower pressure drops necessary to maintain a given mobile phase velocity compared to the room temperature RT conditions. When significant decreases in separation time are desired, heating the enhanced-fluidity (ET-EF) mixture is an option that provides high efficiency and should be considered. As is found in SFC, with these low viscosity mixtures, longer columns and/or coupled columns are viable means of increasing the theoretical plates available for a separation.\textsuperscript{10,11,12}
LIST OF REFERENCES

CHAPTER V

RETENTION VARIATION IN REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING ENHANCED-FLUIDITY MOBILE PHASES

INTRODUCTION

In reversed phase HPLC, the interaction of the solute with the mobile phase is the major factor governing retention. Solvophobic theory assumes that the mobile phase is highly ordered by the tendency of the polar molecules of the mobile phase to self associate and that the stationary phase is a uniform layer of covalently bound alkyl chains that do not differentiate between solute molecules. Several reviews of solvophobic theory are in the literature.\textsuperscript{1,2,3,4} According to the solvophobic model, retention is strongly controlled by the unfavorable interaction of the non polar portion of the solute with the surrounding polar (i.e. H\textsubscript{2}O) molecules in the mobile phase. This results in the release of free energy on the transfer of the solute from the polar mobile phase to the non polar alkyl chains of the stationary phase. The amount of free energy released is determined by the size of the solute molecule and the energy necessary to create a cavity for the solute within the ordered mobile phase. Consistent with this model the logarithm of the capacity factor (log k') has been shown to increase linearly with carbon number within solutes of a homologous series in reversed phase HPLC.
However, if the solutes possess polar functional groups the dipolar or hydrogen bonding interactions of these groups with the polar mobile phase will retard the transfer of the solute from the mobile phase.

Mobile phases with lower surface tension require less energy for cavity formation. The solvophobic model predicts that retention decreases for mobile phases with low surface tension. The surface tension of aqueous based mobile phases is often decreased by the addition of an organic component.

Based on the solvophobic theory, the most common method for varying the retention of neutral solutes in reversed phase HPLC is to change the organic solvent or vary the volume fraction of the organic component in a binary aqueous-organic mobile phase. In many studies a linear relationship between the logarithm of the capacity factor (log k') and the volume fraction of the organic component of the mobile phase, ϕ, was found.5,6,7,8,9,10

\[
\log k' = k_w + S\phi
\]  

(1)

where \( k_w \) is the capacity factor of the solute in a pure H₂O mobile phase, and S is a solute dependent factor related to the solvent strength of the organic solvent. Deviation from a linear relationship between log k' and ϕ been noted when the volume fraction of the organic component is less than 0.1-0.2 or greater than 0.8-0.9, depending on the organic solvent.3 Equation (2) has also been used to describe the variation in retention with aqueous-organic mobile phases.3,4,5,9,10,11,12
Equation 2 is applicable to a wider range of solvent compositions and aqueous-organic combinations than equation 1. However, even equation 2 does not adequately describe retention with eluents consisting of less than 0.1 volume fraction of organic solvent. Under these conditions the mobile phase exerts a hydrophobic effect on the stationary phase and the alkyl chains collapse to form a rigid surface that is poorly wetted by the mobile phase.3,13,14

Snyder et al. have argued that equation 1 can be accepted as a reliable first approximation for describing retention variation over a broad composition range with aqueous-organic mobile phases.9,15 They also showed that the S values determined from this relationship are valuable descriptors of the solvent strength of organic solvents.9,15 The S values determined from the regression of log $k'$ vs. $\phi$ are used to determine mobile phase compositions and to develop programs in gradient elution for methods development in reversed phase HPLC.4,9,15

Information about the variation in retention with the addition of CO$_2$ to the mobile phase is important for the use of enhanced fluidity mobile phases in reversed phase HPLC. Because liquid CO$_2$ is a compressible fluid it is more reasonable to express mobile phase mixtures containing liquid CO$_2$ in terms of mole fraction of the
components as has been done throughout this dissertation. Therefore, equations (1) and (2) were modified for our purposes as:

\[ \log k' = \log k_w + S \chi \]  

(3)

\[ \log k' = \log k_w + a \chi + b \chi^2 \]  

(4)

where \( \chi \) is the mole fraction of CO\(_2\) or organic component in the mobile phase.

In this chapter the variation in retention with the addition of CO\(_2\) to a methanol-H\(_2\)O mobile phase is compared to the variation in retention with a methanol-H\(_2\)O mobile phase where the mole fraction of methanol is varied. This comparison is made for three different solute sets of interest: selected polycyclic aromatic hydrocarbons, probucol and related compounds, and a vitamin test mixture.

Polycyclic aromatic hydrocarbons (PAH) rank as one of the largest groups of environmental chemical carcinogens.\(^{16}\) This ranking is a function of the potency of PAHs and their widespread distribution in the environment. PAHs have been found in air, H\(_2\)O, sediments, fossil fuels, tobacco smoke, and food. Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), and High Performance Liquid Chromatography (HPLC) have all been used extensively in the analysis of PAHs from environmental samples.\(^{17}\) The studies in this chapter and the examples in Chapter IV
demonstrate that enhanced-fluidity HPLC is also a viable technique for the analysis of polycyclic aromatic hydrocarbons.

Probucol is a fat-soluble antioxidant and has been shown to lower serum cholesterol concentrations in humans. Structural analogs of probucol have also been investigated for the same purpose. Santonin and Coutant developed GC and HPLC methods for the analysis of probucol and analogs. The HPLC method was preferred because probucol decomposition occurred by GC unless careful temperature control was used in the separation. The HPLC method of Santonin et al. was a reversed-phase separation using acetonitrile/hexane/0.1 M ammonium acetate.

High performance liquid chromatography has been the most commonly used technique for the analysis of fat soluble vitamins. Several in depth reviews on the chromatographic analysis of vitamins are available. HPLC in both the reversed-phase and normal-phase modes is used for the analysis of fat-soluble vitamins. Packed column and open tubular supercritical fluid chromatography were investigated for the analysis of fat soluble vitamins. Reversed-phase HPLC is frequently used when simultaneous determination of several different vitamins in one chromatographic run is desired. Normal phase HPLC and SFC have been unsuccessful resolving vitamins D₂ and D₃. Vitamins D₂ and D₃ have only been resolved with reversed phase conditions.

As demonstrated in Chapter IV by using enhanced-fluidity liquids as eluents increased speed of analysis or increased efficiency due to the increased diffusion rates in the mobile phase was achieved. Therefore, a separation of compounds that would
EXPERIMENTAL

The chromatographic system was the same as in Chapter IV. The mobile phase components, and experimental procedures were also the same and have been previously described in Chapter IV.

In this chapter four methanol/H$_2$O mixtures of 0.70/0.30, 0.80/0.20, 0.89/0.11 and three enhanced-fluidity mixtures, 0.63/0.27/0.10, 0.56/0.24/0.20, and 0.49/0.21/0.30 mole fraction methanol/H$_2$O/CO$_2$ were studied. Capacity factors were measured in triplicate for each of the three solute sets. Methanol was the injection solvent and was used as the nonretained marker with all mobile phase conditions except when 1.00/0.00 mole fraction methanol/H$_2$O was used as the mobile phase. When 1.00/0.00 mole fraction methanol/H$_2$O was used as the mobile phase a 95/5 v/v methanol/H$_2$O mixture was used as the injection solvent and produced a reliable nonretained marker with these conditions.

The primary polycyclic aromatic hydrocarbon test mixture was a Supelco test mix of 16 polyaromatic hydrocarbon compounds (catalog # 4-8905). This test mix was received in a methylene chloride/benzene solvent. This solvent was evaporated to dryness and replaced with methanol. The final concentration of each individual component of this test mix was 0.030 mg/ml. A methanol solution containing 0.71
μl/ml benzene, 0.079 mg/ml naphthalene, 0.021 mg/ml anthracene, 0.082 mg/ml pyrene, 0.036 mg/ml benz[a]anthracene, 0.071 mg/ml benzo[e]pyrene, 0.039 mg/ml benzo[a]pyrene, and 0.046 mg/ml benzo[ghi]perylene was also used.

The probucol and analog standard test mixture was a methanol solution containing 0.12 mg/ml compound 1, 0.084 mg/ml compound 2, 0.11 mg/ml probucol, 0.10 mg/ml compound 4, 0.096 mg/ml compound 5, and 0.11 mg/ml compound 6. Figure 46 shows the compound number and structures. The probucol and analog standards were a gift from Marion Merrell Dow, Inc. (Cincinnati, OH).

A standard test mixture of vitamins was a methanol solution containing 0.02 mg/ml trans retinol (vitamin A), 0.14 mg/ml butalated hydroxy toluene (BHT), 0.21 mg/ml trans retinal (vitamin A aldehyde), 0.15 mg/ml ergocalciferol (vitamin D$_2$), 0.16 mg/ml cholecalciferol (vitamin D$_3$), 0.17 mg/ml retinol acetate (vitamin A acetate), 0.13 mg/ml ±α-tocopherol (vitamin E), 0.10 mg/ml ±α-tocopherol acetate (vitamin E acetate), and 0.12 mg/ml vitamin K$_1$. Figure 47 shows the structures of trans retinol, trans retinal, retinol acetate, and vitamin K$_1$. Figure 48 shows the structures of ergocalciferol, cholecalciferol, ±α-tocopherol, and ±α-tocopherol acetate. All vitamin standards were purchased from Sigma Chemical Company (St. Louis, MO) with the exception of retinol which was purchased from Aldrich (Milwaukee, WI).
Figure 46. Number and structures of compounds in the probucol and analog standard.
Figure 47. Structures of trans retinal (vitamin A), trans retinol (vitamin A aldehyde), retinol acetate (vitamin A acetate), and vitamin K₁.
Figure 48. Structures of ergocalciferol (vitamin D$_2$), cholecalciferol (vitamin D$_3$), ±α-tocopherol (vitamin E), and ±α-tocopherol acetate (vitamin E acetate).
RESULTS

In this chapter the 0.70/0.30 mole fraction methanol/H₂O mobile phase mixture is used as the reference mobile phase condition. Retention variation is studied as CO₂ is added to this mixture from 0.00-0.30 mole fraction CO₂. This variation is compared to methanol/H₂O mobile phases where the mole fraction of methanol is varied by 0.30 mole fraction over the 0.70/0.30 - 1.00/0.00 mole fraction methanol/H₂O range.

Polycyclic Aromatic Hydrocarbons

Table 20 lists the experimentally determined capacity factors, k', for sixteen polycyclic aromatic hydrocarbons with four methanol/H₂O mobile phases covering the composition range of 0.70/0.30 - 1.00/0.00 mole fraction methanol/H₂O. As mentioned previously, log k' has been shown to increase linearly with carbon number within solutes of homologous series reversed phase HPLC. As an initial characterization of the major retention mechanism using methanol/H₂O and enhanced fluidity mobile phases in our experiments the log k' vs. the carbon number of the PAH solutes used in this study were plotted. Figure 49 shows that the log k' vs carbon number resulted in a linear relationship for both the methanol/H₂O and the enhanced fluidity mobile phase with this solute set. This is evidence that solute solvophobicity is the major factor governing retention in these systems.

Figure 50 shows a plot of the variation in log k' with mole fraction methanol in the mobile phase with linear fits to the experimental data. The S values resulting from the linear regressions of log k' vs. mole fraction (χ) methanol in the mobile phase are
Table 20. Experimentally determined capacity factors and S values for polycyclic aromatic hydrocarbons with methanol/H₂O mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mole Fraction Methanol/H₂O Mobile Phase</th>
<th>Capacity Factor (k')</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.70/0.30</td>
<td>0.80/0.20</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.47</td>
<td>0.32</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.99</td>
<td>0.59</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>1.21</td>
<td>0.70</td>
</tr>
<tr>
<td>Fluorene</td>
<td>2.05</td>
<td>1.12</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.21</td>
<td>1.18</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.55</td>
<td>1.33</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>3.29</td>
<td>1.68</td>
</tr>
<tr>
<td>Pyrene</td>
<td>3.80</td>
<td>1.95</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>6.04</td>
<td>2.74</td>
</tr>
<tr>
<td>Chrysene</td>
<td>6.04</td>
<td>2.81</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>8.92</td>
<td>4.11</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>9.64</td>
<td>4.22</td>
</tr>
</tbody>
</table>
Table 20. (continued)

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mole Fraction Methanol/H₂O Mobile Phase</th>
<th>Capacity Factor (k')</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.70/0.30</td>
<td>0.80/0.20</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>10.67</td>
<td>4.54</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>11.78</td>
<td>5.17</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>16.64</td>
<td>6.47</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>18.06</td>
<td>7.77</td>
</tr>
<tr>
<td>Indeno[123-c,d]pyrene</td>
<td>19.43</td>
<td>7.99</td>
</tr>
</tbody>
</table>
Figure 49. Variation in log k' with PAH carbon number for 0.70/0.30 mole fraction methanol/H₂O (✦) and 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ (▲) mobile phases. The lines are linear fits through the experimental data.
Figure 50. Variation in log k' with mole fraction methanol in the mobile phase for anthracene (●), pyrene (▲), chrysene (○), benzo[b]fluoranthene (▼), benzo[e]pyrene (♦), and benzo[g,h,i]perylene (■). The lines are linear fits through the experimental data.
also listed in Table 20. Linear fits to the experimental data resulted in an average \( r^2 \) value of 0.9960 while fits to the quadratic equation (equation 4) resulted in an average \( r^2 \) of 0.9993.

Chen et al reported \( S \) values for several of the PAHs used in this study on six different \( C_{18} \) columns with methanol water eluents. The \( S \) values ranged from 2.68 - 2.84 for benzene, 3.57 - 3.83 for naphthalene, 4.40 - 4.69 for phenanthrene, 4.49 - 4.61 for anthracene, and 5.20 - 5.28 for chrysene. S values for the same solutes from this study based on variation of volume fraction on methanol were 3.03 for benzene, 3.62 for naphthalene, 4.25 for phenanthrene, 4.36 for anthracene and 4.97 for chrysene. Comparison of the \( S \) values between the two studies is good with the general trend of increasing \( S \) with solute size.

Table 21 lists the experimentally determined capacity factors \( (k') \) for the same sixteen PAHs with a methanol/H\(_2\)O mobile phase of 0.70/30 mole fraction or 2.3/1.0 mole ratio where the mole fraction of CO\(_2\) in the mobile phase is varied from 0.00 - 0.30 mole fraction. Figure 51 shows a plot of the variation in log \( k' \) with mole fraction CO\(_2\) in the mobile phase with linear fits to the experimental data. The \( S \) values resulting from the linear regressions of log \( k' \) vs. mole fraction \((\chi)\) CO\(_2\) in the mobile phase are also listed in Table 21. Linear fits to the experimental data resulted in an average \( r^2 \) value of 0.9567 while fits to the quadratic equation (equation 4) resulted in an average \( r^2 \) of 0.9998.
Table 21. Experimentally determined capacity factors and S values for polycyclic aromatic hydrocarbons with methanol/H₂O/CO₂ mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>0.70/0.30/0.00</th>
<th>0.63/0.27/0.10</th>
<th>0.56/0.24/0.20</th>
<th>0.49/0.21/0.30</th>
<th>S Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>0.47</td>
<td>0.43</td>
<td>0.41</td>
<td>0.46</td>
<td>0.07</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.99</td>
<td>0.79</td>
<td>0.69</td>
<td>0.67</td>
<td>0.57</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>1.21</td>
<td>0.94</td>
<td>0.80</td>
<td>0.75</td>
<td>0.68</td>
</tr>
<tr>
<td>Fluorene</td>
<td>2.05</td>
<td>1.47</td>
<td>1.16</td>
<td>1.04</td>
<td>0.99</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.21</td>
<td>1.58</td>
<td>1.27</td>
<td>1.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.55</td>
<td>1.77</td>
<td>1.40</td>
<td>1.27</td>
<td>1.01</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>3.29</td>
<td>2.22</td>
<td>1.74</td>
<td>1.57</td>
<td>1.07</td>
</tr>
<tr>
<td>Pyrene</td>
<td>3.80</td>
<td>2.57</td>
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<td>1.85</td>
<td>1.04</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>6.04</td>
<td>3.68</td>
<td>2.77</td>
<td>2.46</td>
<td>1.29</td>
</tr>
<tr>
<td>Chrysene</td>
<td>6.04</td>
<td>3.74</td>
<td>2.84</td>
<td>2.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Benzo[e]pyrene</td>
<td>8.92</td>
<td>5.48</td>
<td>4.26</td>
<td>3.85</td>
<td>1.20</td>
</tr>
<tr>
<td>Benzo[h]fluoranthene</td>
<td>9.64</td>
<td>5.62</td>
<td>4.18</td>
<td>3.76</td>
<td>1.36</td>
</tr>
</tbody>
</table>
Table 21. (continued)

<table>
<thead>
<tr>
<th>Solute</th>
<th>Capacity Factor (k')</th>
<th>Mole Fraction Methanol/H₂O/CO₂ Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.70/0.30/0.00</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>10.67</td>
<td>6.04</td>
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<td>Benzo[a]pyrene</td>
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<td>6.87</td>
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<td>Dibenzo[a,h]anthracene</td>
<td>16.64</td>
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<td>Benzo[g,h,i]perylene</td>
<td>18.06</td>
<td>10.48</td>
</tr>
<tr>
<td>Indeno[123-c,d]pyrene</td>
<td>19.43</td>
<td>10.58</td>
</tr>
</tbody>
</table>
Figure 51. Variation in log k' with mole fraction CO₂ in the mobile phase for anthracene (●), pyrene (▲), chrysene (○), benzo[b]fluoranthene (▼), benzo[e]pyrene (♦), and benzo[g,h,i]perylenne (■). The lines are linear fits through the experimental data.
Figure 52 is a chromatogram for the analysis of polycyclic aromatic compounds on the US EPA target compound list using the 0.49/0.21/0.30 methanol/H$_2$O/CO$_2$ enhanced fluidity mobile phase. An interesting comparison can be made with Figure 52. This isocratic separation with the enhanced-fluidity mobile phase is just as efficient and has similar total analysis times to many gradient-elution separations of the same test mix.\textsuperscript{28} The resolution and efficiency of this separation is attributed to the selectivity and increased solute diffusion provided by the enhanced-fluidity mobile phase.

**Probucol and Related Compounds**

Table 22 lists the experimentally determined capacity factors, $k'$, for six compounds in the probucol and related compound standard with four methanol/H$_2$O mobile phases covering the composition range of 0.70/0.30 - 1.00/0.00 mole fraction methanol/H$_2$O. Figure 53 shows a plot of the variation in log $k'$ with mole fraction methanol in the mobile phase with linear fits to the experimental data. The $S$ values resulting from the linear regressions of log $k'$ vs. mole fraction ($\chi$) methanol in the mobile phase are also listed in Table 22. Linear fits to the experimental data resulted in an average $r^2$ value of 0.9990 while fits to the quadratic equation (equation 4) resulted in an average $r^2$ of $>0.9999$.

Table 23 lists the experimentally determined capacity factors for the same six compounds with a methanol/H$_2$O mobile phase of 0.70/30 mole fraction or 2.3/1.0 mole ratio where the mole fraction of CO$_2$ in the mobile phase is varied from
Figure 52. Chromatogram at 204 atm with 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ mobile phase. (s) solvent; (1) benzene; (2) naphthalene; (3) acenaphthalene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluorene; (8) pyrene; (9) benz[a]anthracene; (10) chrysene; (11) benzo[b]fluoranthene; (12) benzo[k] fluoranthene; (13) benzo[a]pyrene; (14) dibenzo[a,h]anthracene; (15) indeno[123-c,d]pyrene; (16) benzo[g,h,i]perylene.
Table 22. Experimentally determined capacity factors and S values for probucol and related compounds with methanol/H$_2$O mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Mole Fraction Methanol/H$_2$O Mobile Phase</th>
<th>Capacity Factor ( $k'$ )</th>
<th>S Value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>0.70/0.30</td>
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<td></td>
</tr>
<tr>
<td>1</td>
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<td>9.38</td>
<td>4.89</td>
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<td>2</td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td>14.76</td>
<td>4.98</td>
</tr>
<tr>
<td>4 Probucol</td>
<td></td>
<td>18.86</td>
<td>5.20</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>33.21</td>
<td>5.52</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>6.38</td>
<td>2.15</td>
</tr>
</tbody>
</table>
Figure 53. Variation in log k' with mole fraction methanol in the mobile phase for compound 1 (•), 2 (▲), 3 (●), 4 probucol (▼), and 5 (♦). The lines are linear fits through the experimental data.
Table 23. Experimentally determined capacity factors and S values for probucol and related compounds with methanol/H₂O/CO₂ mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>0.70/0.30/0.00</th>
<th>0.63/0.27/0.10</th>
<th>0.56/0.24/0.20</th>
<th>0.49/0.21/0.30</th>
<th>S Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.38</td>
<td>3.50</td>
<td>1.54</td>
<td>0.80</td>
<td>3.57</td>
</tr>
<tr>
<td>2</td>
<td>13.15</td>
<td>5.73</td>
<td>2.77</td>
<td>1.60</td>
<td>3.06</td>
</tr>
<tr>
<td>3</td>
<td>14.76</td>
<td>5.56</td>
<td>2.43</td>
<td>1.29</td>
<td>3.53</td>
</tr>
<tr>
<td>4 Probucol</td>
<td>18.86</td>
<td>6.52</td>
<td>2.70</td>
<td>1.37</td>
<td>3.79</td>
</tr>
<tr>
<td>5</td>
<td>33.21</td>
<td>10.09</td>
<td>3.76</td>
<td>1.78</td>
<td>4.24</td>
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<td>6</td>
<td>8.13</td>
<td>3.75</td>
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</tbody>
</table>
0.00 - 0.30 mole fraction. Figure 54 shows a plot of the variation in log $k'$ with mole fraction $\text{CO}_2$ in the mobile phase with linear fits to the experimental data. The $S$ values resulting from the linear regressions of log $k'$ vs. mole fraction ($\chi$) $\text{CO}_2$ in the mobile phase are also listed in Table 23. Linear fits to the experimental data resulted in an average $r^2$ value of 0.9955 while fits to the quadratic equation (equation 4) resulted in an average $r^2$ of $>0.9999$.

Figure 55 is a separation of probucol and 5 structurally related compounds using the 0.49/0.21/0.30 methanol/H$_2$O/CO$_2$ enhanced fluidity mobile phase. Figure 46 shows the structures of probucol and analogs. Baseline resolution was achieved for all compounds with the exception of compounds 2 and 3 (see Table 20) in under 11 minutes.

**Vitamins**

Table 24 lists the experimentally determined capacity factors for eight compounds in the vitamin test mixture with three methanol/H$_2$O mobile phases covering the composition range of 0.80/0.20 - 1.00/0.00 mole fraction methanol/H$_2$O. Figure 56 shows a plot of the variation in log $k'$ with mole fraction methanol in the mobile phase with linear fits to the experimental data. The $S$ values resulting from the linear regressions of log $k'$ vs. mole fraction ($\chi$) methanol in the mobile phase are also listed in Table 24. The average $r^2$ values when the experimental data were fit to the linear equation were 0.9921. Data from the vitamin test mixture were not fit to the quadratic equation because only three data points were available for each fit.
Figure 54. Variation in log $k'$ with mole fraction CO$_2$ in the mobile phase for compound 1 (♦), 2 (▲), 3 (●), 4 probucol (▼), and 5 (◇). The lines are linear fits through the experimental data.
Figure 55. Chromatogram at 204 atm with 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ mobile phase. (s) solvent; (1) compound 1; (2) compound 3; (3) compound 4; (4) compound 2; (5) compound 5; (6) compound 6.
Table 24. Experimentally determined capacity factors and S values for compounds in the vitamin test mixture with methanol/H₂O mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Capacity Factor (k')</th>
<th>Mole Fraction Methanol/H₂O Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butalated Hydroxy Toluene (BHT)</td>
<td></td>
<td>1.27/0.89/0.20</td>
</tr>
<tr>
<td>Trans Retinal (Vitamin A Aldehyde)</td>
<td></td>
<td>5.25/2.59/0.89</td>
</tr>
<tr>
<td>Retinol Acetate (Vitamin A Acetate)</td>
<td></td>
<td>5.52/2.74/0.89</td>
</tr>
<tr>
<td>Ergocalciferol (Vitamin D₂)</td>
<td></td>
<td>17.30/7.39/0.89</td>
</tr>
<tr>
<td>Cholecalciferol (Vitamin D₃)</td>
<td></td>
<td>18.71/7.98/0.89</td>
</tr>
<tr>
<td>±α-Tocopherol (Vitamin E)</td>
<td></td>
<td>28.14/11.43/0.89</td>
</tr>
<tr>
<td>±α-Tocopherol Acetate (Vitamin E Acetate)</td>
<td></td>
<td>12.57/3.67/0.89</td>
</tr>
<tr>
<td>Vitamin K₁</td>
<td></td>
<td>17.88/5.34/0.89</td>
</tr>
</tbody>
</table>
Figure 56. Variation in log $k'$ with mole fraction methanol in the mobile phase for BHT ($\oplus$), trans retinal ($\Delta$), retinol acetate ($\bullet$), ergocalciferol ($\nabla$), cholecalciferol ($\blacklozenge$), and $\pm\alpha$-tocopherol ($\blacksquare$). The lines are linear fits through the experimental data.
Table 25 lists the experimentally determined capacity factors \( k' \) for the same eight compounds with a methanol/H\textsubscript{2}O mobile phase of 2.3/1.0 mole ratio where the mole fraction of CO\textsubscript{2} in the mobile phase is varied from 0.10 - 0.30 mole fraction. Figure 57 shows a plot of the variation in log \( k' \) with mole fraction CO\textsubscript{2} in the mobile phase with linear fits to the experimental data. The S values resulting from the linear regressions of log \( k' \) vs. mole fraction (\( \chi \)) CO\textsubscript{2} in the mobile phase are also listed in Table 25. The average \( r^2 \) values when the experimental data were fit to the linear equation were 0.9976.

Figure 58 is a separation of fat soluble vitamins with the 0.49/0.21/0.30 methanol/H\textsubscript{2}O/C0\textsubscript{2} enhanced fluidity mobile phase. Baseline resolution for most compounds is achieved in under 20 minutes. Partial resolution of vitamin D\textsubscript{2} and D\textsubscript{3} is also achieved, this is attributed to the separation taking place under reversed-phase conditions.

**DISCUSSION**

**Deviation from Linearity**

The linear relationship between log \( k' \) and \( \Phi \) provided an adequate description of the variation in retention with addition of methanol for the solutes in the PAH, probucol and vitamin test mixes. The linear relationship also described the variation in retention with addition of CO\textsubscript{2} to the methanol/H\textsubscript{2}O mobile phase for the solutes in the probucol and vitamin test mixes. The linear relationship does not describe well the
Table 25. Experimentally determined capacity factors and S values for compounds in the vitamin test mixture with methanol/H₂O/CO₂ mobile phases.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Capacity Factor (k')</th>
<th>Mole Fraction Methanol/H₂O/CO₂ Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.63/0.27/0.10</td>
</tr>
<tr>
<td>Butalated Hydroxy Toluene (BHT)</td>
<td>1.73</td>
<td>1.16</td>
</tr>
<tr>
<td>Trans Retinal (Vitamin A Aldehyde)</td>
<td>3.69</td>
<td>2.00</td>
</tr>
<tr>
<td>Retinol Acetate (Vitamin A Acetate)</td>
<td>7.22</td>
<td>3.63</td>
</tr>
<tr>
<td>Ergocalciferol (Vitamin D₂)</td>
<td>17.58</td>
<td>5.35</td>
</tr>
<tr>
<td>Cholecalciferol (Vitamin D₃)</td>
<td>18.76</td>
<td>5.66</td>
</tr>
<tr>
<td>±α-Tocopherol (Vitamin E)</td>
<td>28.72</td>
<td>7.75</td>
</tr>
<tr>
<td>±α-Tocopherol Acetate (Vitamin E Acetate)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin K₁</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 57. Variation in log k' with mole fraction CO₂ in the mobile phase for BHT (♦), trans retinal (▲), retinol acetate (●), ergocalciferol (▼), cholecalciferol (♣), and ±α-tocopherol (■). The lines are linear fits through the experimental data.
Figure 58. Chromatogram at 204 atm with 0.49/0.21/0.30 mole fraction methanol/H₂O/CO₂ mobile phase. (s) solvent; (1) trans-retinol; (2) BHT; (3) trans-retinal; (4) ergocalciferol; (5) cholecalciferol; (6) retinol acetate; (7) ±α-tocopherol; (8) ±α-tocopherol acetate; (9) vitamin K₁.
variation in retention with addition of CO₂ to the mobile phase for solutes in the PAH
test mix. Linear fits of the variation of log k' with CO₂ for the PAH compounds
resulted in a significantly lower average correlation coefficient, $r^2 = 0.9567$, than linear
fits to any of the other data. All other linear fits to the variation in log k' with methanol
and with CO₂ for compounds in the PAH, probucol, vitamin and standard resulted in
average $r^2$ values of $\geq 0.9921$.

As mentioned previously, most agree that deviations from linearity at less than
0.1 volume fraction of organic component is due to the collapse of the alkyl chains of
the stationary phase as the mobile phase exerts a hydrophobic effect on the stationary
phase as mentioned previously. ³, ¹⁴, ¹³

The experiments in described in this chapter were performed with high volume
fractions (0.86 - 1.00) of organic components. At low H₂O volume fractions in the
mobile phase departures from linearity have been often explained on the basis of "dual"
retention mechanisms due to competitive silanophilic interactions with the solutes.⁵,³

The dual retention mehanism based on silanophilic interactions with the solutes
as an explanation for the non linearity in this data is unlikely for several reasons. In the
methanol/H₂O/CO₂ mobile phase mixtures, H₂O is always present in the mobile phase
and would be expected to hydrate the silanol groups and prevent solute-silanol
interactions. However, it is the linear fits of the variation of log k' with CO₂ for the
PAH compounds that deviate from linearity. In addition, the solutes in the probucol
and vitamin test mixes have hydroxyl, ketone, and ester substituent groups. These
solute should more readily participate in silanophilic interactions than the PAH compounds. If the retention mechanism was strongly influenced by solute-silanol interactions, the variation in log k' with mobile phase conditions for the probucol and vitamins solutes would be expected to deviate most significantly from linearity. This is was not observed in this study.

A previous study shows retention data for three PAH solutes (naphthalene, phenanthrene, and pyrene) that varies similarly to this data with the addition of CO₂ to the methanol/H₂O mobile phase. In the same work solvochromatic studies with the same methanol/H₂O/CO₂ mixtures used in this study revealed that the addition of CO₂ to a methanol/H₂O mobile phase changed the methanol/water solvent structure substantially. Over the 0.10 - 0.30 mole fraction range the solvent the dipolarity/polarizability (π*) and hydrogen bond acidity (α) decreased by approximately 10% while the hydrogen bond basicity (β) of the solvent increased markedly. It was concluded that monomeric methanol was being released from the methanol-H₂O hydrogen bond association to cause the observed increase in basicity.

In addition, the same study also suggested that the addition of CO₂ to the methanol/H₂O mobile phase alters the C₁₈ stationary phase. NMR studies have demonstrated that C₁₈ chains are highly hindered with methanol/water mobile phases. It was proposed that a methanol-H₂O hydrogen bond surface layer sheathes the C₁₈ stationary phase causing the C₁₈ chains to be highly hindered and that the addition of CO₂ changes the structure of the C₁₈ stationary phase by breaking the hydrogen bond
surface layer structure. Other studies have shown that differences in the structure of 
C\textsubscript{18} stationary phases dramatically affects the retention of PAH solutes\textsuperscript{32,33}

The limited data in this chapter and lack of previous information on the trends in retention of solutes with methanol/H\textsubscript{2}O/CO\textsubscript{2} mobile phases limits conclusions that can be drawn. However, in light of the previous information on the change of mobile phase and stationary phase structure with CO\textsubscript{2} addition, it is suggested that the change in stationary phase structure with CO\textsubscript{2} is the major factor causing the nonlinear variation in log k\textsuperscript{1} with addition of CO\textsubscript{2} to the mobile phase for the PAH solutes.

\textbf{S as a Function of Solute Structure}

When methanol is varied by volume fraction, the S value of methanol has been reported to be in the range of 2 - 4\textsuperscript{15}. However, studies have shown the S value to increase with solute size within a homologous series by \textasciitilde 0.40 per aliphatic carbon unit and by \textasciitilde 0.10 - 0.12 per aromatic carbon unit\textsuperscript{9,10,15}. In our methanol/H\textsubscript{2}O experiments, when the S value was calculated using volume fraction of methanol, the S value was 3.03 and 3.62 for benzene and naphthalene, respectively, and the S values continue to increase linearly with solute size by \textasciitilde 0.16 per aromatic carbon unit. It has also been noted that S values increase with increased solute retention\textsuperscript{34}. In general, this is also the case with the S values determined in this study with the variation in log k\textsuperscript{1} with methanol and CO\textsubscript{2} experiments. However, these two conclusions can not be taken independent of each other. As discussed in the introduction and shown in Figure 49
there is linear correlation between the size of the solute and retention in reversed phase HPLC.

In the experiments in this study it has been observed that solutes of similar structure resulted in similar S values for both the variation in log k' with methanol and with CO₂. In the PAH test mixture benz[a]anthracene and chrysene are structurally similar and also have similar S values of 2.70 and 2.64, respectively, when the mole fraction of methanol is varied and S values of 1.29 and 1.23 when the mole fraction of CO₂ in the mobile phase is varied. Other examples of similar S values for PAH solutes with similar structures are benzo[b]fluoranthene and benzo[k]fluoranthene that have S values of 2.80 and 2.85, respectively, with variation in methanol and 1.36 and 1.41 when the mole fraction of CO₂ in the mobile phase is varied. In the vitamin test mixture ergocalciferol (D₂) and cholecalciferol (D₃) are structurally similar and have S values of 4.49 and 4.46, respectively with variation in methanol, and 4.88 and 4.92 with variation in CO₂. It is not surprising that molecules that molecules with similar structures would have similar S values since the free energy necessary for cavity of formation within the mobile phase should be the similar for solutes with similar structures.

An interesting observation in the S values determined by variation in methanol and CO₂ and also observed by others is that "compact" solutes have smaller S values than "extended" solutes.²⁴,²⁵ Within the PAH sample, several examples of this phenomema exist among structural isomers. For example, pyrene would be considered
a compact solute and its S values are smaller than the S values for extended solutes benz[a]anthracene and chrysene. The structural isomers benzo[e]pyrene, benzo[a]pyrene, and dibenzo[a,h]pyrene would be considered compact, less compact, and extended, respectively, the trend S values increases in the same order. While this trend is convincing, extended solutes are most often retained longer than their compact isomers in reversed-phase HPLC. There are however, examples of extended solutes that are less retained and that have larger S values than more retained compact solutes. For example, dibenzo[a,h]anthracene would be considered an extended solute and it is less retained than either benzo[g,h,i]perylene, and indeno[123-cd]pyrene (more compact solutes of higher molecular weight) however, the S value of dibenzo[a,h]anthracene is considerably larger than either benzo[g,h,i]perylene, and indeno[123-cd]pyrene.

Taking in to consideration the retention, structures, and S values for all solutes in this study the major factor determining the magnitude of the S values for variation in methanol and variation in CO₂ is solute retention. Both increasing solute size and extended solute structure (with a few exceptions discussed previously) affect the magnitude of the S values by increasing retention.

**S Values and Methods Development**

S values are used to describe solvent strength and are used to determine gradient elution programs in methods development.⁴,⁹,¹⁵ When comparing the S values of solutes derived from the variation in log k' with mole fraction methanol to the S
values of solutes derived from the variation in log k' with addition CO₂ to a 2.3/1.0 mole ratio methanol/H₂O mixture, the S values are significantly greater with variation in log k' with mole fraction methanol for solutes in both the PAH and probucol test mixtures. Retention is affected more dramatically by the variation of the mole fraction of methanol in the mobile phase than by the addition of CO₂. This means that in developing a gradient elution method for these solute sets a much steeper gradient would need to be employed to elute all the solutes within the same window of time when using CO₂ as the modifier than when using methanol. However, with the vitamin test mix the S values for ergocalciferol, cholecalciferol, and ±α-tocopherol are similar with the variation of mole fraction of methanol and with CO₂.

As discussed previously, different solutes have different S values. The size and the shape of the molecule are two factors that determine S values. When employing a gradient to resolve the bands of two closely eluting solutes it is important to know the relative difference in the S, values of two adjacent bands. The relative difference is the difference in the S values divided by their average, ΔS/ΔS. ΔS/ΔS is directly related to the resolution of adjacent bands. In evaluating different mobile phase gradient methods for the resolution of two closely eluting bands, the mobile phase system with the greatest ΔS/ΔS would be preferred. For example, the benz[a]anthracene and chrysene bands are difficult to resolve, ΔS/ΔS for the methanol/H₂O system is 0.022 where ΔS/ΔS for the addition of CO₂ to the methanol/H₂O mobile phase is 0.048. Another example of two solute bands that are difficult resolve is ergocalciferol (vitamin D₂) and
cholecalciferol (vitamin D₃), the ΔS/ΔS for the methanol/H₂O system is 0.0067 where ΔS/ΔS for the addition of CO₂ to the methanol/H₂O mobile phase is 0.0082. In these cases the methanol/H₂O/CO₂ mobile phase would be the mobile phase system of choice to resolve these two solutes.

**CONCLUSIONS**

Enhanced fluidity mobile phases can be used in reversed phase HPLC to separate different compounds of interest. The linear relationship of variation log k' with mole fraction CO₂ described the retention for the solutes in the probucol and vitamin test mixes. The retention of the PAH solutes is not adequately described by the variation in log k' with mole fraction CO₂. It is suggested that differences in stationary phase structure may account for the non linearity observed for the variation in log k' with mole fraction CO₂.

The S values in this study were found to be increase with the size, the length, and the retention of the solute. These factors cannot be taken independently. Solutes of higher molecular weight and “extended” solutes also generally increase the retention in reversed phase HPLC. However, an example of an extended, less retained solute that has a higher S value than more retained solutes was described.

Finally the implications of S values in gradient elution HPLC were described. Higher resolution of sets of closely eluting bands of interest is predicted in specific
examples when using methanol/H₂O/CO₂ mobile phases in gradient elution reversed phase HPLC.
LIST OF REFERENCES


34. Snyder, L. R.; Quarry, M. A.; Glajch, J. L. Chromatographia 1987, 24, 33.
CHAPTER VI

NORMAL PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING ENHANCED-FLUIDITY LIQUID MOBILE/phases

INTRODUCTION

In this chapter initial studies of enhanced-fluidity mobile phases in normal-phase HPLC with a hexane/CO₂ mobile phase is described. Mobile phases in normal phase chromatography are typically mixtures of a nonpolar solvent, such as hexane, and one or more polar solvents. The polar solvents are added to control the selectivity of the separation. The addition of CO₂ to normal phase mobile phases should be viewed as a fluidity modifier. Especially for the separation of polar solutes, the addition of CO₂ will not markedly affect the selectivity of the separation. As a benchmark characterization, an evaluation of the hexane/CO₂ mixture with the understanding that more polar modifiers must be added to this mixture for the separation of polar solutes. An attractive feature of the use of liquid CO₂ as a co-solvent in normal phase HPLC is that when CO₂ is included in mixtures it functions as a homogenizing agent and often allows complete miscibility of solvent pair with substantially different polarity.¹ In addition, the disposal cost of many nonpolar solvents is an increasing concern. The use of large
proportions of carbon dioxide as a mobile phase co-solvent will further reduce the use
and disposal of "environmentally unfriendly" solvents.

EXPERIMENTAL

Instrumentation

The chromatographic system consists of an ISCO LC-2600 syringe pump
(ISCO, Lincoln, NE), a Valco W-series high pressure injection valve with an injection
volume of 60 nL (Valco Instruments, Houston, TX), a Deltabond Cyano (3-
cyanopropyl polysiloxane stationary phase), 250 mm x 1 mm column packed with 5 μm
diameter particles containing 300 Å pores with stationary phase loading of ca. 4%
carbon (Keystone Scientific, Bellefonte, PA) and a Spectra-Physics UV2000 UV/vis
absorbance detector equipped with a capillary flow cell mounting (model 9550-0155).
A piece of 50 μm i.d. fused silica tubing (Polymicro Technologies, Phoenix AZ) was
connected to the end of the column with a zero dead volume fitting, a flow cell was
created by removing the polyimide coating from a 5 mm length of the tubing and
centering it in the capillary flow cell mounting. The detector excitation wavelength was
210 nm. A Setra 204 series pressure transducer (Setra Systems Inc. Acton MA) was
placed in-line after the detector and before post-detection restrictor. The outlet
pressure was monitored because the column pressure must be maintained above a
minimum pressure to prevent the hexane/CO₂ mobile phase mixture from separating
into two phases (liquid-gas). For example at 25 °C and 0.6 mole fraction of CO₂ the
hexane/CO₂ separates into two phases at pressures lower than 43 atm. Figure 59 is a hexane/CO₂ phase diagram that delineates the single liquid phase region at 25°C. All experiments in this study were performed under conditions in which the hexane/CO₂ mixture was in a single liquid phase. The flow control for the chromatographic system was maintained by an appropriate length of 8, 15, or 20 μm-i.d. fused silica tubing. The column inlet pressure was maintained at 136 atm throughout this study except when hexane was the mobile phase. When n-hexane was used as the mobile phase higher pressures were necessary to obtain linear velocities above 0.22 cm/sec. Data were collected on a strip chart recorder (Linear Instruments, Reno NV).

Column efficiency, N, was calculated manually via the method developed by Foley and Dorsey³ (equation 1):

\[
N = \frac{41.7 \left( \frac{t_r}{W_{0.1}} \right)^2}{(B/A + 1.25)}
\]  

(1)

Where \( t_r \) is retention time, \( W_{0.1} \) is the width of the chromatographic band at 10% of the peak's height and \( B/A \) is an empirical asymmetry factor. \( A \) and \( B \) are referenced to the peak maximum with \( A + B = W_{0.1} \). This calculation is based on the exponentially modified Gaussian model (equation 2):

\[
N = \frac{t_r^2}{(\sigma^2 + \tau^2)}
\]  

(2)
Figure 59. Hexane/CO₂ phase diagram at 25°C.²
where $\sigma$ is the standard deviation of the Gaussian function and $\tau$ is the time constant of the exponential. Two independent studies recently showed that Foley and Dorsey's method is the most accurate means to determine chromatographic column efficiency manually.\textsuperscript{5,6}

The solvent strength was characterized by measuring the Kamlet-Taft $\pi^*$ parameter for the hexane/CO$_2$ mixtures.\textsuperscript{7} To determine the $\pi^*$ parameter the solvatochromic shift of ortho-nitroanisole's UV/Vis spectrum was measured.\textsuperscript{8} A DMS-100 UV/Vis (Varian, Sunnyvale, CA) was used in these studies. The $\pi^*$ parameter was calculated using equation 3:\textsuperscript{7}

$$\pi^* = \frac{(v_{max} - v_o)}{s}$$  \hspace{1cm} (3)

where $v_{max}$ is the frequency of the absorbance maximum of o-nitroanisole, when dissolved in the solvent of interest; $v_o$ is the frequency of the absorbance maximum for the molecular probe in a reference solvent (typically cyclohexane), and $s$ is a proportionality constant that limits the values of $\pi^*$ to a range of 0 to 1 for common solvents. The literature value of $s$ for o-nitroanisole, -2.428 ± 0.195 kK, was used to calculate $\pi^*$ in these experiments.\textsuperscript{9} Cyclohexane was used as the reference solvent. The absorption spectrum was obtained using a home-made, stainless steel, high-pressure, optical flow cell with an internal volume of 10 mL and an optical pathlength of 3.5 cm. The optical path was terminated on each end with cylindrical quartz windows that were 1.125 in. dia. x 0.69 in thick (ESCO Prod. Inc., Oak Ridge, NJ).
The optical cell was sealed with Teflon o-rings. The concentration of o-nitroanisole used in these studies was approximately $1 \times 10^{-4}$M.

**Materials**

The test analytes used in this study were phenetole, methyl benzoate, nitrobenzene, and dimethyl phthalate. The concentration of analytes were 4830 ppm phenetole, 5470 ppm methyl benzoate, 5950 ppm dimethyl phthalate, and 3590 ppm nitrobenzene in pentane. Pentane was unretained. Supercritical fluid grade CO$_2$ from Scott Specialty Gases (Plumsteadville, PA) and 99+ % hexane from Aldrich Chemical Co. (Milwaukee, WI) were used as purchased. Hexane/CO$_2$ mixtures were prepared using two high pressure syringe pumps. A known volume of hexane was placed in one pump. Liquid CO$_2$ at 136 atm and 25 °C was held in another pump. Using the known density of CO$_2$ at these conditions the appropriate volume of CO$_2$ was calculated and then delivered to the pump holding the hexane to make a given hexane/CO$_2$ mixture. The mixture was then pressurized to 136 atm and allowed to equilibrate at 25 °C for at least 12 hours to ensure complete mixing of the solution.

**RESULTS AND DISCUSSION**

**Viscosity and Pressure Drop**

The viscosity of the mobile phase mixtures (Figure 60) was estimated using the estimation methods of Lucas, Teja and Rice, and Grunberg and Nissan.$^{10}$ These estimation methods accurately predict viscosities of non-aqueous mixtures at low
temperatures within a 5% error. The mixture viscosity decreases substantially with increasing proportions of CO₂. As expected when the viscosity of the mobile phase decreases, the pressure drop across the column also decreases for the same linear velocity. Table 26 shows the pressure drop measured across the system for comparable linear velocities with different mobile phase compositions. The pressure drop across the column decreased by a factor of approximately 6.5 when the mobile phase mixture was varied from 100 % n-hexane to 100 % CO₂.

**Diffusion Coefficients**

Various empirical expressions have been used to describe the relationship between the viscosity of a solvent and solute diffusivity. Equation 4 has proven to be the most accurate relationship for predicting diffusion coefficients from solvent viscosities for nonaqueous and mixed solvents over a wide range of temperatures and viscosities.⁹,¹¹

\[
D_m = A \eta^p
\]  

(4)

\(D_m\) is the diffusion coefficient of a given solute, \(\eta\) is the viscosity of the solvent and \(A\) and \(p\) are parameters that are characteristic of the solute. \(A\) and \(p\) can be readily determined from the radius of the solute.¹² Using Bondi radii¹³ for the studied solutes, the \(p\) parameter is approximately 0.9. Therefore from equation 4, the diffusion coefficients for the studied solutes are expected to vary approximately inversely with the viscosity of the mobile phase mixture (Figure 60).
Figure 60. Estimated viscosity of mobile phases at 25°C and 136 atm: (+) Grunberg and Nissan method; (●) Teja and Rice method.
Table 26. Variation in pressure drop across the chromatographic column with mobile phase conditions.

<table>
<thead>
<tr>
<th>Mole Fraction CO₂</th>
<th>Linear Velocity (cm/s)</th>
<th>Pressure Drop (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.121</td>
<td>61.0</td>
</tr>
<tr>
<td>0.33</td>
<td>0.118</td>
<td>53.3</td>
</tr>
<tr>
<td>0.50</td>
<td>0.121</td>
<td>41.3</td>
</tr>
<tr>
<td>0.70</td>
<td>0.121</td>
<td>15.1</td>
</tr>
<tr>
<td>1.00</td>
<td>0.116</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Retention

Figure 61 shows the variation in capacity factor with mobile phase composition. The capacity factors of nitrobenzene and dimethyl phthalate decreased with increasing proportions of liquid carbon dioxide added to the mobile phase. Minimum capacity factor values were reached at 0.70 mole fraction CO₂. Also as the mole fraction of CO₂ is increased the selectivity of the separation decreases. Figure 62A is a chromatogram of the test solutes with n-hexane as the mobile phase; while Figure 62B is a chromatogram of the test solutes with 0.50/0.50 mole fraction n-hexane/CO₂ as the mobile phase at the same linear velocity. This comparison illustrates the reduction in retention and selectivity with 0.50/0.50 mole fraction n-hexane/CO₂ mobile phase.

From the data in Figures 61 and 62, the addition of CO₂ to hexane clearly increases the mobile phase solvent strength. Retention of solutes is expected to decrease as the polarity of the solvent increases. The Kamlet-Taft π⁺ parameter was measured for the hexane/CO₂ mixtures. π⁺ is a measure of solvent polarizibility and dipolarity. Figure 63 shows that as more CO₂ is added to the mixture the π⁺ of the mixture also increases. Interestingly, the π⁺ parameter of the mixture begins to reach an asymptote near the 0.70 mole fraction CO₂ composition which is where the capacity factor levels off as well. Electron donor-acceptor complexing of CO₂ with unsaturated compounds, such as alkenes and benzene,¹⁴,¹⁵ is well documented. Therefore, another possible reason for the diminished retention with the addition of CO₂ is that similar
Figure 61. Variation in capacity factor ($k'$) with mobile phase composition: (▲) phenetole; (●) methyl benzoate; (■) nitrobenzene; (●) dimethyl phthalate. 95% confidence intervals are within the size of the marker. Lines are added as a guide to the eye.
Figure 62. Chromatograms at 136 atm and 25°C (A) with n-hexane as the mobile phase and (B) with 0.50 mole fraction CO₂ in hexane as the mobile phase. (1) phenetole; (2) methyl benzoate; (3) nitrobenzene; (4) dimethyl phthalate
Figure 63. Variation in Kamlet-Taft $\pi^*$ Parameter as a function of mobile phase composition. Error bars indicate 95% confidence intervals.
complexation with the cyano-functionality of the stationary phase might occur. More studies are required to understand the retention variation.

**Band Dispersion and Efficiency**

The non-coupled van Deemter equation shows the relationship between the column band dispersion or plate height, $H$, and linear velocity of the mobile phase, $u$.

$$H = A + \frac{B}{u} + Cu$$

(5)

$A$ and $B$ are measures of the band dispersion from multiple flow paths and longitudinal diffusion, respectively, and $C$ measures the summation of band dispersion caused by the combined resistance to mass transfer in the mobile phase, stagnant mobile phase that is present in the pores of the particles, and the stationary phase. In packed columns, $B = \gamma D_m$, where $\gamma$ is an obstruction factor and $D_m$ is the diffusion coefficient of the solute in the mobile phase. The resistance to mass transfer in the mobile and stagnant mobile phase in the particle pores can be expressed as $C_{m,sm} = f(k')/D_m$, where $k'$ is the capacity factor.

In most HPLC separations, the linear velocity used is such that the $C$-terms are the major cause of band dispersion in the column. Also, when microporous particles are used, the $C_{sm}$ term (due to dispersion in the stagnant mobile phase in the pores) often predominates.\(^6\) If the packing particles are spherical, the following expression describes the variables that control the $C_{sm}$ term:
where $\phi$ is the fraction of total mobile phase in the intraparticle space, $\gamma$ is the tortuosity factor. Since the diffusion coefficient of the solute is inversely related to solvent viscosity, from equation 6, the $C_{sm}$ term is expected to decrease with decreasing mobile phase viscosity. Over the limited range of viscosities possible with common liquid mobile phases in normal-phase liquid chromatography using bonded phases, this is clearly the experimentally-observed trend. \(^{17}\) Therefore, if diffusion in the stagnant mobile phase controls band dispersion in the present system, then from equation 6, the addition of CO$_2$ to hexane should cause decreased band dispersion due to increased solute diffusion coefficients as long as the capacity factors do not increase with the addition of CO$_2$.

Also when the first derivative of the van Deemter equation is taken with respect to $u$, set equal to zero, and the definitions of $B$ and $C$ are substituted, we obtain the following expression:

$$u_{opt} \propto \frac{D_m}{\sqrt{f(k')}}$$  \hspace{1cm} (7)
This relationship predicts that if diffusion is the rate-limiting step in the mass transfer process, then as the diffusion coefficient of a solute increases a corresponding shift in the optimum linear velocity should be occur when all other factors remain the same.

To evaluate the effect of mobile phase composition on band dispersion, the variation of plate height with linear velocity was determined for nitrobenzene and dimethyl phthalate for mobile phase compositions of 0.0, 0.33, 0.50, 0.70, and 1.0 mole fraction of CO₂ in n-hexane at 26 °C and 136 atm. Equation 1 was used to calculate the plate heights from the experimental peak shapes.

Figures 64 and 65 show the reduced plate height versus linear velocity (0.00 - 0.40 cm/s) for nitrobenzene and dimethyl phthalate, respectively. For both analytes, there is no observable shift in the optimum velocity as a function of added CO₂ to the mobile phase. For high linear velocities, the slope of the reduced plate height versus linear velocity plots for dimethyl phthalate decreased when CO₂ was added to hexane for the following mixtures: 0.00 mole fraction CO₂ > 0.33 mole fraction CO₂ > 0.50 mole fraction CO₂. For nitrobenzene, the slopes of these curves for the same mobile phase compositions were statistically the same. By comparing Figure 64 and Figure 65 and knowing that the capacity factor of dimethyl phthalate decreased significantly with increased CO₂ while that of nitrobenzene decreased minimally (see Figure 61), it is clear that the decrease in plate height with added CO₂ for dimethyl phthalate was primarily due to the capacity factor change not diffusion coefficient decrease. Also, interestingly, instead of seeing further enhancements in efficiency when the added CO₂ was greater
Figure 64. Variation of reduced plate height of nitrobenzene as a function of mobile phase linear velocity at 136 atm for different mobile phase compositions: (■) n-hexane; (●) 0.33 mole fraction CO₂ in hexane; (▲) 0.50 mole fraction CO₂ in hexane; 0.50 mole fraction CO₂ in hexane; (+) 0.70 mole fraction CO₂ in hexane; (♦) CO₂. Average % relative standard deviation of the data was 5%.
Figure 65. Variation of reduced plate height of dimethyl phthalate as a function of mobile phase linear velocity at 136 atm for different mobile phase compositions: (■) n-hexane; (●) 0.33 mole fraction CO₂ in hexane; (∆) 0.50 mole fraction CO₂ in hexane; 0.50 mole fraction CO₂ in hexane; (+) 0.70 mole fraction CO₂ in hexane; (♦) CO₂. Average % relative standard deviation of the data was 5%.
than 0.50 mole fraction, the reduced plate height dramatically increases for mobile phase compositions of 0.70 and 1.00 mole fraction CO₂ in n-hexane.

Figure 66 shows the variation in asymmetry factor, B/A, with mobile phase composition over the linear velocity range 0.00 - 0.12 cm/s. The observed trend in the plate height measurements correlates closely with the variation in peak asymmetry factor over the same mobile phase compositions. Peak tailing is lowest for nitrobenzene and dimethyl phthalate with mobile phase compositions of 0.33, 0.50 mole fraction CO₂ in n-hexane and increases markedly for 0.70 and 1.00 mole fraction of CO₂ in neat n-hexane. The asymmetry factor was also measured for the two solutes over the 0.16-0.24 cm/s linear velocity range. The shape of the B/A versus mole fraction CO₂ curve was the same as that observed in Figure 66 for the lower flow rate conditions. The major difference between the measured asymmetry factor for the two linear velocities ranges is that the magnitude of the asymmetry factor is greater for the higher linear velocity conditions. For example, the asymmetry factor for 0.70 mole fraction CO₂ in n-hexane at the higher linear velocities was 1.82 and 1.67 for nitrobenzene and dimethyl phthalate, respectively, which is substantially higher than the B/A values observed at the lower linear velocities. The asymmetry factor for the 0, 0.33, and 0.50 mole fractions of CO₂ in n-hexane mobile phase compositions did not vary substantially with linear velocity. The measured peak asymmetry did not vary with solute concentration. From a comparison of the band dispersion data in Figures 64 and 65 with the peak asymmetry data in Figure 66 and a careful look at the data used to
Figure 66. Variation in peak asymmetry (B/A) with mobile phase composition: (+) nitrobenzene; (■) dimethyl phthalate. Error bars indicate 95% confidence limits.
calculate plate heights, it is clear the peak asymmetry caused the variation in the measured plate height.

Many possible causes for the observed variations in plate height and peak asymmetry exist. The surfaces of chemically-bonded stationary phases are often heterogeneous. For moderately polar compounds, such as those used in this study, the 3-cyanopropylpolysiloxane bonded phases have been found to behave chromatographically like a deactivated silica gel column. Residual surface silanols were believed to control the retention substantially under those conditions. From the present data and our prior experience with using supercritical CO₂ as an eluent, we speculate that the addition of liquid CO₂ to the mobile phase initially lowers the asymmetry of the chromatographic band because it is more polar than n-hexane. However after more than 0.50 mole fraction CO₂ is added to hexane, the stationary phase may begin to expand slightly (as is often the case with supercritical CO₂) and then more surface silanols are exposed on the support which causes the increased band broadening with mixture composition of 0.7 and 1.0 mole fraction CO₂.

Because of the mixed retention mode (combined interaction of silanols and cyano groups with solutes) of the cyano phase, amines are often added to the mobile phase to complex with the surface silanol groups. The addition of a more polar modifier to the eluent should provide better capping of the surface silanols, then the expected increase in efficiency should be more readily apparent when CO₂ is added to the mixture.
CONCLUSIONS

In Chapter IV it was demonstrated that by using enhanced-fluidity liquid mixtures as eluents in reversed-phase separations the pressure drop across a chromatographic column was diminished. In addition, the efficiency and speed of separation increased with enhanced-fluidity mobile phases. This study was an initial step to analyze the attributes of enhanced-fluidity liquid mixtures for normal phase chromatography. The viscosity of the eluent was lowered by adding CO$_2$ to n-hexane which resulted in substantially a decreased pressure drop across the chromatographic column and increased solute diffusion in the eluent.

However, gains in efficiency were only observed for specific mixtures of hexane/CO$_2$. This trend correlates closely with the observed trend in peak asymmetry, where enhanced-fluidity mixtures of 0.33 and 0.50 mole fraction CO$_2$ in n-hexane provided chromatograms with the most symmetric peaks. Variations in solute interactions in the bulk mobile phase or the stationary phase when CO$_2$ was added probably caused changes in peak asymmetry and also controlled the measured efficiency. Resistance to mass transfer in the bulk mobile phase or in the stagnant mobile phase of the pores was not the controlling force that caused the chromatographic band shape for some of the hexane/CO$_2$ mixtures. Further studies are necessary to determine whether small proportions of polar cosolvent can stop the variation of peak asymmetry with added CO$_2$. Only then will the lowered solvent viscosity have a measurable effect on the chromatographic efficiency.
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