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CHARACTERIZATION AND CHROMATOGRAPHIC APPLICATIONS OF ENHANCED-FLUIDITY SOLVENTS

DISSertation

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

By

Isabelle Souvignet

*****

The Ohio State University

1996

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To Eliane,
ACKNOWLEDGMENTS

I would like to give a special thank to my advisor, Dr. Susan Olesik for the opportunity of working in her research group but also for her advices and kindness during my graduate school period.

I would like also to thank a lot of people that made my stay at Ohio State very enjoyable: the present and past members of Dr. Olesik's group and in particular Huimin Yuan and Dana Albaiu for their friendship and shopping advice!, the people of the Chemistry Department, especially Dr. Gustafson and Ruth Anderson and the french "connection" (Cyril Barrière, Hélène Péreira, Patrick Bernardelli and many more..). I would like to give an huge thank to my parents, grand-parents for their emotional supppport, to Vincent Leroy for his love and friendship and to Eliane for her encouragements.
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CHAPTER I

FUNDAMENTAL PROPERTIES OF THE MOBILE PHASE IN SEPARATION SCIENCE

INTRODUCTION

Properties of supercritical fluids. A supercritical fluid is defined as a fluid above its critical pressure ($P_c$) and temperature ($T_c$). As is shown in Figure 1, the critical point sets the minimum pressure and temperature beyond which the phase boundary between the liquid and the gas phases disappear. At conditions above the critical point, a single phase exists. The critical parameters vary widely upon the nature of the fluid. Polar compounds such as methanol, water or ammonia have high critical temperatures and pressures. The critical pressures and temperatures of some compounds are listed in Table 1.

In the supercritical state, the fluid is neither a gas nor a liquid. Its properties are intermediate between those of a liquid and a gas. The density ($\rho$), viscosity and diffusivities are among the properties that are of interest to the chromatographic or extraction science. Table 2 lists a comparison of the differences of the fluid properties between its different states. The viscosity of a supercritical fluid is about 100 times
Figure 1. Pressure-temperature phase diagram of a substance [1].
Table 1. Critical parameters of some commonly-used solvents [2].

<table>
<thead>
<tr>
<th>Fluid</th>
<th>$T_c$ (K)</th>
<th>$P_c$ (atm)</th>
<th>Dipole Moment (D)</th>
<th>$V_c$ (ml mol$^{-1}$)</th>
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<tr>
<td>CO$_2$</td>
<td>304.1</td>
<td>72.9</td>
<td>0</td>
<td>94.8</td>
</tr>
<tr>
<td>N$_2$O</td>
<td>309.6</td>
<td>72.5</td>
<td>0.17</td>
<td>97.8</td>
</tr>
<tr>
<td>NH$_3$</td>
<td>405.5</td>
<td>112.5</td>
<td>1.47</td>
<td>72.5</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>512.2</td>
<td>79.9</td>
<td>1.7</td>
<td>117.9</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>647.3</td>
<td>221.2</td>
<td>1.8</td>
<td>57.1</td>
</tr>
<tr>
<td>Fluid</td>
<td>Gas</td>
<td>Liquid</td>
<td>Supercritical Fluid</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>--------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Density (g cm(^{-3}))</td>
<td>$10^{-3}$</td>
<td>1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Diffusivity (cm(^2) s(^{-1}))</td>
<td>$10^{-1}$</td>
<td>$5 \times 10^{-6}$</td>
<td>$10^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Viscosity (g cm(^{-1}) s(^{-1}))</td>
<td>$10^{-4}$</td>
<td>$10^{-2}$</td>
<td>$10^{-4}$</td>
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greater than those of a liquid. Its density is between 0.1 to 0.8 of those of its liquid state.

The density of a fluid controls its solvent power. In the supercritical state, the fluid density can be easily manipulated through the pressure or the temperature. An increase in the density is achieved by an isothermal increase in the pressure. Under isobaric condition, an increase in the temperature leads to a decrease in the density. The change in pressure is the most commonly-used way to modify the fluid density in the supercritical state. Unfortunately, the low isothermal compressibility associated with the liquid state prevents any significant change of the density for reasonable changes in the pressure. As a consequence, the variation in the fluid density is usually achieved through a change in its compost in the liquid state. The shape of the isotherm of the curve of the density versus the pressure depends upon pressure and temperature of the supercritical fluid. In the vicinity of the critical point, the curve presents an horizontal tangent. Small variations in the pressure leads to significant variations in the fluid density. In the liquid or gas-like region (at high temperature) of the supercritical fluid, the curve is more linear. Unusual properties (solubility, partial molar volume) are usually associated with the supercritical state in the vicinity of its critical point. Substantial clustering of the solvent molecules around the solute was evidenced [4], [5]. The aggregate of the solvent molecules was held responsible for the large magnitude of the partial molar volume of the solutes as well as the large increase in the solubility. This region of the supercritical state is then worth studying in order to comprehend the solvation effects in supercritical fluids.

Despite the nice attributes that seem to derive from the use of the supercritical fluids, the pressure and temperature seem to be the only two factors available to tune the
properties of the fluid. The polarity and the density of the supercritical fluids commonly-used is not large enough to broaden the application of the supercritical fluids. Moreover, the polar fluids that could provide substantial solvent strength have high critical parameters [1]. The addition of an organic solvent to a supercritical fluid was thought to be a way to solve the problem. Unfortunately, there is a maximum amount of organic solvent that can be added to a supercritical fluid. This amount is limited by the mixture’s phase diagram and also by the critical parameters of the resulting supercritical fluid mixtures.

The ideal fluid mixture would be a fluid that would combine the positive features attributed to both the liquid and the supercritical phases.

The enhanced-fluidity liquid mixture: an alternative option to the use of liquids and supercritical fluids? An “enhanced-fluidity” liquid is a liquid mixture composed of an organic solvent with a liquefied gas. The organic solvent is usually an associated liquid such as an alcohol but could be any organic liquid (tetrahydrofuran). The liquefied gas is in most cases carbon dioxide (CO₂). CO₂ is extensively used because of its low cost, chemical inertness and ease of use. The organic solvent provides for the sufficient solvent strength whereas the liquefied gas is the key factor to tune the viscosity of the mixture. The difference between an enhanced-fluidity liquid and a supercritical fluid mixtures are of two kinds. In an enhanced-fluidity mixture, there is a larger proportion of the organic solvent than in usual supercritical fluid mixtures. The temperature and pressure required are milder than in the supercritical state since the mixture is in the liquid state. Our research group has been working on the application of
such enhanced-fluidity liquid mixtures.

Preliminary data show the broad range of application of such fluid mixtures. They have been used as mobile phases both in reversed and normal liquid chromatography [6], [7], [8]. It was shown that the addition of CO₂ to an organic liquid (methanol or hexane) or a mixture of organic liquid (methanol/water) leads to a lower plate height and decreased analysis time. The enhanced-fluidity liquid mixtures have also shown their application as extraction fluids for the extraction of phenols and nitroaromatic compounds from ODS (C18) matrices [9]. The enhanced-fluidity liquid mixtures of tetrahydrofuran (THF) and CO₂ were also used as mobile phases in the size exclusion chromatography (SEC) of polystyrene [10]. Significant increases in the optimum velocity and the speed of analysis were observed compared to using pure THF. The pressure drop across the column was also lowered.

In order to take full advantage of the properties available from these mixtures, the characterization of the fundamental properties of these enhanced-fluidity liquid mixtures is required.

GOALS OF RESEARCH

The topics addressed in this dissertation can be divided in two parts. The first one is dedicated to the study of solvation in supercritical CO₂ whereas the second part is mainly focused on the characterization of the properties of some enhanced-fluidity liquid mixtures and their application in size exclusion chromatography.
Chapter II deals with the solvation of anthracene and naphthalene in supercritical CO₂. The vicinity of the critical point in terms of pressure range was probed. This study provides a new way to look at solvation in supercritical fluid. The average critical distance between the two solutes was estimated from spectroscopic measurements. Although the literature is abundant about the properties associated with the critical state, no studies dealing with distances between solutes were undertaken. This study gives an estimation of how far apart two solutes can be and if the pressure has any influence.

Chapter III and IV deal with the characterization of some properties of the enhanced-fluidity liquid mixtures. Chapter III focuses on the properties of a commonly-used liquid mixture, i.e., methanol/CO₂. The density and the macroscopic properties associated with it, such as the partial molar volume, the isothermal compressibility and the excess volume of mixing, will be evaluated. The density is a measure of the solvent strength of the fluid. The isothermal compressibility gives an indication of how easily the density can be altered by a change in the pressure. The magnitude of the partial molar volume of a solute dissolved in the mixture is an indication of its solubility. The effect of the mole percent of methanol as well as the pressure was investigated. How do the properties associated with an enhanced-fluidity liquid vary when the composition is changed? The mole percent of the organic liquid defines its solvent strength. Nevertheless, when the proportion of organic liquid increases, the viscosity of the mixture increases. There might be a trade-off between having the properties of one constituent without having to sacrifice the properties attributed with the other one. The data of these studies should allow the selection of the methanol/CO₂ mixture that has
the optimum properties that are highest solvent strength, ease of density tunability with the pressure and lowest viscosity.

Chapter IV studies the transport properties associated with the use of ternary mixtures of ethanol/water/CO₂. The viscosity of a fluid affects the molecular diffusion coefficient of the solutes. The decrease in the viscosity of a fluid mixture is usually achieved by an increase in the temperature [11], [12]. However, significant increase in the diffusivity of the solutes can only be achieved at temperatures greater than 100 °C. The present study give information on the effect of the temperature and/or mole fraction of dissolved CO₂ on the fluidity of the ethanol/water/CO₂ mixtures.

Chapter V illustrates one of the applications of the tetrahydrofuran/CO₂ mixtures as a mobile phase for liquid chromatography at the critical point of adsorption. This type of chromatography is applied to the characterization of the polymers according to their chemical heterogeneities. The use of an enhanced-fluidity liquid as a mobile phase offers many tunable parameters. The solvent strength can be adjusted by a selective control of the mole percent of CO₂ in the mixture. The higher isothermal compressibility of these mixtures allow for an easy tuning of the mobile phase’s density. The influence of the mobile phase composition and the pressure on the chromatography of homopolymers and copolymers of polystyrene were investigated.

The Appendix section is composed of the preliminary results concerning the application of the spectroscopic ruler to the determination of the distance between anthracene and naphthalene in methanol/CO₂ mixtures.
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CHAPTER II

ESTIMATION OF AVERAGE DISTANCES BETWEEN ANTHRACENE AND
NAPHTHALENE IN SUPERCRITICAL CO$_2$

INTRODUCTION

Numerous studies have been performed to elucidate the solvation process in a supercritical fluid. A number of approaches have already been attempted to highlight the solvent-solute interaction in a supercritical solvent.

Computational studies. The calculation of cluster size was successfully accomplished via fluctuation analysis [1]. An aggregation of over 100 molecules of solvent around naphthalene and tetrabromomethane was found in the vicinity of the critical point of supercritical CO$_2$. This excess of solvent density compared to the solvent bulk was also demonstrated by spectroscopic studies [2], [3]. Computer simulations involving calculation of solute-solvent and solvent-solvent pair correlation functions provide a dynamic picture of the clustering phenomenon. Petsche and coworkers investigated by computer simulation the long-ranged effects in both attractive and repulsive supercritical fluid mixtures [4]. The dynamics of solvent molecules clustering whose lifetime is in the picosecond time scale was clearly demonstrated as well as the extent of solute-solvent interactions.
**Macroscopic studies.** In the near critical region, macroscopic properties such as partial molar volume and solubilities of nonvolatile solutes are strong functions of the density of the fluid [5], [6], [7]. Large, negative partial molar volumes of naphthalene and camphor in supercritical carbon dioxide and ethylene were observed in the vicinity of the critical point [8]. The collapse of solvent molecules around the solute was proposed to explain the sign and magnitude of the partial molar volume. Moreover studies of the pressure effect on the solubility show that both macroscopic properties are correlated. The "divergence" of these two parameters is also linked with the isothermal compressibility of the fluid. In the vicinity of the critical point, the compressibility of a supercritical fluid is assumed to be infinite and thus allows for quite unusual solvation properties [9].

**Spectroscopic studies.** Spectroscopic methods aim at the characterization of the solvent at the molecular level and are complimentary to the macroscopic studies previously cited. Among the spectroscopic methods frequently used to probe the solute's surrounding, solvatochromic studies are often a method of choice [2], [3]. These studies were done at experimental conditions mainly near the critical point of the solvent where the difference between local and bulk density is greater. During the solvation process, the clustering of solvent molecules around the solute affects its spectroscopic properties. A change in the maximum absorption peak in UV, IR or in the NMR chemical shift is linked to the change in the solute's environment. The magnitude of the change is correlated to the strength of the solute-solvent interaction. It is also an indication of the solvent strength of the medium.
A shift in the wavelength of maximum absorption in the UV spectrum of the solute is often used as a means of evaluating the average number of solvent molecules in the solvent sphere [10]. Most solutes studied are polar compounds such as phenol blue, 2-nitroanisole and benzonitrile derivatives. Large shifts were observed in favorable solvation conditions, such as polar compounds dissolved in polar supercritical fluids. The solvatochromic shifts are the results of solute-solvent interactions through London dispersive forces, dipole-dipole and dipole-dipole-induced interactions. For poor solvation conditions, such as the solvation of polyaromatic compounds in polar supercritical fluids, the changes in the wavelength of maximum absorption with pressure or density variation are typically small and so solvatochroism is not always of practical use to study these systems. Moreover minimal information on solute-solvent interactions is obtained beyond the cybotactic region because only short-ranged interactions are probed under past conditions [11].

Fluorescence spectroscopy is also a powerful tool to investigate the local density around the solute. Its large sensitivity provides the opportunity to probe solute-solvent at the molecular level. Fluorescence studies of aromatic or organic compounds in various supercritical fluids or fluid mixtures were performed to characterize the solvating parameters of the fluid, both close to and away from the critical point [12], [13]. Local ordering of supercritical solvent around solutes was noted from the relative intensity ratios in the fluorescence spectra and from the analysis of time-resolved fluorescence studies [14], [15]. Aggregates of solvent molecules around a solute can distort its electronic cloud
and thus affect the symmetry of its excited state. The intensity of an emission band that is symmetry forbidden can then be enhanced. Brennecke et al. used this perturbation to probe the solute-solvent interactions in the case of polycyclic aromatics in supercritical fluids. The enhancement of a forbidden band (the 0-0 transition) in the vicinity of the critical point where the isothermal compressibility is high demonstrates solvent aggregation [16].

The interest in the solvation process occurring in these fluids has not been limited to the supercritical state. Characterization of fluids in the supercritical but also subcritical regions gives important information regarding the difference in solvation of these two states. The solvation of 6-propionyl-2-(dimethylamine)-naphthalene (PRODAN) was probed in sub and supercritical nitrous oxide [12]. Fluorescence anisotropy coupled with time resolved fluorescence spectroscopy was used to calculate the volume of the entity formed by the solvent molecules and the solute. Clustering of nitrous oxide molecules around PRODAN was clearly demonstrated in the vicinity of the critical point. At a reduced temperature ($T_r = T/T_c$) of 1.01 and reduced pressures ($P_r = P/P_c$) in the 1 to 1.2 range, the increase in the solvent density was 2.5 times the bulk density. This solvation behavior was not present in the subcritical state of nitrous oxide.

Nevertheless, to this date, no studies dealing with distances between solutes in supercritical fluid have been reported. The spectroscopic method derived from Förster's theory could be applied to investigate both solute-solute and solute-solvent interactions in a supercritical fluid. It will provide insight on the solvation shell. This theory also named
the "spectroscopic ruler" aims at determining the spatial distance between two chromophores in a given medium. This present work deals with the application of this theory to elucidate the behavior of two different solutes, naphthalene and anthracene, in supercritical CO₂. This study is limited to the near critical region of CO₂ since it is where the most drastic change in solvent structure around the solute is expected to occur.

The existence of an energy transfer between molecules has first been reported by Perrin [17] and Förster [18]. Förster's theory deals with the resonance energy transfer between two chromophores. The efficiency at which such transfer occurs is proportional to the average distance between the two molecules or moieties of a molecule. This radiationless transfer occurs via dipole-dipole interaction between an energy donor and an energy acceptor molecule. The dipole-dipole interaction is the interaction which has the longest range among multipole-multipole interactions. Stryer and Haughland suggested that this energy transfer could be used as a "spectroscopic ruler" to measure distances between molecules in the range of 10-80 Å [19]. The "spectroscopic ruler" has been used in a number of applications. For example, the configurations of macromolecules and flexible biopolymers (glycopeptide, complex oligosaccharide, protein) were successfully determined using this method [20], [21], [22].

The choices of systems to study are dictated by the theory itself. For this type of energy transfer to take place three conditions must be met [23]. 1) The energy donor must fluoresce. (The higher the quantum yield of the donor, the higher the efficiency of the energy transfer). 2) The fluorescence spectrum of the donor and the absorbance spectrum
of the acceptor must overlap. 3) For efficient energy transfer, the separation between the two chromophores should be no greater than the range of 10-100 Å. Naphthalene and anthracene fluorescence spectrum and absorbance spectra, respectively, readily meet conditions 1 and 2 and condition 3 is easily met by aromatic molecules [24]. Also the end-to-end distance of flexible organic polymer chains was previously determined by using the "spectroscopic ruler method" with naphthalene (the donor) attached to one end of the polymer and anthracene (the acceptor) attached to the other end of the chain [25]. Europium and terbium have also been used as energy donors for labeling of biological macromolecules [26], [27]. The use of such lanthanide chelates offers many advantages such as long and monoexponential lifetime as well as large value of critical distance, $R_0$. These properties make them particularly attractive to measure accurately and easily the energy transfer over long distances ($R > 100$ Å). Nevertheless, no solubility data as well as no phase diagram in supercritical fluids exists for these compounds which prevented their use as the energy donor and acceptor molecules in supercritical CO$_2$. Moreover, the choice of naphthalene and anthracene as the energy donor/acceptor couple of the energy transfer in this study is also supported by the considerable literature available on these compounds. Also the macroscopic and microscopic properties in supercritical carbon dioxide such as solubilities and partial molar volumes are well documented. They were therefore deemed ideal compounds for these studies on the solvent structure found in supercritical CO$_2$. 

THEORY SECTION

The energy transfer probability is directly linked to the distance between the energy donor and acceptor according to the generalized formula [24]:

\[ w = \left( \frac{R_0}{R} \right)^m \frac{1}{\tau_{\text{M}1}} \]  

where \( w \) is the energy transfer probability, \( \tau_{\text{M}1} \) is the fluorescence decay time of the energy donor when the energy acceptor is absent, \( R \) is the average distance between the two molecules, \( R_0 \) is the critical distance and \( m \) is a constant that depends on the nature of the interactions between the energy donor and acceptor.

The energy transfer can be the result of multipole-multipole interactions between the energy donor and acceptor. In the case where the energy transfer is due to dipole-dipole interactions, the constant \( m \) in equation (1) is equal to 6. For higher-order multipole-multipole interactions, \( m > 6 \).

The critical distance is the distance between the energy donor and the energy acceptor at which the probability of donor decay by energy transfer is the same as the probability of its decay by other mechanisms. At a distance equal to the critical distance, the efficiency of energy transfer equal to 50\%. The critical distance, \( R_0 \), is given by [24]:

\[ R_0 = \left( \frac{9000 \ (Lm10)K^2 \Phi_E \int f_{\text{M}1}(v) \ e_{\text{M}2}(v) \ dv/v^4}{128 \pi^3 h^5 N} \right)^{(1/6)} \]  

(2)
where $K^2$ is the orientation of the dipole vectors of both molecules, $\Phi_F$ is the fluorescence quantum yield of the energy donor, $n$ is the refractive index of the solvent, $N$ is Avogadro's number, $f_{M1}$ and $e_{M2}$ are the normalized fluorescence of the energy donor and the molar extinction coefficient of the energy acceptor at each wavenumber $\nu$, respectively.

The orientation of the dipoles, $K^2$ is given by [28]:

$$K^2 = (\cos \Phi_{DA} - 3\Phi_D \Phi_A)^2$$  \hspace{1cm} (3)

where $\Phi_{DA}$ is the angle between the transition moment vectors of the donor and acceptor molecules, $\Phi_D$ and $\Phi_A$ are the angles between these respective vectors and the vector joining the donor and acceptor molecules. The value of $K^2$ spans between zero and four, which corresponds to the donor emission and acceptor absorption dipoles being perpendicular and parallel, respectively. In most cases, the value of $K^2$ equals to $2/3$ is taken under the assumption that both dipoles sample all orientations during the interval of the excited state, i.e., dynamic averaging condition [18]. This is a logical assumption to make for the conditions studied herein. Values of $K^2$ different than $2/3$ are most commonly found when the chromophores are attached to macromolecules that limit their motion [19].
Determination of the efficiency of the energy transfer.

Several methods (donor quenching, sensitized fluorescence, time resolved energy transfer, fluorescence polarization) allow the determination of the efficiency of the energy transfer, $E$. The center-to-center intermolecular distance between two chromophores, $R$, can be calculated from the efficiency of the energy transfer, $E$ and the critical distance, $R_0$, according to the following equation [18]:

$$ E = \frac{R_0^6}{(R_0^6 + R^6)} \quad (4) $$

By using the energy quenching of the energy donor method, the efficiency of the energy transfer is expressed as [29]:

$$ E = 1 - \frac{F_{DA}}{F_D} \quad (5) $$

where $F_D$ is the integrated fluorescence of the donor alone, and $F_{DA}$ is the integrated fluorescence of the donor in the presence of the acceptor.

The time resolved energy transfer method is a multiexponential fitting of the donor fluorescence decay according to the following equation [29]:

$$ I_{DA}(t) = \sum_{k=1}^{m} A_k \int P_k(r) \sum_{l=1}^{n} \alpha_l \exp \left[ \left( -\frac{1}{\tau_1} \right) \left( 1 + \left( \frac{R_0}{R} \right)^6 \right) \right] dr \quad (6) $$
where \( m \) is the total number of populations, \( n \) is the refractive index of the solvent, \( A_k \) is the relative concentration of the \( k \)th population, \( P(r) \) is the probability distribution modeled either by a Gaussian or a Lorentzian function, \( \alpha \) and \( \tau \) are the amplitude and lifetime of the donor in the absence of the energy acceptor, \( R_0 \) is the critical distance and \( dr \) is the differential of the distance.

The fluorescence polarization data may be used to determine the average distance, \( R \), by the following equation [30]:

\[
R = \left( \frac{15sI0^3}{4\pi N \left( \frac{1}{p^m} + \frac{1}{3} \right)} \right)^{1/6} (2a)^{1/2}
\]  

(7)

where \( s \) is the slope of the reciprocal of the polarization versus the molar concentration, \( 1/p^m \) the intercept of the curve, \( N \) the Avogadro's number and \( a \) the average radius of the molecule.

The experimental results described below were obtained using two different methods namely the sensitized fluorescence and the time resolved energy transfer methods. The average intermolecular distance, \( R \), between two molecules undergoing an electronic excitation transfer is expressed by the equation (4). The critical energy transfer distance (in Angstroms) is defined by [28]:

\[
R_0 = 9.78 \times 10^3 (K^2 \Phi \eta J^n \eta^{-4})^{1/6}
\]  

(8)

where \( K^2 \) is the dipole-dipole orientation factor, \( \Phi \) is the quantum yield of the energy
donor in the absence of transfer, \( J \) is the spectral overlap integral in units of cm\(^3\) M\(^{-1}\) and \( n \) is the refractive index of the solvent. The extent of overlap between the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor is described by the following equation [31]:

\[
J = \frac{\int \lambda^4 \varepsilon_\lambda I_\lambda d\lambda}{\int I_\lambda d\lambda}
\]  
(9)

where

\[
\int I_\lambda d\lambda = 1
\]  
(10)

\( J \) is the overlap integral between the normalized fluorescence intensity of the donor group (naphthalene) at the wavelength \( \lambda \), \( I_\lambda \), and the molar extinction coefficient of the acceptor group (anthracene), \( \varepsilon_\lambda \) (in cm\(^{-1}\).M\(^{-1}\)). The overlap integral varies with the solvent primarily due to the sensitivity of the fluorescence emission of the donor to its environment [19].

The efficiency of the energy donor absorption is given by [25]:

\[
P = \frac{1-10^{-A_\sigma(\lambda_1)}}{2-10^{-A_\sigma(\lambda_1)}-10^{-A_\sigma(\lambda_2)}}
\]  
(11)

where \( A_\sigma(\lambda_1) \) and \( A_\sigma(\lambda_2) \) are the absorbency of the energy donor and acceptor molecules at the wavelength \( \lambda_1 \), respectively.
The energy transfer efficiency, $E$, is given by the following equation [31], [32]:

$$E = \frac{1}{P'T} \left( \frac{I_{AF}^R(\lambda_1)(1-10^{-A(\lambda_2)})}{I_{AF}^R(\lambda_2)(1-10^{-A(\lambda_1)})} - 1 + P \right)$$  \hspace{1cm} (12)$$

where $\lambda_1$ is the wavelength at which predominantly the energy donor group absorbs, $\lambda_2$ is the wavelength at which only the energy acceptor group absorbs, $T$ is the termination efficiency in the case of a polymer or the ratio of the energy acceptor to the energy donor molecules, and $P$ is the efficiency at which the energy donor molecule absorbs at $\lambda_1$. $I_{AF}^R(\lambda_1)$ and $I_{AF}^R(\lambda_2)$ are the acceptor fluorescence intensities measured in the ratio mode by exciting the sample containing both the energy donor and the energy acceptor molecules at wavelength $\lambda_1$ and $\lambda_2$, respectively.

**EXPERIMENTAL SECTION**

UV-visible absorption spectrum of anthracene and naphthalene in supercritical fluid CO$_2$.

**Materials.** Naphthalene ($\geq 98\%$ purity, Mallinckrodt, Inc., Paris, KT) and anthracene (Baker grade from J. T Baker Chemical Co., Philipsburg, NJ) and research SFC/SFE grade CO$_2$ (99.999\% pure, Matheson, Newark, CA) were used without further purification.

**Absorption spectra.** Absorption spectra of each compound in supercritical CO$_2$ were obtained with a DMS-100S (Varian, Sunnyvale, CA) UV-visible spectrophotometer.
used in the dual beam mode (see Figure 2). Absorption spectra of each solute in supercritical CO$_2$ were obtained by using a home made stainless steel high pressure cell with an internal volume of 10 ml and optical path length of 3.5 cm. Stainless steel inlet and outlet connections were perpendicular to the optical axis and allowed the supercritical CO$_2$ to flow through the cell. A 1.125 in diameter x 0.69 in thick quartz window (ESCO Prod. Inc., Oak Ridge, NJ) was placed on each side of the cylindrical high pressure cell. High pressure Teflon O-rings (3/4 in i.d. x 15/16 in o.d.) were used to make the high pressure seal against each window. The flow cell was heated with a coaxial heating coil (Aeoqoaax™, Ari Industries, Inc., Addison, IL.) that was soldered to the body of the cell. This heater coil consists of a stainless steel sheath with magnesium oxide insulation separating the sheath and the heater wire. A model CN9000 series temperature controller (Omega, Stamford, CT) equipped with a type J thermocouple was used to monitor and control the cell temperature to within ± 0.1°C. The supercritical fluid was pumped through the cell by a high pressure syringe (Model 260D, ISCO, Lincoln, NE). A pressure transducer (Model 204, Setra, Acton, MA) coupled to the syringe pump provided pressure measurements with accuracy and precision of ± 0.37 atm.

**Procedure.** Qualitative UV spectra of anthracene and naphthalene in supercritical fluid CO$_2$ were first obtained to determine $\lambda_1$, and $\lambda_2$, wavelength where only the energy acceptor absorbs. Quantitative UV-visible spectra were obtained by the following procedure. The apparent absorbance of supercritical CO$_2$ was recorded at constant temperature (34°C) over a range of pressures (60 atm - 140 atm) relative to an air
Figure 2. Schematic view of the UV-visible experimental setup.

A, pressure transducer; B₁, B₂, valves; C, reference cell;
D, sample cell; E, spectrophotometer.
reference. Next the quantitative UV-visible spectrum of each solute was recorded. Two stock solutions of each solute were prepared in pentane, $4.096 \times 10^3$ M and $1.268 \times 10^3$ M for naphthalene and anthracene, respectively. The high pressure cell was closed on one side with one window in place. Aliquots of these solutions (0.3 ml and 0.5 ml, for anthracene and naphthalene, respectively) were placed onto the surface of a quartz window. The pentane was evaporated by a gentle flow of argon (99.9995% pure, Linde Specialty Gases, Columbus, OH) in the cell. A white residue of the solute was left on the quartz window. The other quartz window was put in place and the optical axis of the cell was closed. The cell was filled with CO$_2$ via the stainless steel outlet connecting the syringe pump to the cell. The solute concentrations in carbon dioxide for the various experiments in this study were $3.804 \times 10^{-3}$ M and $2.048 \times 10^{-4}$ M for anthracene and naphthalene, respectively. The concentrations are well below the solubility limits which are $6 \times 10^{-2}$ M and $2.7 \times 10^{-4}$ M for naphthalene and anthracene in supercritical CO$_2$, respectively, in the pressure range studied [7], [33]. Nevertheless, the solubility of anthracene may be enhanced in the mixture of anthracene-naphthalene-CO$_2$. Naphthalene is very soluble in supercritical CO$_2$ and acted as an “solubility entrainer” in many solid-solid-CO$_2$ mixtures [34]. The high pressure cell was then placed in an ice bath for 30 min to allow the CO$_2$ to liquefy. The liquid CO$_2$ dissolved the solid solute from the quartz window. Because the kinetics of solute dissolution are faster in liquid CO$_2$ than in supercritical CO$_2$, this step was added to decrease the analysis time per sample. Once the solute was completely solubilized, the temperature of the cell was raised to 34 °C and the
desired pressure was set. Temperature and pressure were allowed to equilibrate in the cell for at least 1 hour. The amount of time needed for equilibration was determined by initially monitoring the variation in the measured absorbance with time. According to Rößling et al. one hour is sufficient to allow for a complete dissolution of the solutes in supercritical CO$_2$ [33]. This procedure was used to determine the UV-visible absorption spectrum (200-400 nm range) of anthracene in supercritical CO$_2$ and the absorbance of naphthalene at 280 nm and 360 nm relative to an air reference. The absorbance values of the solutes were further corrected for the absorption of the supercritical CO$_2$. The CO$_2$ solvent apparent absorbance was obtained by placing it in the sample side of the spectrophotometer. The same experimental procedure was applied as for the determination of the spectra of the solutes. The scan rate was set to 100 nm/min, the time constant was 0.3 s. The measurements were done statically (flow rate of the pump equals to zero ml/min). At least three replicates of each measurement were performed.

The sensitized fluorescence method: fluorescence measurements.

Instrumentation. The fluorescence spectra obtained in supercritical CO$_2$ were performed with a PE-LS5 fluorimeter equipped with a PE3700 Data Station (Perkin-Elmer, Norwalk, CT). The fluorescence intensities were recorded in the ratio mode. The measurements were done in steady state fluorescence. The experimental setup is drawn in Figure 3. The excitation and emission slit widths were set to 5 nm and 3 nm, respectively, throughout the experiments. A high pressure sapphire tube 0.18 cm i.d.
x 0.33 o.d. and 13 cm long was used as the optical cell (Saphikon, Milford, NH). The total internal volume of the tube was 0.3548 ml and 0.2992 ml for the experiments performed in supercritical and liquid CO₂, respectively. The volume of the sapphire tube was determined by filling it with methanol. The tube was weighed and the volume was deduced from the density of the methanol. For the slit conditions used, the rectangular spot size of the source beam in the optical cell chamber was 8 mm long x 4 mm wide. Therefore with the sapphire tube centered in the optical beam, this corresponds to an illuminated fluid volume of 0.020 ml. The optical cell was sheathed by an open window copper cartridge built in house. The open window copper cartridge was coupled to a Variac heater. The cartridge was then heated with ceramic-insulated heater wire (ACE Glass, Louisville, KT). Its temperature was monitored and controlled with the same Omega CN9000 temperature controller used in the UV absorbance measurements. This allowed temperature control of the optical cell to within ± 0.1 °C. The open window copper cartridge facilitated heating the optical cell and did not block the light beam. A Swagelok tee connection was secured at the top of the sapphire tube to allow connections to the high pressure pump to fill the tube with CO₂ and to a vacuum pump to evacuate the tube when needed. The connection to the vacuum line was then closed under pressure of CO₂. The connection between the sapphire high pressure cell and the syringe pump was a 430 μm o.d. x 320 μm i.d. fused silca tube (Polymicro Technologies, Phoenix, AZ) that was approximately 130 cm long.
Figure 3. Schematic view of the fluorescence experimental setup.

A, pressure transducer; B₁, B₂, valves; C, sapphire tube;
D, rubber O-ring; E, heating cartridge;
F, fluorescence cuvette; G, fluorimeter.
Procedure. The following procedure was applied to get a fluorescence spectrum both in liquid and supercritical CO₂. The sapphire tube was evacuated (ca. 1 x 10⁻³ torr). The connection of the optical cell to the delivery of CO₂ was opened, allowing a positive pressure of CO₂ in the cell. The outlet to the vacuum line was then closed under the pressure of CO₂. The optical cell was placed in the copper cartridge. The copper cartridge assembly was placed in a conventional fluorimetric cell (in order to insulate thermally the fluorimeter from the optical cell). A rubber O-ring was put at the top of the high pressure sapphire tube to stabilize its position in the fluorimeter hole. The experimental procedure for the determination of the fluorescence spectrum of anthracene and/or naphthalene was as follows. A volume of 10 μl of a stock solution of 10.05 x 10⁻⁵ M of naphthalene in pentane and/or 10 μl of a solution 10.23 x 10⁻⁵ M of anthracene in pentane was injected into the sapphire tube. The fluorescence experiments performed on the mixture of naphthalene and anthracene in supercritical CO₂ were done at the concentrations of 2.88 x 10⁻⁶ M of anthracene and 2.83 x 10⁻⁶ M of naphthalene. The concentrations of anthracene and naphthalene in liquid CO₂ were 3.63 x 10⁻⁶ M and 3.28 x 10⁻⁶ M, respectively. The concentrations were also chosen in order to be in the solubility range of these solutes in supercritical CO₂, but also to provide a T value (equation 12) equal to unity. The T value was 1.011 in this study. The tube was evacuated (ca. 1 x 10⁻³ torr) to evaporate the solvent and minimize the oxygen quenching in the fluorimetric experiment. The outlet to the vacuum line was then shut off under a positive pressure of CO₂ in the tube. The dissolution of the solid from the walls of the
tube was ensured by liquefaction of CO₂ in an ice bath. The temperature was then raised to 34° C. The fluorimetric experiments were performed under various pressures of CO₂ (75-90 atm). Each fluorimetric experiment was performed at two excitations wavelengths, 280 and 360 nm. The excitation wavelengths were selected based on the UV-visible absorption spectrum of anthracene and naphthalene in supercritical or liquid CO₂. At 280 nm, negligible absorption of anthracene occurs, while at 360 nm, anthracene absorbs predominantly. The monitored emission wavelengths ranged from 300 to 500 nm for an excitation wavelength set to 280 nm. All spectra had a magnification of the fluorescence equal to 10, due to the decrease in the fluorescence intensity with the temperature. The measurements were done statically with a pump flow rate equal to zero ml/min.

The time resolved energy transfer method: fluorescence measurements.

Instrumentation. The fluorescence measurements were determined using the high pressure flow cell previously described. The concentrations of anthracene and naphthalene in the mixture were 1.646 x 10⁻⁴ M and 1.650 x 10⁻⁴, respectively.

Determination of the fluorescence lifetime of naphthalene in supercritical CO₂.

Instrumentation. The determination of the fluorescence lifetime measurements was done using the high pressure flow cell previously described. All fluorescence lifetimes were determined using a time-correlated single-photon counting (TCSPC) instrument. The diagram is drawn in Figure 4. Briefly, the second-harmonic output of
Figure 4. Diagram of the time-correlated single-photon counting instrument.
a Mode-Locked Nd:YLF laser (Coherent Antares) was used to pump a cavity-dumped dye laser (Coherent 700 Series / Rhodamine 6-G; 1 MHz; 25 mW) and the pulse train was directed to a beam splitter. A portion of the train was routed to a fast photodiode that registered a "start" signal on a Time-to-Amplitude Converter (TAC–Tennelec TC-864) after passing through a Constant Fraction Discriminator (CFD–Tennelec TC-455). This established the time position of each pulse with great precision. The "start" pulses initiated the charging of a capacitor in the TAC. The remainder of the pulse train was frequency-doubled in a non-linear crystal to generate the excitation beam. Fluorescence was collected front-face from a stainless steel 10 cm cylindrical cell with quartz optical windows. The fluorescence was detected through a subtractive-double monochromator centered at 335 nm (American Holographic DB-10S). The bandpass was either 2 nm or 5 nm. The detector was a Microchannel Plate Photomultiplier Tube (Hamamatsu R-2809U-07). The signals arising from single photons were amplified and passed through a second CFD. The CFD output pulses then served as the "stop" signals for the capacitor in the TAC. The TAC capacitance was transferred to a Multi-Channel Analyzer (MCA–Tennelec PCA-II), where the data were binned and presented as a histogram. This histogram served as a representation of the fluorescence decay.

Data analysis consisted of a fit to a sum of two exponentials (weighed in accordance with Poisson statistics) with the PeakFit™ software package from Jandel Scientific. A typical fluorescence decay of naphthalene is shown in Figure 5. One exponential function dominates in the curve fitting. The standard convolute-and-compare
Figure 5. Fluorescence decay of naphthalene in supercritical CO$_2$, 83.3 atm, 34 °C.
data analysis technique was not used in these experiments due to distortions in the instrumental response function (measured at the laser wavelength) relative to the “true” instrument response function that was convoluted with the sample fluorescence decay. These distortions had their origin in stray light reflections that were an inevitable consequence of the front-face detection geometry employed with the stainless-steel supercritical fluid cell. The contribution of the instrumental response function was negligible in any case, however, as the shortest naphthalene fluorescence lifetime was on the order of 55 ns, while the instrumental response function had a full width at half-maximum of ~60 ps. Figure 6 is the typical instrument response observed for the supercritical CO₂ experiment.

RESULTS AND DISCUSSION

Determination of the energy transfer by the sensitized fluorescence method.

Our first studies of energy transfer between the solutes naphthalene (an energy donor) and anthracene (an energy acceptor) in CO₂ were attempted in the liquid state of CO₂ to obtain a qualitative view of the phenomenon. In this case, the temperature was maintained at room temperature and the pressure scanned from 68 to 200 atm. Figures 7 and 8 show a qualitative UV-visible absorption spectrum of each solute in liquid CO₂. According to the UV-visible spectrum of both anthracene and naphthalene in supercritical CO₂, λ₁ was chosen to be 280 nm whereas λ₂ was set to 360 nm. Anthracene absorbs predominantly in the 300-400 nm wavelength region whereas the absorption of the
Figure 6. Instrument response of the fluorescence experiments in supercritical CO$_2$, 34 °C.
Figure 7. Qualitative UV-visible absorption spectrum of naphthalene in CO$_2$. 
Figure 8. Qualitative UV-visible absorption spectrum of anthracene in CO$_2$. 
naphthalene is greater than that of the anthracene in the 230-300 nm area. The excitation of the sample containing both the energy donor and the energy acceptor at 280 nm should excite the naphthalene molecule. Upon excitation, fluorescence will occur. Part of this fluorescence could be absorbed by the anthracene molecule if the wavelengths of the fluorescence emission are in its absorption region. Förster’s theory states that the excitation energy transfer between two molecules should result in the quenching of the fluorescence intensity of the energy donor and may lead to the enhancement of the fluorescence intensity of the energy acceptor. To demonstrate this effect, a series of fluorescence experiments were carried out on a solution containing the energy acceptor alone and a solution containing both the energy donor and acceptor molecules (in a 50/50 molar ratio). In both cases, the wavelength of excitation was set to 280 nm, where naphthalene (the energy donor) absorbs predominantly. The fluorescence emission wavelength of anthracene was monitored at 484 nm. An increase in fluorescence emission of anthracene is noticed in the solution containing both the energy acceptor and the energy donor as seen in Figure 9. The same experimental procedure was performed in supercritical CO₂ at 34° C and over a pressure range of 68.0 to 86.8 atm. The fluorescence quenching of the naphthalene results in the enhancement of the fluorescence intensity of the anthracene. The existence of this energy transfer occurring between anthracene and naphthalene in supercritical fluid could be predicted from their respective UV-visible spectrum (which is similar to Figures 7 and 8). Over a wide range of CO₂ densities, there is an overlap between the energy acceptor absorbance spectrum
Figure 9. Enhancement of the fluorescence intensity of anthracene in liquid CO$_2$, 26 °C. Excitation: 280 nm, Emission: 484 nm. (□) anthracene, (■) anthracene/naphthalene (50/50 mole ratio).
(300 - 375 nm) and the fluorescence spectrum of the energy donor.

**Determination of the critical distance, \( R_0 \).**

The critical distance is defined as the intermolecular distance at which the rate of the excitation transfer of the energy donor becomes the same as the rate of the decay of its excited state. The determination of both the fluorescence quantum yield of the energy donor, i.e., naphthalene and the overlap integral between the energy donor and acceptor were experimentally determined in supercritical CO\(_2\). The effect of pressure on these parameters was also be investigated.

**Calculation of the fluorescence quantum yield of naphthalene in supercritical CO\(_2\).**

The fluorescence quantum yield, \( \Phi_F \) was determined according to the following equation from the fluorescence lifetime measurements [35]:

\[
\Phi_F = \frac{\tau_{\text{exp}}}{\tau_{\text{nat}}}
\]

where \( \tau_{\text{exp}} \) and \( \tau_{\text{nat}} \) are the experimental and natural fluorescence lifetime, respectively. The value of the natural fluorescence lifetime of naphthalene was calculated from the experimental fluorescence quantum yield and lifetime of naphthalene in hexane [36]. The fluorescence lifetimes of naphthalene in supercritical CO\(_2\) were experimentally determined from its fluorescence decay. Table 3 summarizes the parameters used in the
Table 3. Biexponential fitting of naphthalene fluorescence decay time.

<table>
<thead>
<tr>
<th>Pressure (atm)</th>
<th>68.04</th>
<th>71.5</th>
<th>77.9</th>
<th>78.2</th>
<th>81.7</th>
<th>83.3</th>
<th>85.1</th>
<th>86.8</th>
<th>liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp(1)</td>
<td>7870</td>
<td>8575</td>
<td>8427</td>
<td>8333</td>
<td>7794</td>
<td>7525</td>
<td>7241</td>
<td>6857</td>
<td>6369</td>
</tr>
<tr>
<td>τ(1) (ns)</td>
<td>73.03</td>
<td>69.44</td>
<td>64.05</td>
<td>61.98</td>
<td>61.17</td>
<td>59.09</td>
<td>57.91</td>
<td>57.76</td>
<td>54.93</td>
</tr>
<tr>
<td>Amp(2)</td>
<td>1013</td>
<td>1075</td>
<td>1001</td>
<td>1240</td>
<td>1604</td>
<td>1686</td>
<td>2329</td>
<td>2413</td>
<td>2643</td>
</tr>
<tr>
<td>τ(2) (ns)</td>
<td>7.82</td>
<td>9.35</td>
<td>7.88</td>
<td>8.55</td>
<td>8.18</td>
<td>8.73</td>
<td>6.83</td>
<td>7.81</td>
<td>8.05</td>
</tr>
<tr>
<td>σ(1)</td>
<td>0.382</td>
<td>0.355</td>
<td>0.270</td>
<td>0.202</td>
<td>0.284</td>
<td>0.282</td>
<td>0.231</td>
<td>0.267</td>
<td>0.257</td>
</tr>
<tr>
<td>σ(2)</td>
<td>1.58</td>
<td>1.84</td>
<td>1.80</td>
<td>1.55</td>
<td>1.16</td>
<td>1.10</td>
<td>0.72</td>
<td>0.72</td>
<td>0.63</td>
</tr>
<tr>
<td>% Amp(1)</td>
<td>88.59</td>
<td>88.86</td>
<td>89.39</td>
<td>87.04</td>
<td>82.93</td>
<td>81.69</td>
<td>75.66</td>
<td>73.97</td>
<td>70.67</td>
</tr>
</tbody>
</table>

Liquid: CO₂, 26 °C, 95.3 atm

Amp(i): amplitude of the ith exponential.

τ(i): lifetime of the ith exponential.

σ(i): standard deviation of the fit of the ith exponential.

The fitting equation used was the following:

\[ y = Amp(1) \exp\left(\frac{-t}{\tau(1)}\right) + Amp(2) \exp\left(\frac{-t}{\tau(2)}\right) \]  \hspace{2cm} (14)
fitting of the naphthalene fluorescence decay data. These data are the average values of 3 replicates. The decays were fitted according to a biexponential function. The curve fittings had a correlation coefficient, $R^2$, greater than 0.9991 in all cases. Nevertheless, as seen in Table 3, a major contribution was attributed to only one exponential. The relative contribution of the major component appears to decrease with increasing the pressure. This is probably due to the fact that the absolute fluorescence intensity of the naphthalene decreases with increasing pressure. The count rate in the peak channel (counts/sec) due to the naphthalene is therefore lower at higher pressures. The count rate due to the sapphire fluorescence is not affected by the pressure, so for a given collection time, a larger fraction of the counts in the peak channel are attributable to the sapphire fluorescence at high pressures. The effect of pressure on the naphthalene fluorescence lifetime and hence on the quantum yield are illustrated in Figures 10 and 11, respectively. There is a quenching of the naphthalene fluorescence as the density of the fluid increases. As the pressure of the fluid increases, collisional quenching contributes to the faster decay of the fluorescence. The pressure range investigated correspond to the region where the clusters of CO$_2$ are building around the solute [1]. When the pressure is moved past the critical point, the fluorescence lifetime levels off. It reaches the same value as that obtained in pentane. This demonstrates that properties of supercritical fluids equivalent to a liquid could be achieved by a sufficient increase in the pressure of the system at constant temperature.
Figure 10. Pressure effect on the fluorescence lifetime of naphthalene in supercritical CO₂, 34 °C.

Liquid: 26 °C, 95.3 atm.
Figure 11. Pressure effect on the fluorescence quantum yield of naphthalene in supercritical CO$_2$, 34°C.

Liquid: 26 °C, 95.3 atm.
Previous investigations of solute fluorescence lifetimes in supercritical fluids were published. They demonstrate that a minute change in pressure can lead to a large change in the fluorescence lifetime of a solute in a supercritical fluid. A threefold decrease in the fluorescence lifetime of 6-propionyl-2-(dimethylamine)naphthalene in supercritical nitrous oxide was observed in the vicinity of the critical point, i.e., $T_R = 1.01$ and $0.952 < P_R < 1.10$ [12]. For the same pressure range, this pressure effect was not observed in the subcritical region of nitrous oxide [12]. The decrease in fluorescence lifetime with increased pressure does not prevail for all supercritical fluids. Fox and coworkers observed an increase in the fluorescence lifetime of 9-cyanoanthracene (9CA) in supercritical ethane as its density increases [37]. The reduced pressure range probed was from 0.69 to 5.78. Even if the trend is not the same, the density augmentation around (9CA) was one of the reasons that accounted for the unusual change in the fluorescence lifetime with the pressure.

**Calculation of the critical distance, $R_o$.**

The determination of the overlap integral, $J$, as well as the the fluorescence quantum yield of the energy donor are required. The magnitude of the overlap integral is mainly dependent upon the magnitude of the fluorescence intensity of the energy donor and the molar extinction coefficient of the energy acceptor over the wavelength range where the overlap occurs (equations 9 and 10). The molar extinction coefficients for anthracene at various pressures were obtained by measuring the UV-visible spectrum of a known concentration of anthracene as described in the experimental section.
These spectra were obtained by placing a high pressure cell on one side of the dual beam spectrophotometer and another high pressure cell in the reference beam. Prior any experiments were performed, a UV-visible spectrum was taken with the 2 cells empty. The cells were positioned in a way to get no absorbance. However, the cell in the reference beam was only “air filled”. The CO₂ solvent apparent absorbance was obtained by placing it in the sample side of the spectrophotometer. The absorbance spectrum of anthracene was then the difference between the two spectra measured in this manner. Figures 12 and 13 show the variation in the observed CO₂ background at 280 and 360 nm as a function of the pressure. At least seven replicate measurements of complete CO₂ spectra as a function of pressure were obtained. CO₂ does not absorb light in the near UV or visible region of the electromagnetic spectrum [38]. Absorption is first observed at wavelength less than 175.0 nm [39]. Therefore, the observed variation in the CO₂ background with pressure is due to the variation in light scattering with pressure. For the conditions studied, the light scattering is expected to be described approximately as Rayleigh scattering that varies with the inverse fourth power of the wavelength [40]. This accounts for the large difference in the CO₂ background signal at 280 nm and 360 nm. A large increase in the light scattering was observed at approximately 80 atm at both 280 nm and 360 nm. In supercritical CO₂ at 34 °C, the isothermal compressibility is highest at approximately 80 atm [1]. This is also the pressure where clusters of CO₂ reach their maximum size in neat solution [1]. From light scattering experiments, Cataldi and Drickamer previously predicted clustering in pure supercritical solvents [39].
Figure 12. Variation of the apparent absorbance of CO$_2$ with pressure.

34 °C, 280 nm.

The error bars not shown on the graph are smaller than the dots.
Figure 13. Variation of the apparent absorbance of CO$_2$ with pressure.

34 °C, 360 nm.

The error bars not shown on the graph are smaller than the dots.
The increased clustering at 80 atm would readily account for the observed variation in light scattering in supercritical CO₂ at 34 °C.

The relative intensity of each peak of the UV-visible absorption spectrum of anthracene in supercritical CO₂ was determined at different pressures. These experimental results were then treated by PeakFit software in order to determine the value of the molar absorption coefficient of the anthracene at any wavelength between 300 and 375 nm. (The peak fits had a correlation coefficient >0.99).

The value of the quantum yield of the energy donor in the absence of an energy acceptor was experimentally determined as mentioned previously. Literature values of the refractive indices of carbon dioxide at 632.8 nm as a function of pressure at 34 °C were also used [41], [42]. A value of 2/3 was taken for K² accordingly to the literature [28]. The validity of this assumption is often in doubt in the case of macromolecules because the dynamic averaging is prevented to some extent by the structure of the macromolecule. In the present case, dynamic averaging is believed to occur during the time interval associated with the energy transfer. In a free liquid solution this is a valid assumption [19]. Cage effects on reaction rates are known to occur in supercritical solvents although it is not unusually strong compared to liquid solvents [43].

The dependence of the J term on the pressure of the CO₂ is a result from the direct effect of pressure change on both the molar absorption coefficient of the anthracene and to a lesser extent the fluorescence spectrum of naphthalene. Figure 14 shows that there is no significant effect of the pressure on the naphthalene fluorescence spectra.
Figure 14. Effect of pressure on the fluorescence of naphthalene in supercritical CO$_2$, 34 °C. Excitation: 280 nm.

(+) 80.0 atm, (■) 81.8 atm, (□) 86.8 atm, (○) 83.3 atm.
Finally using the absorbance and fluorescence spectra of anthracene and naphthalene respectively, the area representing the overlap integral, J, was calculated using PeakFit™ software. Table 4 lists the experimental values of J and R₀ as a function of the pressure of CO₂. Figure 15 illustrates the variation of the critical distance, R₀, with the pressure. The critical distance increased with increasing CO₂ pressure, and the average critical distance across the observed CO₂ pressure range is similar to that found for aromatic compounds in various liquid solvents (ca. 20Å) [19]. As it should be noticed in Figure 15, a significant variation of R₀ with the pressure is observed.

### Calculation of the average intermolecular distance, R.

The molar extinction coefficients of anthracene and naphthalene at 280 nm and 360 nm were determined in order to calculate the parameters P and E according to equations 11 and 12, respectively. The fraction, P, of the total absorbed radiation at λ₁(280 nm) due to the energy donor (naphthalene) was calculated from the absorbance of anthracene and naphthalene at 280 nm. Figures 16 and 17 are the molar extinction coefficients of anthracene and naphthalene at 280 nm. The curve for the naphthalene has the same general shape as found for anthracene.

There is a substantial variation of the molar extinction coefficient of the solute with the pressure. The density dependence of the molar absorption coefficient in supercritical fluids was observed both in infrared and in UV-visible absorption spectroscopies [44], [45]. A 1.2 increase in the UV-visible molar absorption coefficient of
Table 4. Pressure effect on the overlap integral, $J$ and critical distance, $R_0$.

<table>
<thead>
<tr>
<th>Pressure (atm)</th>
<th>$J$ (cm$^6$ mol$^{-1}$ 10$^{13}$)</th>
<th>$n^*$</th>
<th>$R_0$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>77.9</td>
<td>2.32±0.83</td>
<td>1.0657</td>
<td>19.43±1.31</td>
</tr>
<tr>
<td>78.2</td>
<td>7.80±1.32</td>
<td>1.0657</td>
<td>22.86±0.72</td>
</tr>
<tr>
<td>81.7</td>
<td>7.95±0.28</td>
<td>1.0805</td>
<td>22.51±0.11</td>
</tr>
<tr>
<td>83.3</td>
<td>12.5±1.81</td>
<td>1.0955</td>
<td>23.92±0.76</td>
</tr>
<tr>
<td>85.1</td>
<td>13.9±2.01</td>
<td>1.1105</td>
<td>23.71±0.04</td>
</tr>
<tr>
<td>86.8</td>
<td>16.2±2.01</td>
<td>1.1219</td>
<td>24.60±0.16</td>
</tr>
</tbody>
</table>

*refractive indices values obtained from references [41].
Figure 15. Effect of pressure on the critical distance, $R_0$, in supercritical CO$_2$, 34 °C.
Figure 16. Effect of pressure on the molar absorption coefficient of anthracene in supercritical CO$_2$, 34 $^\circ$C, 280 nm.
Figure 17. Effect of pressure on the molar absorption coefficient of naphthalene in supercritical CO₂, 34 °C, 280 nm.
2-methyl-2-nitrosopropane in supercritical CO₂ (60 °C) over 0 < \rho < 2 was observed. In the case of naphthalene at 280 nm, there is a 1.25 increase in its molar extinction coefficient over the same reduced density range. The region of the curve where the slope changes the most (near a pressure of 80 atm at 34 °C) corresponds to conditions where there is a clustering of the CO₂ solvent molecules [8], [1]. These observed changes in the molar extinction coefficients, \( \epsilon \), are not artifacts caused by the variation in the \( \lambda_{\text{max}} \) for each absorption band with pressure because the observed shift in \( \lambda_{\text{max}} \) is not related to the shift in wavelength. At pressures greater than 80 atm, the molar absorptivity values level off although there is a shift in the wavelength of maximum absorption. Also the wavelengths chosen for this analysis, 280 nm and 360 nm, were selected so they are not near the \( \lambda_{\text{max}} \) of the absorbance band and therefore their molar extinction coefficient, \( \epsilon \), is not as sensitive to wavelength shifts as the wavelengths near the maximum. This corresponds to the pressure at which the partial molar volume of a solute in supercritical fluid undergoes its largest negative change [1]. The effect of pressure upon the molar extinction coefficient does not reflect totally the clustering effect of CO₂ around the solute but parallels the curve of the pressure effect upon the dielectric constant of the fluid (although there is a shift in the pressure at the inflection point) (see Figure 18) [46].

Figure 19 shows the variation in P as a function of CO₂ pressure. Naphthalene is clearly the predominant absorber at 280 nm. However the molar extinction coefficient of anthracene clearly varies more substantially with pressure than does that of naphthalene which results in the observed variation in the P term with pressure. The curves relating
Figure 18  Effect of pressure on the dielectric constant of CO$_2$

(□) 32.1 °C, (▲) 40.3 °C from reference [46].
Figure 19. Efficiency of the energy donor absorption, $P$, in supercritical CO$_2$, 34 °C.
the molar extinction coefficients of anthracene and naphthalene in supercritical CO$_2$
parallel closely the curve of density versus the pressure and correlates with the previously
reported density effects upon UV-visible absorption found by Sun [47]. Nevertheless, the
clustering of solvent molecules around the solute does not totally explain the shape of the
curve. As a matter of fact, after having reached its maximum size at a pressure of 80 atm
(at 35 °C), the cluster size decreases with pressure. The calculation of the efficiency of
energy transfer, E, was done through the determination of the molar extinction coefficient
of anthracene at 360 nm. Figures 20 and 21 are the pressure effect on the molar
coefficient of anthracene at 360 nm and the E term, respectively. The center-to-center
intermolecular distance, R, between anthracene and naphthalene was calculated according
to equation (4). Figure 22 is the pressure effect on the intermolecular distance, R. Over
the pressure range of 78-82 atm, the measured distance between the two solutes decreased
with increasing pressure. The pressure range of 78-82 atm also corresponds to the region
where the isothermal compressibility of CO$_2$ decreases drastically. This decreased
compressibility causes the cluster size of the CO$_2$ molecules around the analytes to lower
proportionately. If, as we believe, naphthalene and anthracene are enveloped by separate
solvation shells, then the measured decrease in internuclear distance in this region would
be primarily due to the cluster size decrease. The solutes seem to be in the closest
proximity at a pressure larger than 80 atm due to the clustering effect of CO$_2$. 
Figure 20. Effect of pressure on the molar absorption coefficient of anthracene in supercritical CO₂, 34 °C, 360 nm.
Figure 21. Efficiency of the energy transfer, $E$, in supercritical CO$_2$, $34\, ^\circ$C.
Figure 22. Average intermolecular distance, $R$, in supercritical CO$_2$, 34 °C.

(+) time resolved energy transfer, (■) sensitized fluorescence.
The existence of enhanced solute-solute interactions for pyrene in supercritical CO$_2$ has been reported at a concentration as low as $6 \times 10^{-7}$ M [14]. This result was illustrated by the existence of pyrene excimer. The excimer formation results from the higher probability for an excited molecule to get close enough to a non excited molecule in the CO$_2$ cluster. The clustering of solvent molecules is loose enough to accommodate several molecules of pyrene. Nevertheless the existence of such excimer occurred in the low reduced density region, especially $\rho_r < 1$ whereas the probability of excimer formation decreases as the reduced density of the solvent get larger than 1. At a reduced density, $\rho_r > 1$, the clustering effect of the solvent around the solute weakens as demonstrated by the study of Sastry and Mukhopadhyay [48]. The solute-solute clustering in the case of pyrene is in agreement with the molecular dynamics study of Wu [49].

Despite the fact that the concentrations of anthracene and naphthalene in this study are 10 times larger, no solute-solute clusters were observed via the detection of excimers. There is no appearance of red shifted absorption band upon decreasing the density of the fluid for either the anthracene or naphthalene solutes. The fluorescence spectrum of both compounds did not show any extra band at longer wavelength. The absence of excimer formation in the case of naphthalene (at a mole fraction of $3.5 \times 10^{-5}$) was also observed by Brennecke et al. [14]. Chandra and Lim studied excimer fluorescence in the case of several polycyclic aromatic hydrocarbons [50]. Their first assumption was that the excimer formation leads to a sandwich type excimer with a D$_{2h}$ configuration. For the studied polycyclic compounds, the interplanar distances of the sandwich configuration
varied from 3 to 4 Å. For the molecule of naphthalene, this interplanar distance was set to 3 Å. They observed that the fluorescence from the lowest excited state is dipole forbidden in these highly symmetric excimers. Excimer fluorescence could only occur by distortion of the symmetry. The absence of excimer formation reported by our data therefore does not exclude the possibility of solute-solute interactions with a sandwich type configuration. However, our measured intermolecular distances are far too large to correspond to the sandwich spacing in the excimer. Moreover we have an equimolar mixture of molecules of almost the same size. The probability of finding two alike molecules at a given distance should be the same as the one of finding two unlike molecules. In other words, if the supercritical solvent is able to assist the solute-solute clustering and if this process occurs, the intermolecular distance between the energy donor and the energy acceptor should be the shortest at low reduced density ($\rho_r < 1$) for a pressure smaller than 80 atm. The curve of the intermolecular distance versus pressure does not show the expected pattern but the opposite. The largest intermolecular distances are found at pressures where we would have expected the molecules being the closest via assisted solvent clustering. This result dismisses the believe in solute-solute clustering mechanism in this set of experimental conditions. Following the theory of Sastry and Mukhopadhyay which states that there exists an equilibrium between clusters of mixed solutes ("closely packed clusters") and clusters of pure solute, the predominance of clusters of pure solutes occurs in our case.
The variation of the intermolecular distance with the pressure could be explained by the change in the free volume of the solution [51]. In the near critical pressure region \((0.5 < \rho_r < 1.5)\) there is a decrease in the free volume and although the cluster size does not vary drastically, the solutes are getting closer due to the isothermal compressibility of the fluid. The values of the intermolecular distance between the solutes as well as the comparison with the literature may lead to the conclusion that solute-solute clustering does not occur for the anthracene and naphthalene solutes in these conditions. The curve of the intermolecular distance versus the pressure follows the trend of aggregation of solvent molecules around the solute. At reduced density greater than one, the cluster size decreases. The molecules are thus getting closer and closer.

**Determination of the energy transfer by multiexponential analysis of time resolved fluorescence decay data.**

The decays for naphthalene and anthracene could not be fit successfully to a sum of less than four exponentials. Figure 23 is a typical fluorescence decay of naphthalene and an equimolar mixture of anthracene and naphthalene. The energy transfer from the naphthalene to the anthracene leads to a reduction in the fluorescence lifetime of the energy donor. The efficiency of the energy transfer was from the ratio of the fluorescence lifetimes of the naphthalene with and without the energy acceptor according to equation (5). Table 5 lists the pressure effect on the experimental fluorescence decays of naphthalene.
Figure 23. Fluorescence decays of naphthalene and naphthalene/anthracene in supercritical CO$_2$, 34 °C.
Table 5. Fluorescence lifetime of naphthalene in supercritical CO$_2$.

<table>
<thead>
<tr>
<th>Pressure (atm)</th>
<th>$\tau^a$ (ns)</th>
<th>$\tau^b$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>77.9</td>
<td>61.23±0.41</td>
<td>17.28±0.74</td>
</tr>
<tr>
<td>78.2</td>
<td>59.42±0.46</td>
<td>17.67±1.32</td>
</tr>
<tr>
<td>81.7</td>
<td>58.50±0.89</td>
<td>17.00±0.27</td>
</tr>
<tr>
<td>83.3</td>
<td>56.23±1.53</td>
<td>16.94±2.05</td>
</tr>
<tr>
<td>85.1</td>
<td>55.51±1.17</td>
<td>13.83±1.07</td>
</tr>
<tr>
<td>86.8</td>
<td>54.54±0.31</td>
<td>16.50±0.59</td>
</tr>
</tbody>
</table>

$\tau^a$ (ns): fluorescence lifetime of naphthalene without the energy acceptor (anthracene).

$\tau^b$ (ns): fluorescence lifetime of naphthalene with the energy acceptor (anthracene).
Solvatochromic shifts in supercritical CO₂.

The magnitude of the solute-solvent interactions is probed through the studies of the solvent-induced spectral shift of the solute. Spectroscopic measurements are usually used to investigate these interactions. The frequency shift and intensity of an electronic or vibrational band in UV-visible or infrared spectroscopy and the chemical shift and spin-spin coupling in magnetic resonance spectroscopy give valuable information [52], [53]. The magnitude of the spectral shifts depend on the nature of the interactions between the solute and the solvent. Large shifts are due to specific interactions. They are mainly chemical interactions such as hydrogen bonding, proton or charge transfer, ionization or isomerization equilibria. Smaller shifts are observed when there are no specific interactions such as dipole-dipole-induced, dipole-dipole.

As mentioned earlier, the strength of solute-solvent interactions in supercritical solvents is usually investigated through the studies of the absorbance and fluorescence of polar solutes that exhibit solvatochromic frequency shifts in their spectra with polarity change. The magnitude of the frequency shift can be related to the solvating ability of the supercritical solvent. Yonker et al. compared the wavelength shift for 2-nitroanisole in polar and apolar supercritical fluids, NH₃ and CO₂, respectively [54]. The wavelength shift for the solute probe was 2 nm in CO₂ whereas it was equal to 12 nm in NH₃. Large solvatochromic effects are usually seen in the solvation of polar molecules in polar solvent. In this case, the dipole-dipole and dipole-dipole induced interactions contribute also to the frequency shift. For the polyaromatic compounds in carbon dioxide, the London forces
are the main contributing effects. Smith et al. reported the similarity between the bathochromic shift of 2-nitroanisole probe in various nonpolar supercritical fluids and their $\pi^*$ values (Kamlet and Taft parameter) that measures the polarity-polarizability [2]. As the density of the fluid increases so does its polarizability per volume. Regarding the pressure effect, the larger the variation in the $\pi^*$ value of the solvent is the larger the bathochromic shift of the solute probe is.

In this study, the solvatochromic behavior of anthracene in supercritical CO$_2$ was investigated. The wavelength shifts of two of its absorption bands as a function of the reduced density of CO$_2$ (critical density $\rho_c = 0.472$ g/ml) are plotted in Figure 24 and 25, respectively. The errors for these values correspond to a standard deviation of 95%. The larger wavelength shifts are observed at the reduced densities greater than 1.2. The wavelength shifts are small for $0.5 < \rho_r < 1.2$, except in the vicinity of the critical point ($\rho_c \approx 1$) where an "abnormal behavior" appears. The shifts in the electronic absorption transition are attributed to the difference in the energy of interaction with the solvent molecules between the ground and excited state of the solute. The magnitude of this spectral shift is dependent upon several factors such as the change in dipole moment and polarizability upon the transition, the size of the molecules and the interactions of the solute probe with the solvent. The importance of the supercritical fluid density on the solvation properties of the solute was evidenced from the Figures 24 and 25. A discussion of this effect is proposed in the next section.
Figure 24. Wavelength shifts of the peak of maximum absorption for anthracene as a function of the reduced density of CO$_2$, 34°C.

The error bars not shown on the graph are smaller than the dots.
Figure 25. Wavelength shifts of the peak of maximum absorption for anthracene as a function of the reduced density of CO$_2$, 34°C.

The error bars not shown on the graph are smaller than the dots.
The solvatochromic behavior of a solute in a solvent can be described by the Onsager Reaction Field theory [55]. The theory is based upon the continuum model of dipole solvation. The solute is assumed to behave as a point dipole at the center of a solvent cavity. The distribution of the solvent molecules around the solute creates an electrostatic field, $E_R$, experienced by the solute. The magnitude of the electrostatic field is expressed as follows:

$$E_R = \frac{\mu}{a^3} \cdot \frac{2(n^2-1)}{(2n^2+1)}$$

where $\mu$, $a$ and $n$ are the dipole moment of the solute in the ground state, the radius of the spherical solute cavity, the refractive index, respectively.

A modification of the Onsager Reaction Field theory was developed by McRae and Bayliss [56]. In addition to the internal field acting on the solute molecule, the transition dipole of the solute induces a polarization of the solvent molecules [57]. During the absorption or emission of light, there is a difference between the charge distributions in the ground and excited states of the solute. Upon the transition, the uneven charge distribution creates an electrostatic field that induces a change in the orientation of the permanent dipole of the solvent molecules. This effect is favored since the lifetime of the excited state ($\sim 10^{-8}$ s) is large compared to the solvent dielectric relaxation time ($\sim 10^{-11}$ s) [57]. This effect called the polarization shift is dominant for nonpolar solutes dissolved in nonpolar solvents but it can obscured by additional intermolecular interactions.
in the case of the solvation of a polar solute in a polar solvent. The McRae-Bayliss adds additional terms to the equation developed by Onsager.

The spectral change of the solute is related to the solvent macroscopic properties by the following equation [58]:

\[ \Delta \nu = (AL + B) \left( \frac{n^2 - 1}{2n^2 + 1} \right) + C \left( \frac{e-1}{e+2} - \frac{n^2-1}{n^2+2} \right) + F \left( \frac{e-1}{e+2} - \frac{n^2-1}{n^2+2} \right)^2 \] (16)

where \( \Delta \nu \) is the frequency shift (compared to a gas-phase reference) in reciprocal centimeters, A and B are the polarizability effect constants, C and D are the dipolar effect constants, L is a "weighted mean wavelength", \( e \) is the dielectric constant of the solvent, \( n \) is the index of refraction. The first term with the constants A and L represents the induced dipole-induced dipole interaction. The second term with the constant B represents the solute dipole-solvent induced dipole interaction. The third and fourth terms represent the dipole-dipole and the solvent dipole-solute induced dipole interactions. For a solvent that has no permanent dipole moment such as CO\(_2\), \( e=n^2 \) [59] and equation (16) is then reduced to:

\[ \Delta \nu = (AL + B) \frac{n^2 - 1}{2n^2 + 1} \] (17)

The application of this theory to supercritical fluids where local densities around the solute are known to exist may not be valid. Measured frequency shifts for UV
absorption maxima of solutes in supercritical solvents have been compared to those predicted from the McRae-Bayliss model. The attempt to correlate the bathochromic shift observed for 4-(dimethylamino)benzonitrile in nonpolar supercritical ethane and polar trifluoromethane with the Onsager reaction field theory failed [60], [61]. Near the critical point, the observed frequency shifts are markedly higher than predicted by the McRae-Bayliss model. It is now well documented that this discrepancy is caused by substantial clustering around the solute occurring in this highly compressible region of the diagram. The solvatochromic shifts of polar dyes have been so thoroughly studied near the critical point of supercritical solvents that the shift is often used to predict the extent of clustering around a given solute [61].

An attempt has been made in this study to see if the behavior of supercritical fluids has some analogies with the behavior of low compressibility solvents. Onsager’s reaction field theory could not be applied since the frequency shift derived is proportional to the change in the dipole moment of the solute upon excitation [40]. In the case of anthracene, there is no variation of the dipole moment upon excitation [62]. There is however a change in its polarizability upon excitation [63]. In the case of polyacene molecules, an increase in the polarizability upon an electronic transition occurs. The changes in the polarizability upon the transition [S₀→S₁] for anthracene and naphthalene are 15.7 and 9.8 (Å³) respectively in nonpolar solvents [64] (from electrochroism measurements). The measured frequency shift in the anthracene S₀ to S₁ electronic manifold is therefore a measure of the solvent effect on the polarizability change, Δα, in anthracene upon
excitation. Moreover an increase of the strength in dispersive interactions is likely to occur if there is a concomitant change between the polarizability of the excited and ground state. For the case of liquid solvents, Bakhshiev et al. derived the frequency shift of the absorption maximum due solely to dispersive interactions between a solute and a solvent using equation (18) which is an extrapolation of the London equation [65].

The frequency shift of maximum absorption in a nonpolar medium is expressed as follows:

$$h\Delta \lambda_{\text{disp}} = \frac{2(\alpha_g - \alpha_e)}{3r^3} \frac{(JJ')}{(J+J') (n^2-1)} \frac{1}{(n^2+2)}$$  \hspace{1cm} (18)

where $\alpha_g$, $\alpha_e$ are the polarizability of the solute in the ground and excited states respectively, $r$ is the van der Waals radius of the solvent molecule, $J$ and $J'$ are the ionization potentials of the solute and solvent molecules and $n$ is the refractive index of the medium. The ionization potentials of the solute and solvent were taken as 7.55 and 13.773 eV [66]. An expression relating the frequency shift caused by dispersive interactions similar to equation (18) was also derived by Liptay [63].

If Bakhshiev's equation describes the dispersive interactions between solute and solvent in the supercritical state, then a plot of of the frequency shift, $\Delta v$, versus the refractive index function $(n^2-1)/(n^2+2)$ should be linear with a positive slope. Figure 26 shows the frequency shift (relative to the gas-phase condition) of two electronic bands in the $S_0$-$S_1$ absorption manifold of anthracene as a function of CO$_2$ at 34 °C.
Figure 26. Difference in the frequency of maximum absorbance of anthracene in supercritical CO₂ (34 °C), compared to the gas-phase condition.

(+ ) gas-phase 30,480 cm⁻¹, (■) gas-phase 29,020 cm⁻¹.
The frequencies in the gas-phase were taken as 30,480 cm\(^{-1}\) (328 nm) and 29,020 cm\(^{-1}\) (344 nm) [67]. The values of refractive indexes of CO\(_2\) were calculated from the density of CO\(_2\) based on the Lorenz-Lorentz equation [41]:

\[
\frac{(n^2 - 1)}{(n^2 + 2)} \times V = 6.600 + \frac{1.25}{V} - \frac{2.64}{V^2}
\]  

(19)

where \(n\) is the refractive index and \(V\) is the molar volume in cm\(^3\) g\(^{-1}\). In Figure 26, a bathochromic shift of the frequency occurs for the pressure range of 70 - 80 atm, then a hypsochromic shift occurs from 80-83 atm. As the pressure is increased further, bathochromic shifts occur but two different slopes changes are evident. From approximatively 83-100 atm, the slope in the curve is steep then from approximatively 100-130 atm the curve has a more shallow slope. Figure 27 is a plot of frequency shift, \(\Delta v\), versus the refractive index function \((n^2 - 1)/(n^2 + 2)\) for the studied pressure range. It shows that for the values of the function [\((n^2 - 1)/(n^2 + 2)\)] = 0.06-0.15 which corresponds to the pressure range of 80-83 atm, Bakhshiev’s equation seems to describe approximatively the variation of the frequency shift. Therefore at least in that region, the dispersive interactions in supercritical CO\(_2\) are approximated by the liquid-state theory. This is the same approximate region of the phase diagram where the Onsager reaction field theory seems to work well for polar molecules. However, the bathochromic shift is too high at very low pressures to fit this theory and the hypsochromic shift occurs at 80 atm. Similar discrepancies were observed and were explained as being due to the clustering of
Figure 27. Frequency shifts of the peak of maximum absorption for anthracene in supercritical CO₂ as a function of the refractive index function.

(+) experimental, (■) calculated according to equation (18).
solvent molecules around the solutes [61], [68] (even in nonpolar supercritical fluids). The shifts at the low region of the curve (70-83 atm) are most likely predominately controlled by clustering interactions. Since the polarizability and therefore the strength of the dispersive interaction scales approximatively with density, the initial bathochromic shift is simply a result of the CO₂ local density around anthracene increasing drastically as the compressibility increases. The isothermal compressibility of CO₂ is plotted in Figure 28 according to reference [16]. The cluster size of CO₂ scales with the compressibility. The largest cluster is expected to occur at the highest compressibility. In the region from 80 to 83 atm, the hypsochromic shift is caused by the local density decreasing near anthracene as the compressibility decreases.

The effect of local density on the solute spectral shift were evidenced by Figures 24 and 25. It was also evidenced by the equation derived by Longuet-Higgins et al. and the early experimental study of the pressure effect on the optical absorption spectra from Robertson et al [69], [70].

Several slopes changes are observed. Johnston and coworkers investigated the solvatochromic shifts of solutes in polar (trifluoromethane) and nonpolar supercritical fluids (carbon dioxide and ethane) [71]. They explained the formation of clusters of solvent around the solute as a multiple stage process. Each step is density dependent. The shape of the curves presented in that study reveals also three slopes. In the lower reduced density region (ρ_r < 0.5), there is a slow shift in the wavelength of maximum of absorption of the solute. The cluster of solvents around the solute is building up.
Figure 28. Isothermal compressibility of CO₂, 35 °C from reference [16].
At reduced density lower than 0.5, in the so called gas-like region the experimental wavelength shift is usually higher than expected due to the formation of clusters. The next stage of this process is marked by a plateau region. At reduced density greater than 0.5, the cluster size does not change much and thus the wavelength shift varies accordingly with the change in the bulk properties (refractive index, dielectric constant, change in free volume). The wavelength shift is minimal. The aggregation of solvent molecules has reached its maximum value. As the reduced density of the fluid increases past $\rho_r=1.5$, the properties of the solvent are becoming more liquid like. Moreover the isothermal compressibility of the fluid is lower in this pressure range and a larger increase in the pressure is needed to add more solvent molecules to the solvation sphere around the solute.

In summary, this study as well as the results reported in the literature, seems to indicate that the bulk properties are not accurate enough to describe quantitatively the properties of the solvent in the case of a supercritical fluid. The existence of a density gradient in a supercritical fluid prevents it from being considered as a medium of uniform macroscopic properties.
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CHAPTER III

SOLVENT-SOLVENT AND SOLUTE-SOLVENT INTERACTIONS

IN LIQUID METHANOL/CARBON DIOXIDE MIXTURES

INTRODUCTION

Supercritical fluids are commonly used in a broad range of applications, such as extractions, chromatographic separations, and to some degree in chemical reactions [1], [2]. The advantages of using a supercritical fluid medium in all of the above applications derive from the unique properties linked with this state. In the highly compressible state, i.e., at temperature and pressure in the vicinity of the critical point, small change in the pressure leads to a large change in the fluid density. It is then possible to adjust many of the properties of the fluid as well as of its solute by changing the density. The solvent strength and the solubility are among the properties easily tunable. Density variation is very often used in chromatography where the mobile phase is compressible (supercritical fluid chromatography). The pressure dependence of the solubility of the analytes is a way of controlling the selectivity in supercritical fluid extraction. Change in pressure can also be very useful in tuning reaction in supercritical fluid media. The concentration of the reacting species can be easily adjusted and hence the reaction rate modified. The reaction rate constant itself can be varied by a change in the solvent strength of the fluid.
Numerous workers reported a large enhancement factor for the chemical reaction rate in supercritical fluids compared to liquids. Brennecke and coworkers studied the reaction of benzophenol with isopropanol in supercritical carbon dioxide and reported a five-fold increase in the bimolecular rate constant over its value in liquid isopropanol [3]. The rate constant for the thermal decomposition of α-chlorobenzyl methyl ether in supercritical 1, 1 difluoromethane underwent a two order of magnitude increase near the vicinity of the critical point of the solvent [4].

Limitations of the use of pure supercritical fluids.

The use of supercritical fluids is somewhat restricted. Some of the main drawbacks of using supercritical fluids are that the most commonly-used fluids, such as CO₂, ethylene or SF₆ are nonpolar and/or have a low polarizability. CO₂ has a polarizability value (26.5 x 10⁻²⁵ cm³) that is smaller than most of the n-alkanes (except CH₄) [5]. To reach a value of the Hildebrand solubility parameter similar to that of liquid isopentane, the pressure of CO₂ must be increased to 200 atm at 35 °C. The lack of a permanent dipole moment of CO₂ prevents its use as a solvent for polar compounds as well as for high molecular weight apolar compounds. Jay and coworkers demonstrated the limited solvent strength of CO₂ when using it both in the supercritical state and in the vicinity of the critical point of CO₂ [6]. The solubility of β-carotene was measured in traditional organic solvents as well as near and above their critical state. β-carotene had a low solubility in CO₂ as compared with the values obtained in some near critical fluids such as ethylene and SO₂. The data of Jay et al. highlighted that the solvent strength is influenced both by the polarizability
and the dipole moment of the fluid. This is particularly true when the interactions between
the components are mainly dispersive. This latter case occurs for the solvation of apolar
analytes. The use of nitrous oxide whose critical parameters are similar to those of CO₂
is not advised. Despite its slightly higher dipole moment that would make it a better
solvent than CO₂, explosion hazards prohibit its use. These limitations restrict the type
of applications that could be performed in supercritical medium.

Applications of the cosolvent/CO₂ fluid mixtures.

In order to circumvent the lack of polarity, more polar solvents such as water,
n-alcohols, ammonia were used as supercritical fluids. Nevertheless, polar supercritical
fluids, are of limited application due to their high critical pressure and temperature (see
Table 1 in Chapter I). Moreover, the use of some of these fluids in the supercritical state
(especially water and ammonia) requires caution because of their high oxidizing power and
high ionic strength.

The increase in solvent strength can also be achieved by the addition of cosolvents,
such as n-alcohols, organic acids, tetrahydrofuran, to pure supercritical fluids. In a pure
supercritical fluid, the pressure or the temperature are the only tunable parameters that can
affect the density of the fluid. The addition of an organic modifier introduces the ability
to have some chemical interactions with the solute (Lewis acid/base, electron
donor/acceptor complexes, hydrogen bond formation). The nature and the concentration
of the organic modifier are some variables that can be changed to change the polarity and
the solvent strength of the fluid mixtures. In chromatography, the organic cosolvent
(modifier) affects the density of the mobile phase and the properties of the stationary phase. The solute/solvent interactions are then modified and the selectivity and retention time of the solute are changed [7]. In extraction, the cosolvent increases the solubility of some solutes. The solubility enhancement is due to chemical interactions between the cosolvent and the solute [5], [8].

Unfortunately, the addition of a cosolvent to a supercritical fluid greatly increases its critical temperature and pressure. A low mole fraction of organic modifier (usually less than 15-20 mole percent) is then preferred when working with supercritical fluid mixtures. The minimum amount of organic solvent that can be added to the supercritical fluid limits the potential to achieve a significant increase in the solvent strength of an apolar supercritical fluid.

**Enhanced-fluidity liquid mixtures as alternative solutions.**

In order to circumvent these drawbacks, we recently studied mixtures of hydrogen-bonded liquids and liquefied gases, such as methanol mixed with CO₂ in the subcritical state. Methanol is very often used as a cosolvent because it can interact as an acid/base with a variety of solutes. It is both a good hydrogen bond donor and hydrogen bond acceptor. [9]. Commonly-used liquids have high solvent strength but they have viscosities that are an order of magnitude higher than supercritical fluids and diffusivities that are two orders of magnitude lower than those of supercritical fluids (see Table 2 of Chapter I). The optimum solvent would possess the positive attributes of both supercritical fluids (fast mass transport due to their high molecular diffusion coefficients) and commonly-used
liquids (high solvent strength). Enhanced-fluidity liquid mixtures have viscosities and diffusivities that are intermediate between that of a supercritical fluid and common liquids. The low viscosity and the associated high diffusivity of these mixtures have proven to increase the efficiency and speed of separation in high performance liquid chromatography (HPLC) [10], and also increase the extraction efficiency of polar solutes from solid matrices. They also maintain approximately the solvent strength of the hydrogen-bonded liquid even when large proportions of CO₂ (such as 60 volume %) are added to the mixture [11].

The properties of mixtures of an organic liquid with a liquefied gas are not well documented. Johnston and coworkers characterized some of the density dependent properties of supercritical mixtures, such as pentane/CO₂ as well as methanol/CO₂. Nevertheless, they focused their studies to cosolvent-supercritical fluid. The temperature and pressure were above the critical pressure and temperature of the fluid mixture as well as near the critical end points of the three components mixture [12]. Substantial variation in analyte solubility was noted near the upper critical endpoint (UCEP) as well as the lower critical end point (LCEP) in the case of naphthalene dissolved in supercritical CO₂. This increase was explained to be due to the corresponding change in the apparent solute partial molar volume [13], [14]. Moreover, they limited their studies to low mole percent of the cosolvent in the supercritical fluid namely < 10 mole %.

The solvation in binary mixtures was studied trough solvatochromic studies. Nevertheless, most of the work focused on the solvation of polar compounds (2-nitroanisole, phenol blue) in supercritical fluid mixtures [15], [16], [17].
The supercritical fluid mixtures of n-alcohols/CO₂ and organic solvent/CO₂ revealed significant clustering of the organic cosolvent molecules around the solute. The condensation of the organic molecules around the solute occurs because there are some interactions between the solute and the cosolvent. The hydrogen bond formation between acridine and methanol explains its solubility enhancement [7]. The addition of only 3.5 mole % of methanol to CO₂ produces an increase in the solubility of acridine similar to a pressure increase from 120 to 350 atm. The same condensation of cosolvent molecules around 2-nitroanisole was observed for binary mixtures of 2-propanol/CO₂ both on the supercritical and subcritical states [17]. Nevertheless, the magnitude of the excess of organic solvent in the cybotactic region was less in the subcritical state than in the supercritical state (in region where the isothermal compressibility of the fluid mixtures was high). In the supercritical state, the local density around the 2-nitroanisole is enriched by a factor of 4 in 2-propanol over the bulk concentration. In subcritical mixture, the enrichment is smaller (2 to 2.5 times enhancement). The preferential solvation phenomenon in the subcritical state was also pressure dependent.

The data published in the literature concerning the density of MeOH/CO₂ were also not comprehensive. The temperature and composition domains are far beyond the ones that are of interest with enhanced-fluidity liquid work. The density values reported are mostly related to the use of MeOH/CO₂ as supercritical fluid mixtures. They are limited to a maximum mole fraction of MeOH of 25 % and for temperatures higher than 40 °C [18], [19]. The only other data available deal with mixtures that are in the liquid-gas phase (pressure below 50 atm) [20]. It is then mandatory to determine experimentally the
density of the methanol/CO₂ fluid mixtures since the density is one parameter in the
equation of state to predict others variables (such as solubility and partial molar volume
of solute).

Estimation of the critical temperature of the methanol/CO₂ mixtures.

Knowledge of the critical point of a fluid mixture is of prime interest to know if
the experimental conditions are in the sub or supercritical domain. Some limitations exist
for the estimation by analytical methods of the critical parameters of the fluid mixtures in
which one of the components is polar and able to form hydrogen bond (methanol). The
most often used equations to estimate the critical temperature of a mixture are the Li and
Chueh-Prausnitz [21]. Unfortunately these equations were derived for mixtures of
hydrocarbons or other nonpolar compounds. They do not predict accurately the critical
temperature and pressure of more polar mixtures. The critical temperature and pressure
of a liquid mixture can be estimated via the set of equations derived from the

The COSTALD correlation is based upon the

expressed as a function of their corresponding reduced parameters. The parameters
most often used are the critical temperature and pressure and the pseudocritical
temperature and pressure for a mixture. Unfortunately, the two parameter equations are
of limited use because they describe with a good accuracy only nonpolar and mixtures of
nonpolar compounds. Third and fourth parameters were also introduced to extend the
COSTALD application to more polar fluids. Numerous studies attempted to estimate the
appropriate additional parameters. Pitzer introduced the acentric factor as third parameter. Tarakad et al. describe the use of the second virial coefficient and radius of gyration as additional parameters [22]. Unfortunately, even the introduction of a third and fourth parameter does not accurately predict the volumetric properties of polar fluids or mixtures of hydrogen bonding compounds. The most widely used set of COSTALD equations are the Yen-Wood and the Hankinson-Thomson [23], [24]. Unfortunately, the Yen-Wood equations require specific parameters for each compound. The modified Hankinson-Thomson does not describe accurately the density of compressed fluid mixtures in which the components are strongly associated such as methanol/CO\textsubscript{2} mixtures. Table 7 is an estimation of the critical temperature of the methanol/CO\textsubscript{2} fluid mixtures calculated via the equations derived from the COSTALD set (equations A through C) (see Table 6). Equation A is the modified Hankinson- Brobst-Thomson equation. The calculation of the critical temperature with these equations leads to an absolute percentage error of 1 to 3%. The calculated critical temperature values vary substantially depending on the equation used. In addition, the calculated values differ from the experimental values as well [25]. The use of analytical equations to estimate the critical parameters must be done with caution in the case of strongly associated mixtures. In our study, the values were used only as an estimation of how far from the critical point the studies were undertaken.

The paucity of data as well as the lack of sufficient accuracy provided by the analytical equations, requires the experimental characterization of the properties of an organic solvent/CO\textsubscript{2} fluid mixture at subcritical conditions. The present study focuses on the determination of some properties of the methanol/CO\textsubscript{2} liquid mixtures derived from
Table 6. Mixing rules equations for the estimation of the critical temperature of the MeOH/CO$_2$ mixtures, $T_{cm}$ [26].

<table>
<thead>
<tr>
<th>Mixing Rule Equation</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation A</td>
<td>$\sum_i \sum_j x_i x_j V_{ij}^* T_{ij}/V_m^*$</td>
</tr>
<tr>
<td>Equation B</td>
<td>$\sum_i V_i^* T_{ci}/\sum_i x_i V_i^*$</td>
</tr>
<tr>
<td>Equation C</td>
<td>$\sum_i x_i V_i^* (T_{ci})^{0.5}/(\sum_i x_i V_i^*)^2$</td>
</tr>
</tbody>
</table>

where \[ T_{ci} V_i^* = (V_i^* T_{ci} V_j^* T_{cj})^{0.5} \]

- $x_i$ mole fraction of the $i$th component
- $V_{ci}$ critical volume of the pure component
- $V_i^*$ characteristic volume of the pure component
- $V_m$ critical volume of the mixture
- $T_{ci}$ critical temperature of the pure component
- $V_m^*$ characteristic volume of the mixture
Table 7. Calculation of the critical temperature (°C) of mixtures as a function of the mole percent of methanol in carbon dioxide.

<table>
<thead>
<tr>
<th>Mole % Methanol</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li [21]</td>
<td>56.70</td>
<td>81.1</td>
<td>104.3</td>
<td>126.7</td>
</tr>
<tr>
<td>Equation A</td>
<td>43.10</td>
<td>57.10</td>
<td>73.00</td>
<td>90.94</td>
</tr>
<tr>
<td>Equation B</td>
<td>57.10</td>
<td>81.70</td>
<td>105.1</td>
<td>127.2</td>
</tr>
<tr>
<td>Equation C</td>
<td>46.4</td>
<td>63.62</td>
<td>82.52</td>
<td>102.8</td>
</tr>
</tbody>
</table>
density measurements. The experimental conditions are far from any of the the critical endpoints of the mixture.

Herein, macroscopic solvent properties, such as the infinite dilution partial molar volume of the solute, the excess volume of mixing and the isothermal compressibility were determined as a function of mixture composition and pressure. These data demonstrate the feasibility of achieving a fairly high solvent strength in a subcritical state.

**EXPERIMENTAL SECTION**

**Materials.** All compounds were used as received. CO₂ was SFE/SFC grade (99.997% with < 1 ppm H₂O) from Air Products (Allentown, PA). Methanol was HPLC-grade (99.99% pure with < 0.01% H₂O) from J.T. Baker (Phillipsburg, NJ). To remove dissolved air from the methanol, the methanol was sonicated before use. Argon and nitrogen were 99.998% and 99.99% pure respectively, as obtained from Liquid Carbonic Speciality Gases (Chicago, IL). Naphthalene was ≥ 98% pure from Mallinckrodt, Inc., (Paris, KT).

**Instrumentation.** Figure 29 shows the instrumentation used in this study. The densities of the mixtures were measured with a oscillating tube density meter (DMA 45, Anton Paar) that had an external high pressure measuring cell (DMA-512, Anton Paar, Warminster, PA). This density measuring apparatus is capable of density measurements with a precision and accuracy of ± 0.0001 g cm⁻³. The measuring cell was wrapped with an insulating blanket. The temperature of the cell was controlled to within ± 0.005 °C with a Techne TU-16D constant temperature bath. However, the
Figure 29. Experimental setup of the density meter.

A, syringe pump; B and E, valves; C, density meter;
D, pressure transducer; F, restrictor.
temperature measuring devices (the digital thermometer on the bath and a calibrated mercury thermometer) were accurate and precise to ±0.01 °C. The pressure in the measuring cell was controlled by an ISCO 260-D syringe pump that contained the mixture of interest. The motor on this pump controls the pressure to a resolution of ± 0.014 atm [27]. The pressure in the measuring cell was monitored with two pressure transducers: the transducer on the syringe pump and a pressure transducer (model 204, Setra Systems, Inc. MA) that was placed on the opposite side of the measuring cell from the syringe pump. The Setra transducer had an accuracy and precision of ± 0.37 atm. The Setra transducer was also calibrated in-factory against NIST standards. A 13 μm i.d. x 12 cm long fused silica (Polymicro, Technologies, Phoenix, AZ) open tube was placed after the pressure transducer, and was used to control the flow rate of the fluid through the measuring cell.

**Mixture Preparation.** The methanol/CO₂ mixtures were made using two ISCO 260-D syringe pumps. The first pump was filled with liquid CO₂ at 204 atm and ambient temperature. The second pump was filled with a specific volume of methanol. From the known density of the CO₂ under the specified conditions, the appropriate volume of CO₂ was calculated to produce a given methanol/CO₂ composition. The correct volume of CO₂ was then delivered to the pump holding the methanol; the mixture was pressurized to 78.2 atm and allowed to equilibrate at ambient temperature for at least 12 hrs to ensure complete mixing. From previous studies, this period of time was more than sufficient to provide complete mixing of the methanol/CO₂ mixtures. Each mixture of a given composition was prepared three times and the density values of these mixtures were measured to provide assurance that the mixtures preparation procedure was reproducible.
The error bars reported for each graph are for the 95% confidence interval levels. The error bars not shown on a graph are within the size of the dot.

**Density meter calibration.** The density meter was calibrated with CO₂ and argon. The calibration was done at 26 °C. Densities of CO₂ were obtained from SF-Solver™ (version 2.5.1, 1991) (ISCO, Inc., Lincoln, NE) a computerized data base. These density values were verified by comparison with data in standard reference tables [28]. Densities of argon for each pressure were calculated from its compressibility factor, Zc [29]. The compressibility factor of a gas can be calculated according to the following equation [30]:

\[
Z_c = Z^{(0)} + Z^{(1)} \omega
\]  

(1)

where \( Z_c \) is the compressibility factor, \( Z^{(0)} \) and \( Z^{(1)} \) are function of \( (T_R, P_R) \), \( \omega \) is the acentric factor. Figure 30 is a plot of the compressibility factor, \( Z_c \) for argon as a function of the pressure. From these values, the density of argon was calculated according to the following equations:

\[
V = \frac{ZRT}{P}
\]  

(3)

\[
\rho = \frac{M}{V}
\]  

(2)

where \( V \) is the molar volume, \( R \) the ideal gas constant, \( T \), the temperature, \( P \) the pressure.
Figure 30. Calculated compressibility factor, $Z_c$, of argon.
Procedures for Density Measurements. The density was measured with a calculating digital density meter Paar DMA-512 measuring cell (Anton Paar K.G.). The measurement principle of the density meter is the change in the oscillation frequency upon filling with a liquid or gas. The oscillation is based upon the suspension of a mass M on a spring.

The natural frequency of the oscillator is then given by:

\[
    f = \frac{1}{2\pi} \sqrt{\frac{c}{(M+\rho V)}}
\]  \hspace{1cm} (4)

where \( f \) is the natural frequency, \( c \) is the spring elasticity constant, \( M \) is the mass of the body, \( \rho \) is the sample density and \( V \) is the volume filled. The period of the oscillation, \( T \), is directly measured by the density meter.

\[
    T = 2\pi \sqrt{\frac{(M+\rho V)}{c}}
\]  \hspace{1cm} (5)

The experimental constants, \( A \) and \( B \), are defined by the following equations:

\[
    A = \frac{4\pi^2 V}{c}
\]  \hspace{1cm} (6)

\[
    B = \frac{4\pi^2 M}{c}
\]  \hspace{1cm} (7)
The period of oscillation, $T$, is then described as:

$$T^2 = A\rho + B \quad (8)$$

Figures 31 and 32 are the plots of the period of oscillation, $T$, for CO$_2$ and argon, respectively.

Figure 33 is the calculated density of argon as a function of pressure. From the density values of CO$_2$ and argon, the constants $A$ and $B$ were calculated at each pressure (Figure 34). Before all density measurements, valve B and E (Figure 29) were opened slowly and the fluid of interest flowed through the measuring cell to purge the measuring cell for at least one hour. Valve E was then closed. The density value was recorded at each pressure when the measured density remained constant ($\pm 0.0001$ g cm$^{-3}$) for at least one hour. Triplicate measurements were taken at every pressure. Before each run of a different methanol/CO$_2$ composition, nitrogen gas was flushed through the measuring cell until the previous mixture was completely removed as verified by the indicated density value.

**Procedure for the partial molar volume measurements.** A solution of naphthalene in methanol with a specified molarity was made. The specific volume of methanol solution needed to make a given methanol/CO$_2$ mixture was placed in a syringe pump. Carbon dioxide was then transferred into the pump. The experimental conditions were the same as for the experimental determination of the densities of the mixed fluids (methanol/CO$_2$). Before any measurements were taken, the solution was allowed to equilibrate for 12 hours in the pump. These experiments were repeated in triplicate.
Figure 31. Calibration of the density meter with CO$_2$.

Oscillation period, T.
Figure 32. Calibration of the density meter with argon.

Oscillation period, $T$.

The size of the error bar is within the size of the dot.
Figure 33  Calculated density of argon.
Figure 34. Calibration constants, A and B of the density meter.

(○) A, (■) B.

The size of the error bar is within the size of the dot.
RESULTS AND DISCUSSION

The calibration of the density meter was verified by comparing the measured density of methanol as a function of pressure to values obtained from an equation of state (equation 9) which was derived from the measurement of the isothermal compressibility of methanol. The pressure dependence of the specific volume is expressed according to the following equation [30]:

$$V_p = V_0 (1 - AP + BP^2 - CP^3)$$  \hspace{1cm} (9)

$$\rho = V_p^{-1}$$ \hspace{1cm} (10)

where $V_p$ (cm$^3$ g$^{-1}$) is the specific volume at pressure $P$, $V_0$ is the specific volume at 0.1 MPa (1.2712 cm$^3$ g$^{-1}$), A, B, C are constants defined for methanol. Figure 35 shows that our measured densities for methanol and those calculated from the equation of state compare well.

Density of methanol/CO$_2$ mixtures.

Next the density of four methanol/CO$_2$ mixtures (10, 20, 30, and 40 mole % methanol in CO$_2$) at 26 °C was studied. Figure 35 shows a plot of density versus pressure for the four methanol/CO$_2$ compositions. The mixtures were allowed to stay under pressure for at least 12 hours to insure that the liquid phases (CO$_2$ and methanol) were under one phase. The minimum pressure studied was 85 atm in order to work in the
Figure 35. Density of methanol/CO$_2$ mixtures.

Mole percent of methanol:

(▲) 100, (▼) 100 calc., (○) 10, (◇) 20, (□) 30, (+) 40.
single phase region [31]. At 26 °C the two-phase region will occur at pressures below 60 atm. The densities of the methanol/CO₂ mixtures were therefore studied from 85-215 atm. Francis previously showed that the densities of liquid methanol/CO₂ mixtures under conditions of 65 atm and 26 °C were greater than that of the two pure components [32]. The density increased as the mole percent of methanol increases from 10 mole % to 20 mole %. However the density of the 20 mole % up to 40 mole % methanol in CO₂ was very similar.

**Excess volume of mixing of methanol/CO₂ mixtures.**

Another volumetric property studied was the change in volume upon mixing (excess molar volume), ΔV. ΔV was calculated using the following equations for each of the composition [33]:

\[
\Delta V = \frac{1}{\rho_{12}^{obs}} - \frac{1}{\rho_{12}^{calc}} = \frac{1}{\rho_1} + p_2 \left( \frac{1}{\rho_2} - \frac{1}{\rho_1} \right)
\]

\[
\frac{1}{\rho_{12}^{calc}} = \frac{1}{\rho_1} + w_2 \left( \frac{1}{\rho_2} - \frac{1}{\rho_1} \right)
\]

where ΔV is the excess molar volume observed when the pure components are mixed, \(\rho_{12}^{obs}\) is the observed density of the mixture, \(\rho_{12}^{calc}\) is the density of the mixture calculated using equation 12, and \(w_2\) is the weight fraction of component 2. The magnitude and sign of ΔV are a function of the interactions between the mixing fluids. Figure 36 shows the
Figure 36. Excess volume of mixing of methanol/CO₂ mixtures.

Mole percent of methanol: (●) 10, (○) 20, (□) 30, (+) 40.
excess molar volume, $\Delta V$, for the four methanol/CO$_2$ mixtures as a function of pressure. The sign and the magnitude of the excess volume indicate a deviation from the ideal mixing. It also suggests that methanol and carbon dioxide interact strongly under the conditions studied. This is somewhat unexpected since positive volume changes when mixing alcohols with non polar solvents have been reported [34]. The excess volumes for the methanol/CO$_2$ mixtures are ten times larger than for mixtures of n-alkanes with 1-alkanols [35]. However, the intermolecular properties of MeOH/CO$_2$ mixtures have been investigated both in the gas phase as well as in the liquid phase at high pressures and temperatures [36]. The authors suggest the formation of MeOH/CO$_2$ aggregate (either of electron donor-acceptor type or H-bonding of the methanol with carbon dioxide which would act as a Lewis acid acceptor). The study of dielectric constant as well as near IR spectra of solutions of n-alcohols in apolar solvents revealed also the presence of some association phenomenon [37], [38]. The ability of n-alcohol molecules to form multimers even at a weight fraction as low as 0.004 was reported [38]. Moreover, the possibility of hydrogen bonding with a specific solvent such as diethyl ether or dioxane (ones that could play in the alcohol-acceptor complex mechanism) was also noticed [39], [40].

The measured volume contraction, $\Delta V$, for the methanol/CO$_2$ mixtures is approximately an order of magnitude greater than that found in strongly interacting liquid mixtures, such as alcohols in water [41], [42]. The association between CO$_2$ and methanol also varies significantly with pressure. As the pressure of the mixture increased, the excess volume increased quickly and then leveled off asymptotically at pressures $> \text{ca. 140 atm}$. A significant change in the excess volume was observed between 10 and 20 mole % methanol
in CO₂. For example, at 90 atm the ΔV is -3 and -5 cm³ mol⁻¹ for 10 and 20 mole % methanol/CO₂, respectively. Also, the effect of composition on the excess volume depends on the pressure range considered. Between 85 - 110 atm, ΔV varies significantly between 10 and 20 mole % methanol in CO₂, but further additions of methanol did not change the ΔV value. Alternatively for pressures > 110 atm (110 - 200 atm), ΔV continued to contract with added methanol over the entire composition range studied (0 - 40 mol %).

In this study, the strong affinity of methanol for carbon dioxide is illustrated by the large negative excess volume (10 times greater than the excess volume of strong H-bonding solutions such as diols/water) [43]. With a weight fraction of methanol in carbon dioxide greater than 0.08, the possibility of association of the alcohol molecules as well as hydrogen bonding with the carbon dioxide is strongly probable. At 10 mole percent of MeOH, the formation of dimers or small aggregates of methanol is favored compared to the formation of multimers of MeOH that would be favored for the 20 to 40 mole percent. This is suggested by the study of the dielectric constant of MeOH/CO₂ mixture as well as the calculation of the Kirkwood correlation factor, g, of these mixtures [44].

The effect of pressure at a given composition may be explained as the following. The literature on the pressure effect of the Kirkwood correlation parameter, g, shows that (∂g/∂P) < 0 for methanol due to the break down of the H-bonds but the dielectric constant of MeOH/CO₂ mixtures increases with the pressure [45]. When the pressure increases, there is formation of more MeOH/CO₂ aggregates and a breakdown of the large
aggregates of alcohol molecules. The formation of MeOH/CO$_2$ pairs that have a higher dipole moment than methanol counter balances the diminution in the electric polarizability of the mixture due to the depletion of the methanol multimers. Hemmaplardh et al. previously documented the association of methanol and carbon dioxide in the gas phase [45]. They proposed the formation of a methanol-CO$_2$ adduct with a standard heat of dissociation of 3.0 kcal mol$^{-1}$ to describe the association. The observed volume contraction with added methanol corresponds qualitatively to what would be expected from an equilibrium process involving an adduct formation between methanol and CO$_2$ as well as its isothermal compressibility.

Determination of the partial molar volume of naphthalene.

The density as well as the excess volume of mixing curves suggest that there are large differences in the molecular-level structure of the 10 mole % methanol in CO$_2$ mixture compared to that of the other mixtures. Potentially these differences in structure may also translate into markedly different solvating capabilities. To begin to evaluate these differences in the molecular-level structure and the possible effect on solvation, the infinite dilution partial molar volume, $v_2^-$, of naphthalene was determined in two methanol/CO$_2$ mixtures, namely 10 and 40 mole % methanol/CO$_2$. Valuable information, such as the pressure effect on the solubility, is gained from the study of the pressure dependence of the partial molar volume of a solute in a fluid [46]. The minimum in the curve of the partial molar volume versus the pressure corresponds to the inflection point of the pressure effect on the solubility of the analyte. Naphthalene was chosen as a test
solute because similar data already exist in the literature for naphthalene in supercritical carbon dioxide [47], [48]. The large negative value in $v_2^\neq$ for naphthalene in supercritical CO$_2$ corresponds to a maximum in the change in solubility versus pressure.

The partial molar volume, $v_2^\neq$, is dependent upon the pressure, temperature and in some cases the composition of the fluid. Estimation of $v_2^\neq$ can also be done analytically. Chueh et al. estimated the partial molar volume in nonpolar liquid mixtures at saturation [49]. They modified and differentiated the Redlich-Kwong equation of state. The partial molar volume of each component in the binary mixture of nonpolar/CO$_2$ compounds was estimated. The calculations showed that the $v_2^\neq$ is very sensitive to the binary interaction parameter $k_{ij}$. This parameter accounts for the molecular interactions between the components of the fluid mixture. $k_{ij}$ has been correctly estimated for nonpolar mixtures, but corrections are necessary in the case of polar mixtures or mixtures in which the components interact by other than dispersive interactions [23].

The partial molar volume can be determined experimentally by a number of methods. Solubility measurements of the solute as a function of pressure can be used [50]. The differentiation $\delta (\ln y)/\delta P$ is a linear function of $v_2^\neq$. However, this method assumes that the partial molar volume does not depend on the composition. This assumption is not always fulfilled especially near the critical point of the mixture [51]. Nevertheless since the experimental conditions of this study are far from the critical points of the methanol/CO$_2$ mixtures, it may be possible to assume that there will be no dependence of the partial molar volume with the mole fraction of the solute. The injection method is another experimental means of determining $v_2^\neq$. With this method, the change of pressure
upon the addition of n moles of solute is monitored [52]. Accuracies similar to numerical estimation are possible. They reported an accuracy of 2% at low pressure (13.6 atm) but there was a significant deviation at higher pressures (P > 68 atm).

In our study, we chose to calculate the partial molar volume from density measurements according to equation (13):

$$v_2^- = v_1 - \left( \frac{\delta v}{\delta y_1} \right)_{T,P}$$

where $v_2^-$ is the infinite dilution partial molar volume of the solute, $v_1$ is the molar volume of the pure solution (methanol/CO$_2$), $v$ is the molar volume of the solution and $y_1$ is the mole fraction of the solute. At low concentrations the last term dominates. The density of a series of dilute solutions of naphthalene in the methanol/CO$_2$ mixtures was measured. Then, from a linear plot of density versus naphthalene concentration, the infinite partial molar volume was determined from the y-intercept. The concentration range of naphthalene used in these experiments varied from $2 \times 10^{-4}$ to $8 \times 10^{-4}$ mole fraction naphthalene which is comparable to the concentration range previously used in the studies of naphthalene in supercritical CO$_2$.

Figure 37 shows the variation of $v_2^-$ of naphthalene as a function of pressure for the two studied compositions, 10 and 40 mole % methanol/CO$_2$. The errors bars on the curves indicate the 95% confidence limits for the data. Higher uncertainties are found for the partial molar volume in the 10 mole % MeOH. This is associated with the larger isothermal compressibility of this fluid mixture as compared with the other ones as it would be demonstrated later in this study. Substantial variation of the $v_2^-$ for naphthalene
Figure 37. Partial molar volume of naphthalene in methanol/CO$_2$ mixtures.

Mole percent of methanol: (□) 10, (■) 40.
with pressure was observed for the 10 mole % methanol in CO₂ mixture. For this composition, very negative values of \( v_2^* \) (for example, -1018 ml mol\(^{-1}\) at 90.9 atm) were observed at low pressures. The partial molar volume can also be expressed by the following equation [53]:

\[
v_2^* = \rho k K_T T - \xi
\]

(14)

where \( \rho \), \( k \), \( K_T \), \( T \) and \( \xi \) are the solvent's density, the Boltzmann's constant, the isothermal compressibility, the temperature and the cluster size, respectively. The cluster size is defined as the excess number of solvent molecules surrounding an infinitively dilute number of solute molecules, with respect to an uniform distribution at the prevailing bulk density. The term, \( \xi \), is positive for attractive mixtures but it is negative for repulsive mixtures [54], [55]. The term \( \rho k T K_T \) being positive, the negative value for the partial molar volume of the solute is attributed to the formation of solvent clusters around the solute. There is a preferential clustering of cosolvent organic molecules around the solute in a mixture of cosolvent/CO₂. The net enrichment of organic solvent molecules around the solute is pressure dependent [17].

**Isothermal compressibility of methanol/CO₂ mixtures.**

The knowledge of the magnitude of the isothermal compressibility, \( K_T \), is important because it will indicate how readily a density change can affect the volumetric properties of the fluid mixture. This parameter can be calculated from an empirical equation of state. Ehrlich et al. outlined some of the limitations of the Redlich-Kwong equation especially
in predicting the isothermal compressibility of mixtures near their critical point [56]. Methanol as most of the organic solvents has a low isothermal compressibility factor value [57]. The addition of a fluid with a higher compressibility factor, such as liquid CO$_2$, should increase the isothermal compressibility of the mixture. The isothermal compressibility of the four methanol/CO$_2$ mixtures studied was calculated according to equation (15):

\[
K_T = -\frac{1}{V} \times \left(\frac{\delta V}{\delta P}\right)_T
\]  

(15)

The isothermal compressibility is related to the magnitude of the cohesive forces in a fluid. The increase in the mole percent of methanol leads to an increase of the hydrogen bonds in the fluid. This results in the decrease in the isothermal compressibility of the MeOH/CO$_2$ mixtures as the mole percent of MeOH increases. Figure 38 shows the variation of the isothermal compressibility of the methanol/CO$_2$ mixtures as a function of pressure.

For comparison, the isothermal compressibility of methanol as a function of pressure is plotted according to the following equation of state (Figure 39) [31]:

\[
K(P) = K(P_0) - AP + BP^2 - CP^3
\]  

(16)

where $K(P_0)$ is the isothermal compressibility at 0.1 MPa. The values of A, B and C are constants specific to methanol. They are $(43.3 \pm 2.2) \times 10^{-13}$ Pa$^{-2}$, $(72.9 \pm 7.7) \times 10^{-15}$ Pa$^{-3}$. 
Figure 38. Isothermal compressibility of methanol/CO₂ mixtures.

Mole percent of methanol: (□) 10, (○) 20, (■) 30, (+) 40.
Figure 39. Isothermal compressibility of methanol according to reference [31].
and (54.0±7.1) x 10^{-17} \text{ Pa}^4, \text{ respectively. As expected, the isothermal compressibility of}
the methanol/CO_2 mixtures is much higher than that of methanol.

From a comparison of Figures 37 and 38, the variation of the partial molar volume
with pressure correlates well with that of the mixtures' isothermal compressibility.
The most compressible mixtures were also those that had the most negative \( \nu_2^- \) values for
naphthalene. At low densities, the isothermal compressibility of the mixture is highest and
the attractive interactions between the solute and the organic solvent are dominant. As the
density of the fluid increases, the repulsive interactions begin to act. At a pressure high
enough (\( P > 140 \text{ atm} \)), the local composition will approach the bulk composition.
Nevertheless, the isothermal compressibility did not account for the change in magnitude
that occurred between 10 and 40 mole % methanol in CO_2 for the partial molar volume
of naphthalene. Equation (17) could explain the behavior of the partial molar volume with
the pressure and with the composition [48]:

\[
\nu_2^- = \nu \beta n (\delta P / \delta n_2)_{T,V}
\]  

(17)

In the lower density region of the fluid (\( P < 90 \text{ atm} \)), the isothermal compressibility
decreases and the term \( n (\delta P / \delta n_2) \) is large and negative. This corresponds to attractive
interactions between the solvent and solute molecules. As the pressure increases, this term
becomes more positive due to repulsive interactions between the solvent and solute
molecules. The density dependence of the partial molar volume was studied via Monte-
Carlo simulation for Lennard-Jones mixtures. The calculations are based on the energy
and size parameters of the solute-solvent pair [58]. The term \( n (\delta P / \delta n_2) \) becomes
predominant over the isothermal compressibility at high density and is a strong function of the composition of the fluid as evidenced by the large change in the magnitude of $v_2^\infty$ which occurs between 10 and 40 mole % of methanol.

In summary, for the 10 mole % methanol/CO$_2$ mixtures, the pressure conditions that provided large negative excess volumes, and large isothermal compressibility had the most negative $v_2^\infty$ values for naphthalene as well. It should be emphasized that these data are observed for liquid mixtures of methanol/CO$_2$. The critical temperatures of the mixtures were calculated according to the equations reported in Table 6. For all of the four compositions of methanol/CO$_2$ studied, the experimental temperature (26 °C) was well below the critical temperature of the mixture (Table 7). The magnitude of the $v_2^\infty$ for naphthalene was large and negative just as observed in supercritical CO$_2$. However, in supercritical CO$_2$, the most negative $v_2^\infty$ is about an order of magnitude smaller than the most negative value observed for these mixtures. The large deviation of the infinite partial molar volume of solutes near the critical temperature of the solvent has been attributed to the increase in long range correlated solvent-solute interactions. This is attributed to the highly compressible nature of the solvent near its critical point. The specific cause of the nonideal behavior of the methanol/CO$_2$ liquid mixtures must be studied further. The experimental temperature, 26 °C, was close to but less than the critical temperature of CO$_2$ (one component of the solvent mixture) which is 31.3 °C [59]. Perhaps a solute-induced phase transition caused the observed deviations in $v_2^\infty$ in the methanol/CO$_2$ mixtures as Sengers et al. originally proposed for the behavior of pure solvents at temperatures near its critical temperature [60]. For the studied liquid mixtures, the value
of the partial molar volume of naphthalene as well as the isothermal compressibility of the methanol/CO₂ mixtures were intermediate between that values found in supercritical fluids and in liquids at ambient conditions.
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CHAPTER IV

DETERMINATION OF THE MOLECULAR DIFFUSION COEFFICIENTS IN ETHANOL/WATER/CARBON DIOXIDE

INTRODUCTION

An understanding of the diffusion process in high pressure liquid mixtures is required if such mixtures are to be used either as mobile phases in chromatography or as extraction fluids. The mass transfer process often controls the column efficiency in chromatography. The molecular diffusion coefficient of solutes is useful for the optimization of a column's performance. Mass transport properties were primarily investigated through the analyses of self-diffusion or binary diffusion coefficients at infinite dilution [1], [2]. Previous studies included the investigation of binary or ternary molecular diffusion coefficients in supercritical fluids [3], [4], [5], [6]. The diffusivity of m-cresol and benzene in supercritical fluids containing polar modifiers such as water, acetonitrile and propylene carbonate were investigated [7]. Sassiat et al. briefly analyzed the effect of the methanol content on the molecular diffusion of benzene in methanol/carbon dioxide mixtures in the subcritical state [5]. Olesik et al. investigated the effect of the temperature increase on the diffusion coefficient for ternary enhanced-fluidity mixtures of methanol/H_2O/CO_2 [8].
Besides the experimental studies, analytical equations can be used to predict the mass transport properties of a solute in a given medium. Nevertheless, the estimation of the molecular diffusion coefficients by analytical equations does not always predict with sufficient accuracy the molecular diffusion coefficient of solutes in ternary fluid mixtures at high pressure.

The paucity of experimental data, as well as, the limited accuracy of the available analytical equations for their estimations leads to a need in their experimental determinations. The present study reports on the experimental determination of the molecular diffusion coefficients of polar (m-cresol, 3-nitrophenol) as well as nonpolar (benzene, anthracene) solutes in ternary mixtures of ethanol/water/carbon dioxide (CO₂). The effect of both the composition of the fluid mixture in terms of mole fraction of CO₂ dissolved and the temperature was studied. The validity of the mass transport equations that are commonly used to describe the diffusion in liquid mixtures will also be investigated.

**EXPERIMENTAL SECTION**

**Materials.** All chemicals were used as received. Ethanol and acetone were HPLC-grade obtained from Fischer Scientific (Pittsburgh, PA). The water was laboratory distilled. CO₂ was SFC/SFE grade (99.997% pure) from Air Products (Allentown, PA). Anthracene and benzene were Baker-grade obtained from J. T Baker Chemical Co. (Phillipsburg, NJ.), 3-nitrophenol (99% pure) and m-cresol (99% pure) were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Benzene and m-cresol were
used as neat solutes whereas anthracene and 3-nitrophenol were diluted in mixture of 
ethanol/water. The mixtures of ethanol/water prepared for the dilution were either 
0.61/0.39 or 0.50/0.50 mole fractions depending on the mobile phase to be studied. The 
concentrations of 3-nitrophenol and anthracene in the mixtures of ethanol/water were 
0.4 \times 10^{-3} \text{ M} \text{ and } 2 \times 10^{-3} \text{ M}, \text{ respectively.}

**Mixture preparation.** The ternary mobile phases were prepared according to the 
following procedure. Ethanol and water were mixed and transferred in an ISCO 260-D 
syringe pump (ISCO, Lincoln, NE). Liquid CO₂ was then delivered to the pump at room 
temperature at 204 atm. The mixture was allowed to equilibrate overnight at 170 atm.

**Chromatographic system.** The connection from the syringe pump to the injector 
was about 60 cm of stainless steel tubing placed in the oven in order to preheat the mobile 
phase. The fluid mixture was delivered to a 17 m of capillary fused silica tubing 
(Polymicro Technologies, Phoenix, AZ) with a 247 \mu m id. The fused silica tube was 
coiled with a radius of coiling of 9 cm. A Valco-W-series high pressure injection valve 
(Valco Instruments Co. Inc., Houston, TX) with a 200 nl injection volume was used. The 
volume of the injector loop contributes to the initial broadening of the sample pulse width 
and must be as small as possible. The oven was an HP-SFC (Hewlett-Packard Company, 
Avondale, PA). The detector was a Spectra 100 UV-visible detector (Spectra-Physics 
Analytical, Fremont, CA). The detector excitation was set at 254 nm and the time 
constant was 0.3 s. The detection was done on-column by removing the polyimide coating 
from the capillary fused silica tubing. The extracolumn contribution to the band 
broadening due to the use of a detector flow cell is then avoided. In order to maintain the
pressure in the chromatographic system, an appropriate length of 30 μm fused silica was used as a post-detector restrictor. The chromatographic data (retention time, peak width, asymmetry factor) were computed by the integrated data station of the chromatographic system. All data were the average of five replicates. All of the chromatographic peaks had an asymmetry factor in the range 0.97 - 1.1. The errors reported for each point are the standard deviations at the 95% confidence level.

RESULTS AND DISCUSSION

The properties of the enhanced-fluidity liquid mixtures of ethanol/water/CO₂ depend on the mixture composition as well as on the pressure and the temperature. The pressure and the temperature are chosen according to the phase diagram of the mixture. The use of the ethanol/water/CO₂ mixture as a mobile phase in chromatography or in extraction requires the fluid to be one-phase. The choice of the mixture composition depends on the phase diagram but also on the viscosity of the mixture. The proportion of the organic solvent in the enhanced-fluidity mixture, i. e., the EtOH/H₂O in the present case, controls the solvent strength as well as the viscosity of the mixture. As the mole fraction of EtOH/H₂O increases in the ternary mixture, the solvent strength increases. Unfortunately, the viscosity of the mixture increases also. The proportion of EtOH/H₂O in the enhanced-fluidity mixture should be kept as low as possible in order to achieve a mixture that would have a sufficient solvent strength and a minimal viscosity.
Viscosity of EtOH/H₂O/CO₂ mixtures.

The ternary mixtures are made by adding the low viscosity fluid (CO₂) to a mixture of EtOH/H₂O. The resultant viscosity will depend not only on the proportion of CO₂ but also on the viscosity of the EtOH/H₂O that we start with.

Ethanol and water form nonideal mixtures due to the existence of hydrogen bonds. The viscosity of the EtOH/H₂O mixtures depends on its composition. The curve of the viscosity versus the composition presents a maximum for a mole fraction of ethanol in water equals to 0.21 [9], [10]. The mixtures with a mole fraction of ethanol greater or lower than 0.21 are then preferred. In this study, EtOH/H₂O mixtures with either 0.61/0.39 or 0.50/0.50 mole ratio were chosen. Mixtures of EtOH/H₂O with a larger proportion of ethanol than water were chosen for the following reasons. Water has a low solubility in liquid CO₂ [11]. Moreover, its solubility in CO₂ decreases as the temperature increases [12]. Unfortunately, the decrease in the viscosity of the ternary mixture of EtOH/H₂O/CO₂ can be achieved by the addition of CO₂ or the increase in the temperature. The proportion of water in the starting EtOH/H₂O mixtures should be lower than the proportion of ethanol. The maximal mole fraction of CO₂ that can be added as well as the temperature range investigated are determined according to the phase diagram of the EtOH/H₂O/CO₂ mixture.

H₂O/CO₂ phase diagram.

The poor solubility of water in CO₂ limits the pressure and temperatures to be used. Kuk et al. reported a solubility value of CO₂ between 4 and 7 g for 100 g of water.
near and above the critical point of CO₂ [12]. Over a pressure range up to 700 atm, and a temperature range from 100 °C down to 12 °C, the mole percent of CO₂ in water is ≤ 3 [13]. At low mole fractions of CO₂ (namely <0.02), the mixtures of water/CO₂ are in the single liquid phase [14]. For mole fractions of CO₂ between 0.02 and 0.998, multiple-phases occur in the phase diagram. The existence of liquid-vapor, liquid-liquid and liquid-liquid-vapor phases depends upon the pressure and the temperature. Under isothermal conditions, the liquid-liquid phase equilibrium occurs at higher pressures than the liquid-vapor equilibrium. At a pressure intermediate between these two regions, the mixture forms a three-phase system. For the mixtures of water/CO₂ in which the mole percent of CO₂ is >99.8 %, a single phase system (vapor or liquid) occurs depending on the pressure and temperature. At low pressures, the vapor phase occurs. Under isothermal condition, an increase in the pressure leads to the formation of a two-phase region (liquid-vapor) at a defined pressure. Further increase in the pressure leads to the formation of a single liquid phase. The existence of a single liquid phase (at moderate pressure and temperature) occurs for the water/CO₂ system in which the mole fraction of CO₂ is either very low (<0.02) or very high (>0.998).

EtOH/H₂O/CO₂ phase diagram.

The phase behavior of ethanol/water/carbon dioxide is available in the literature over a large range of pressures and temperatures (68-205 atm and 25 °C - 60 °C) [15], [16]. The existence of multiple-phase occurs in the ternary mixture of EtOH/H₂O/CO₂. The formation of multiple phases occurs for a pressure below 136 atm over 25 °C-60 °C.
For a starting liquid mixture of ethanol/water such as the mole fractions are 0.61 and 0.39, respectively, the maximum mole fraction of CO₂ to be added is 0.455 in the final mixture at 170 atm and for 25 °C < T < 65 °C [16]. The addition of up to 60 mole % of CO₂ requires a pressure greater than 205 atm over the same temperature range in order to remain in the one-phase region. In the case of a starting liquid mixture of ethanol/water with a higher proportion of water, i.e., 0.50/0.50 mole fraction ethanol/water, the maximum amount of liquid CO₂ is limited to a mole fraction of 0.20 at 170 atm. In the case of a mixture with a lower proportion of water, i.e., 0.78/0.22 mole fraction ethanol/water, a liquid phase with 80 mole % of CO₂ is achieved at a pressure of only 136 atm. If such ternary phases are to be used as mobiles phases in reversed chromatography, the proportion of water should be significant. Ternary mixtures with large proportion of CO₂ can be made and still form a single phase at moderate pressures and temperatures. At 170 atm and for 25 °C < T < 65 °C, a ternary mixture with more than 98 mole % of CO₂ can be made from a starting 0.61/0.39 EtOH/H₂O mixture. Nevertheless, the mixtures with a too high mole proportion of CO₂ (mole fraction greater than 0.60) are not advisable because the solvent strength of the resultant mixture is too low [17], [18].

The band broadening technique.

The determination of the experimental diffusion coefficients, Dₘ, was performed according to the chromatographic band dispersion procedure. This technique was first introduced by Taylor and Aris [19], [20]. Taylor derived a mathematical expression that
models the dispersion of an organic dye into a stream of solvent. The molecular diffusion coefficient was calculated from the observed concentration profile. The theory was later developed by Giddings and Seager who correlated the value of the broadening of a chromatographic peak in an open, uncoated fused silica to the molecular diffusion of the solute [21], [22]. Accurate and rapid determination of molecular diffusion coefficient could be achieved from the peak dispersion in an open capillary tube. The broadening of the chromatographic peak as expressed by its variance ($\sigma^2$) is a function of the characteristics of the column and the mass transfer properties of the solute ($D_m$). With a laminar flow profile, the analyte introduced into the capillary tube is eluted as a Gaussian peak whose variance is described as the following equation [7]:

$$\sigma^2 = \frac{2D_mL}{u} + \frac{d_c L u}{96D_m}$$  \hspace{1cm} (1)

where $L$, $u$ and $d_c$ are the column length, the linear velocity and the diameter of the tube, respectively. The peak variance, $\sigma^2$, is expressed as:

$$\sigma^2 = LH$$  \hspace{1cm} (2)

where $H$ is the chromatographic plate height.

Diffusivities of the solute both in the axial and the radial directions contribute to the overall chromatographic band broadening. The first and second terms of equation (1) account for the diffusion in the axial and radial directions, respectively. The axial diffusion is often negligible if the velocity of the fluid is not too low and if the flow profile
is laminar (Reynolds’ number <2000). These assumptions are tested in the next section.

Errors in the band broadening method.

Various errors in the determination of the molecular diffusion coefficient by the band broadening procedure could arise. The contribution of the axial diffusion can be neglected if the linear velocity is described by the following equation [5]:

\[ u > \frac{70D_m}{r_0} \]  

(3)

The molecular diffusion coefficients of an analyte in an enhanced-fluidity liquid should be intermediate between those found in a supercritical fluids (i.e., \(10^{-4} \text{ cm}^2 \text{ s}^{-1}\)) and those found in liquid mixtures (i.e., \(10^{-3} \text{ cm}^2 \text{ s}^{-1}\)) [23]. Under the assumption that the value of the molecular diffusion coefficient falls in this range, the velocity of the mobile phase must exceed 0.5 and 0.05 cm s\(^{-1}\), for supercritical fluids and liquids, respectively. These values were readily met in our experiments since the average mobile phase velocity was in the range of 2 to 3 cm s\(^{-1}\).

Another source of error that is frequently encountered in the case of a coiled capillary tube is the onset of secondary flow. In a straight tube, only the parabolic flow profile and the diffusion of the solute contribute to the band broadening. In a coiled tube, there is an additional secondary flow that results from centrifugal forces and acts perpendicular to the flow profile. As a consequence, the mass transfer in the axial direction is increased due to the diffusion by convection. The axial dispersion is then
reduced compared to values found for straight tubes. In order to neglect the secondary
flow, the radius of the coil as well as the mobile phase velocity should be restricted.
The mass transport process can be characterized by the function “Dn²Sc” if the conditions
given by the equations (4) and (5) are met:

\[ Dn < 16 \quad (4) \]

\[ \frac{d_{\text{coil}}}{d_{\text{tube}}} > 20 \quad (5) \]

where Dn, Sc, d_{\text{coil}}, d_{\text{tube}} are the Dean number, the Schmidt number, the coil diameter and
the tube diameter, respectively.

The ratio of the dispersion coefficient in a coiled tube to that in a straight tube is
about 1 for values of “Dn²Sc” as given by equation (6) [24], [25]:

\[ Dn²Sc \times 100 \quad (6) \]

The aspect ratio as defined in equation (5) should be as high as possible to avoid the onset
of secondary flow profile. The value of the Dean number in these experiments was 1.14.
The experimental conditions chosen provided a value of less than 100 for the term
“Dn²Sc” and an aspect ratio of 729. Conditions dictated by equations (4) through (6) are
easily met for these experiments. For values of “Dn²Sc” higher than 100, the ratio drops
to 0.25 and the secondary flow profile can not be negligible.
The tailing of the chromatographic peak as evidenced by an asymmetry factor (As) greater than 1.1 causes error due to the poor fitting of the chromatographic peak to a Gaussian curve and hence limited the choice of solutes to be studied. On an uncoated fused silica tube, the tailing is a result of the adsorption of the solute on the residual silanols.

Validation of the method.

The performance of the experimental setup was estimated by the experimental determination of the binary molecular diffusion of m-cresol, benzene and acetone in supercritical carbon dioxide. Table 8 is a comparison of the experimental diffusion coefficients of the solutes in supercritical CO₂ with the values published in the literature. Our data agree well with the published values.

Although benzene and acetone were tested as net compounds, the molecular diffusion coefficients observed are not the self-diffusion coefficients. For example, at 40 °C and 140 atm, the self-diffusion coefficient of benzene is on the order of 1.5 - 3 x 10⁻⁵ cm² s⁻¹ [26], [27]. The viscosity of benzene is larger than the viscosity of supercritical CO₂. If benzene would not diffuse rapidly into the mixture of interest, the experimental molecular diffusion coefficient of benzene in CO₂ would be much smaller than the one obtained. The injection volume used (200 nl) does not seem to affect the data.
Table 8. Comparison of experimental diffusivities of solutes in CO$_2$
(40 °C, 160 atm) with data from the literature.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Solvent</th>
<th>As</th>
<th>$D \times 10^5$, cm$^2$ s$^{-1}$</th>
<th>Lit.$^b$</th>
<th>Lit.$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>neat</td>
<td>0.99</td>
<td>13.6$\pm$0.13</td>
<td>14.5$\pm$0.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>hexane</td>
<td>0.99</td>
<td>10.9$\pm$0.012</td>
<td>11.6$\pm$0.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Acetone</td>
<td>neat</td>
<td>1.00</td>
<td>13.5$\pm$0.064</td>
<td>14.7$\pm$0.4</td>
<td></td>
</tr>
</tbody>
</table>

The average asymmetry factor (As) was calculated at 10% of the peak height.

$^a$ Average value of five replicates.

$^b$ Literature from reference [5]

$^c$ Literature from reference [4]. The overall precision stated was 2.9$\pm$0.6%.
Effect of temperature and fluid mixture composition on the diffusion of solutes.

Figures 40 through 43 show the effects of temperature and fluid composition on the molecular diffusion coefficient of anthracene, benzene, m-cresol and 3-nitrophenol respectively. The mobile phase composition was varied by adding various amount of liquefied CO$_2$ to a 0.61/0.39 mole fraction EtOH/water mixture. The fluid mixtures had between 0 and 0.40 mole fraction of CO$_2$ in the final mixture. Figures 44 through 47 are similar plots but the starting mixture was 0.50/0.50 mole fraction of EtOH/water. The maximum amount of liquefied CO$_2$ added was 0.20.

An increase in the molecular diffusion coefficients of the solutes can be achieved by an increase in the temperature and/or an increase in the mole percent of CO$_2$ in the mixture. An increase in the mole fraction of CO$_2$ as small as 0.1 (under isothermal condition) is equivalent to a 20 °C increase in the temperature. This is observed for all of the solutes studied except for m-cresol. For this solute, an increase in the temperature of 10 °C brought about the same enhancement in its diffusivity than the addition of 10 mole % of CO$_2$ in the mixture composition.

The overall increase in molecular diffusion coefficient depends upon the nature of the solute as well as the mobile phase composition to start with. The nonpolar compounds studied such as benzene and anthracene have higher diffusion coefficients than m-cresol and 3-nitrophenol in the EtOH/H$_2$O/CO$_2$ mixtures. m-cresol and 3-nitrophenol can form hydrogen bonds with water or ethanol in the mixture. Their solvation sphere is expected to be larger than those of benzene and anthracene. The increase in the “apparent
Figure 40. Effect of temperature and mole fraction of CO₂ on the molecular diffusion coefficient of anthracene in EtOH/H₂O (0.61/0.39) mixtures at 170 atm. Mole % of CO₂: (+) 0, (■) 20, (▲) 30, (○) 40

The lines are drawn to facilitate the reading of the curves.
Figure 41. Effect of temperature and mole fraction of CO₂ on the molecular diffusion coefficient of benzene in EtOH/H₂O (0.61/0.39) mixtures at 170 atm.

Mole % of CO₂: (+) 0, (■) 20, (▲) 30, (●) 40.

The lines are drawn to facilitate the reading of the curves.
Figure 42. Effect of temperature and mole fraction of CO\textsubscript{2} on the molecular diffusion coefficient of m-cresol in EtOH/H\textsubscript{2}O (0.61/0.39) mixtures at 170 atm.

Mole % of CO\textsubscript{2}: (+) 0, (■) 20, (▲) 30, (●) 40.

The lines are drawn to facilitate the reading of the curves.
Figure 43. Effect of temperature and mole fraction of CO₂ on the molecular diffusion coefficient of 3-nitrophenol in EtOH/H₂O (0.61/0.39) mixtures at 170 atm.

Mole % of CO₂: (+) 0, (■) 20, (▲) 30, (●) 40.

The lines are drawn to facilitate the reading of the curves.
Figure 44. Effect of temperature and mole fraction of CO$_2$ on the molecular diffusion coefficient of anthracene in EtOH/H$_2$O (0.50/0.50) mixtures at 170 atm.

Mole % of CO$_2$: (+) 0, (■) 20.

The lines are drawn to facilitate the reading of the curves.
Figure 45  Effect of temperature and mole fraction of CO$_2$ on the molecular diffusion coefficient of benzene in EtOH/H$_2$O (0.50/0.50) mixtures at 170 atm.

Mole % of CO$_2$: (+) 0, (■) 20.

The lines are drawn to facilitate the reading of the curves.
Figure 46. Effect of temperature and mole fraction of CO$_2$ on the molecular diffusion coefficient of m-cresol in EtOH/H$_2$O (0.50/0.50) mixtures at 170 atm.

Mole % of CO$_2$: (+) 0, (■) 20.

The lines are drawn to facilitate the reading of the curves.
Figure 47. Effect of temperature and mole fraction of CO₂ on the molecular diffusion coefficient of 3-nitrophenol in EtOH/H₂O (0.50/0.50) mixtures at 170 atm.

Mole % of CO₂: (+) 0, (■) 20.

The lines are drawn to facilitate the reading of the curves.
volume" leads to a decrease in the diffusivities of these compounds in the ethanol/water/CO₂ mixtures. For the mixture of EtOH/H₂O (50/50 mole ratio), the addition of 20 mole percent of CO₂ gives at least a two fold increase in the molecular diffusion coefficient of the solutes.

A larger enhancement is observed for the diffusivity of m-cresol. In the case of the mixture of EtOH/H₂O (61/39 mole ratio), addition of the same mole percent of CO₂ provides an overall smaller enhancement. For the polar compounds (m-cresol and 3-nitrophenol), it is less than 2 (especially for 3-nitrophenol). For benzene and anthracene, the increase in the molecular diffusion coefficient is on the order of 2. This may be due to the lower viscosity of the EtOH/H₂O (50/50 mole ratio) as compared to the mixture EtOH/H₂O (61/39 mole ratio).

The effect of the starting mixture composition can be observed from the comparison with the data of Lee et al. [8]. They studied the effect of temperature and mobile phase composition on the enhancement of the molecular diffusion coefficients of anthracene and benzene in mixtures of MeOH/H₂O/CO₂. The MeOH/H₂O ratio was kept constant at 70/30 mole ration. The effect of the temperature is quite similar for the EtOH/H₂O and MeOH/H₂O mixtures. For the EtOH/H₂O (61/39), an increase in the temperature to 60 °C provides a factor of two increase in the molecular diffusion coefficient of anthracene or benzene. A 4 fold increase is observed for the MeOH/H₂O mixture when the temperature is raised to 120 °C. When carbon dioxide is added to the mixtures, the effect of temperature and mobile phase composition are quite different. Over the same temperature range (25 °C - 60 °C) and mole fraction of CO₂ added
The addition of the low viscosity fluid (CO₂) or the increase in the temperature leads to a change in the viscosity of the fluid mixture. The decrease in the viscosity is responsible for the increase in the diffusion of the solute. Over a wide range of temperature and pressure, the viscosity effect on the solute diffusion coefficients is described by the following equation [28]:

\[ D_m \propto \eta^q \]  

(7)

where \( \eta \) and \( q \) are the viscosity and a constant, respectively. The value of \( q \) varies from -0.5 to -1. The decrease in the viscosity is larger for the enhanced-fluidity liquids containing the highest proportions of CO₂.

The effect of the temperature on the viscosities of the mixtures of ethanol/water (0.61/0.39 mole fraction) were extrapolated from the experimental data published in the literature [29]. The extrapolations were done by fitting the data to a polynomial function of order 6. For each temperature, the regression coefficients for the curves were >0.999. As the temperature increases, the composition of the ethanol/water mixture with the highest viscosity shift to a lower mole percent of ethanol [30]. A mixture of ethanol/water with a mole fraction of ethanol greater than 0.5 is then recommended to start with (in order not to lose the positive effect of the increase of temperature on the molecular diffusion coefficient). The viscosities of ethanol, water and CO₂ at 298.2 K and 1 atm are...
reported in Table 9. In the case of mixtures with low isothermal compressibility (i.e., $10^4 \text{ atm}^{-1}$) such as ethanol/water mixtures, the effect of pressure upon the viscosity is negligible [31].

The viscosities of the ethanol/water/CO$_2$ mixtures in the temperature range from 25 °C to 60 °C and at a pressure of 172.3 atm were calculated. The calculations were done by using the method developed by Chung et al. [28]. This method applies a one-fluid approximation to correlate the properties of the components to those of the mixture. The accuracy of the method is typically of the order of 9%. The densities of the liquid mixtures were estimated according to the modified Hankinson-Brobst equation. Figure 48 illustrates the effect of temperature and composition on the viscosity of a 0.61/0.39 mole fraction ethanol/water mixtures in which various amounts of liquefied CO$_2$ were added. The values of the viscosities of the 0.61/0.39 mole fraction ethanol/water mixtures reported on the graph were taken from the literature [29].

The viscosities of liquefied CO$_2$ at 170 atm for different temperatures were calculated according to the following equation [32]:

$$\ln(\eta \cdot 10^5) = 3.3882 + \frac{1.3423\rho}{(1.909-\rho)}$$

(8)

where $\eta$ and $\rho$ are the viscosity expressed in Poise and the density in g cm$^{-3}$, respectively. The density of CO$_2$ was taken from the databank SFC Solver (ISCO, Lincoln, NE). The accuracy of equation (8) is expected to be on the order of 3% [32]. The decrease in the viscosity achieved by an increase in the temperature up to 60 °C could be easily obtained by the addition of 0.2 to 0.3 (depending of mixture composition) mole fraction
Table 9. Viscosities of liquids at 25 °C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>1.096&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water</td>
<td>0.8904&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>density dependent&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> literature value from reference [10]
<sup>b</sup> literature value from reference [33]
<sup>c</sup> calculated from reference [32]
Figure 48. Temperature effect on the viscosity of EtOH/H₂O (0.61/0.39) mixture with various amount of CO₂.

(+) 25 °C, (■) 30 °C, (□) 40 °C(●) 50 °C, (◇) 60 °C.
of the low viscosity fluid, i.e., CO$_2$ under isothermal condition. The effect of the
temperature on the viscosity is larger for the fluid mixtures with a smaller proportion of
CO$_2$ as compared to those with a larger proportion. This result is expected since the
viscosity depends upon the extent of hydrogen bonds. As the mole fraction of CO$_2$ in the
ethanol/water mixture increases, the fraction of hydrogen bonds between alcohol and water
and alike solvent molecules decreases. The breakdown of the hydrogen bonds by an
increase in the temperature is then more noticeable for mixture with a small proportion
of CO$_2$.

Liquid mass transport theories.

The analytical equations that describe the liquid mass transport in fluids were tested
for their application in enhanced-fluidity liquid mixtures of ethanol/water/carbon dioxide.
The commonly used Stokes-Einstein (S-E) and Wilke-Chang (W-C) mass transport models
were fitted against the experimental data.

The Stokes-Einstein equation. The hydrodynamic Stocke-Einstein equation
served as a basis for the prediction of the molecular diffusion coefficients of a solute in
a liquid. The theory was originally developed for the diffusion of solute molecules that
were larger than the solvent molecules. The solute was seen as diffusing slowly into a
continuous medium. The solvent molecules are associated to the surface of the solute.
This model assumed that only macroscopic properties such as the viscosity, the
temperature and the radius of the diffusing solute, control the diffusion process according
to the following equation [28]:
\[ D_{AB} = \frac{kT}{C\pi\eta r} \]  

(9)

where \( k \) is the Boltzmann constant, \( T \) the absolute temperature, \( C \) a constant, \( \eta \) the viscosity and \( r \) is the hydrodynamic radius of the solute, respectively. The value of the constant \( C \) can be either 6 or 4. If the solvent is treated as a continuous fluid, it is assumed to stick to the surface of the diffusing particle. This condition corresponds to the "stick" boundary condition of fluid flow and the value of \( C \) is 6. Another value of \( C \) is found in the case of the "slip" boundary condition. For this application where the fluid slips over the surface of the diffusing particle, the value of \( C \) is taken as 4. In equation (9), the value of 4 for the constant \( C \) seems to provide the best fit of the experimental data.

Self-diffusion in compressed benzene and tetramethylsilane was successfully described by the S-E equation [34]. The relationship holds also for hydrogen bonded liquid, such as methanol, using the slip boundary limit [35]. The estimation of molecular diffusion coefficients of solutes in hydrocarbons by this equation is poor. It also did not describe accurately either the ternary diffusion coefficients in supercritical fluids [7]. The S-E equation was tested against the experimental diffusion coefficients determined in the present study. The hydrodynamic radii of the solutes were approximated by their Lennard-Jones radii [28]. Values of 2.89 and 2.56 Å were chosen for m-cresol and benzene as their hydrodynamic radii, respectively [34]. The radius of anthracene (4.60 Å) was estimated using the molecular modelling software HyperChem™ (Autodesk, Inc., Sausalito, CA). Figures 49 through 52 show the plots of the experimental diffusion coefficients of the solutes versus the reciprocal viscosity under isothermal conditions.
Figure 49. Stokes-Einstein equation applied to the molecular diffusion coefficient of anthracene in EtOH/H₂O (0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Experimental: (□) 25 °C, (○) 30 °C, (△) 40 °C, (▼) 50 °C, (○) 60 °C.

Stokes-Einstein: (■) 25 °C, (●) 30 °C, (▲) 40 °C, (▼) 50 °C, (◆) 60 °C.
Figure 50. Stokes-Einstein equation applied to the molecular diffusion coefficient of benzene in EtOH/H₂O(0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Experimental: (□) 25 °C, (○) 30 °C, (△) 40 °C, (▽) 50 °C, (◇) 60 °C.

Stokes-Einstein: (■) 25 °C, (●) 30 °C, (▲) 40 °C, (▼) 50 °C, (♦) 60 °C.
Figure 51. Stokes-Einstein equation applied to the molecular diffusion coefficient of m-cresol in EtOH/H₂O (0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Experimental: (□) 25 °C, (○) 30 °C, (△) 40 °C, (▼) 50 °C, (♦) 60 °C.
Stokes-Einstein: (■) 25 °C, (●) 30 °C, (▲) 40 °C, (▼) 50 °C, (◆) 60 °C.
Figure 52. Stokes-Einstein equation applied to the molecular diffusion coefficient of 3-nitrophenol in EtOH/H₂O (0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Experimental: (□) 25 °C, (○) 30 °C, (▲) 40 °C, (▼) 50 °C, (◇) 60 °C.
The lines are drawn to facilitate the reading of the curves. In the case of 3-nitrophenol, the estimation of its molecular diffusion coefficient by the S-E equation was not done because the radius of the molecule could not be calculated. Changes in viscosity of the fluid mixtures were achieved by adding various amount of liquefied CO₂ to a 0.61/0.39 mole fraction of ethanol/water mixture. In the case of m-cresol, benzene and anthracene, the molecular diffusion coefficients predicted by the S-E equation are reported on the graph as well. The Stokes-Einstein equation underestimates the molecular diffusion coefficients for benzene and anthracene although it comes close for m-cresol (at low values of \(1/\eta\)). The deviation of the experimental data from the S-E equation is illustrated by the non linearity as well as the lack of zero point intercept of these plots. The difficulty in estimating the radius of the diffusing species may account for the poor prediction of the S-E equation. Moreover, the plots tend to deviate much more from the S-E prediction at low value of the viscosity, i.e., for mixtures with the highest proportion of CO₂. Previous results show that under isobaric condition, the extent of solvent clustering in an enhanced-fluidity liquid is larger for mixture with a high mole fraction of CO₂ [18]. The radius of the diffusing species is then expected to be larger in mixture of ethanol/water with 0.4 mole fraction of CO₂ compared to a mixture with a smaller amount of CO₂ dissolved in it. The fit of the experimental data to the S-E equation is then better for the highest viscosity mixture than it is for the lowest one. Nevertheless, the S-E equation gives a poor prediction of the molecular diffusion coefficient of the solutes in an enhanced-fluidity mixture. The assumption that the hydrodynamic radius of the diffusing solute is constant over the whole ethanol/water/carbon dioxide is mostly invalid. Nevertheless, the main
problem seems to be in the calculation of the viscosity of the EtOH/H₂O/CO₂ mixture. As the matter of fact, the calculated values of the viscosities are too high. This explain also the underestimation of the molecular diffusion coefficient provided by the S-E equation.

The Wilke-Chang equation. The Wilke-Chang (W-C) equation is a formula derived from the S-E theory and modified in order to fit the diffusion of organic solutes into water [36]. This empirical relationship is defined according to equation (accuracy ca. 10%) (10):

$$D_m = \frac{7.4 \times 10^{-8} (\Phi M)^{0.5} T}{\eta V_A^{0.6}}$$  \hspace{1cm} (10)$$

where $\Phi$, $M$, $T$, $\eta$ and $V_A$ are the association factor, the molecular weight of the solvent, the temperature, the viscosity and the molal volume of the solute at its normal boiling point, respectively.

The molal volumes of the solutes at their normal boiling point were calculated according to the Lebas additive volume method (see Table 10) [28]. The association factor, $\Phi$, was 2.6 for water, 1.5 for ethanol and 1 for carbon dioxide [36]. According to Perkins and Geankoplis, the diffusion in a multicomponent liquid mixtures introduces a modification of the "$\Phi M$" term according to the following equation [10]:

$$\Phi_m M = \sum_i (x_i \Phi_i M_i)$$  \hspace{1cm} (11)$$

where $x_i$ is the mole fraction of the $i$th component in the mixture. The association
Table 10. Calculation of the molal volume of the solute at its boiling point by the LeBas method.

<table>
<thead>
<tr>
<th>Solute</th>
<th>$V_A$ (cm$^3$ mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>96.0</td>
</tr>
<tr>
<td>Anthracene</td>
<td>196.7</td>
</tr>
<tr>
<td>m-cresol</td>
<td>125.6</td>
</tr>
<tr>
<td>3-nitrophenol</td>
<td>114.5</td>
</tr>
</tbody>
</table>
parameter is the effective molecular weight of the solvent with respect to the diffusing process. The W-C equation is expected to provide a better fit of the experimental data since the association parameter, $\Phi M$, accounts for the association between solvent-solvent molecules.

A good estimation of the diffusivities of organic compounds into water or water into hydrocarbon mixtures was found using the W-C equation [37]. Unfortunately, the diffusion of water in hydrocarbon solvents was overestimated because of the difficulty in estimating the accurate volume of the diffusing species. The water molecule was believed to diffuse as a tetramer molecule into the organic solvent.

The experimental diffusion coefficients of benzene, anthracene, m-cresol and 3-nitrophenol in the ethanol/water/CO$_2$ mixtures (starting with a 0.61/0.39 EtOH/CO$_2$) were plotted against the values calculated with the W-C equation. Figures 53 through 56 demonstrate that the W-C equation underestimates the diffusivities of anthracene and benzene but came fairly close to the experimental values found for m-cresol and 3-nitrophenol. It is surprising to notice that the W-C equation provides the best fit at low values of $(1/\eta)$. We would have expected a better fit in the low viscosity part of the curve since the W-C equation fails to describe the diffusivities of solutes in viscous solvents [38], [39]. For example in dodecane ($\eta=1.36$ cP), the W-C equation underestimates the diffusivity of ethane [40].

The Eyring rate theory. In a liquid mixture (including hydrogen bonded liquids), the effect of the temperature on the solute diffusion coefficients is often described by the Eyring rate theory as follows [41], [42], [43]:

\[ \text{Eyring rate theory} \]
Figure 53. Wilke-Chang equation applied to the molecular diffusion coefficient of anthracene in EtOH/H2O(0.61/0.39) mixtures with various amount of CO2 at 170 atm.

Mole % CO2: experimental: (□) 0, (○) 20, (△) 30, (◇) 40.

Wilke-Chang: (■) 0, (●) 20, (▲) 30, (∗) 40.
Figure 54. Wilke-Chang equation applied to the molecular diffusion coefficient of benzene in EtOH/H$_2$O(0.61/0.39) mixtures with various amount of CO$_2$ at 170 atm.

Mole % CO$_2$: experimental: (□) 0, (○) 20, (△) 30, (◇) 40.

Wilke-Chang: (■) 0, (●) 20, (▲) 30, (◆) 40.
Figure 55. Wilke-Chang equation applied to the molecular diffusion coefficient of m-cresol in EtOH/H₂O (0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Mole % CO₂: experimental: (□) 0, (○) 20, (△) 30, (○) 40.

Wilke-Chang: (■) 0, (●) 20, (▲) 30, (◆) 40.
Figure 56. Wilke-Chang equation applied to the molecular diffusion coefficient of 3-nitrophenol in EtOH/H₂O (0.61/0.39) mixtures with various amount of CO₂ at 170 atm.

Mole % CO₂: experimental: (□) 0, (○) 20, (△) 30, (◆) 40.

Wilke-Chang: (■) 0, (●) 20, (▲) 30, (◆) 40.
\[ \ln D_m = A + \frac{B}{T} \]  

where A, B and T are the pre-exponential constant, the activation energy of diffusion, the absolute temperature, respectively.

Figures 57 through Figure 60 are the plots of lnD_m versus the reciprocal of the temperature for the different solutes. The effect of temperature on the diffusion coefficients for the mixtures follows the Eyring rate theory. It seems that the Eyring rate theory works better for the enhanced-fluidity liquid mixtures. At this stage, there is no reasonable explanation that could justify it.
Figure 57. Plot of $\ln D_m$ versus $1/T$ for anthracene.

Mole % CO$_2$: experimental: (□) 0, (●) 20, (▲) 30, (▼) 40.
Figure 58.  Plot of $\ln D_m$ versus $(1/T)$ for benzene.

Mole % $\text{CO}_2$: experimental: $\square$ 0, $\bullet$ 20, $\triangle$ 30, $\blacktriangle$ 40.
Figure 59. Plot of Ln $D_m$ versus $(1/T)$ for m-cresol.

Mole % CO$_2$: experimental: (□) 0, (●) 20, (△) 30, (▼) 40.
Figure 60. Plot of $\ln D_m$ versus $(1/T)$ for 3-nitrophenol.

Mole % CO$_2$: experimental: (□) 0, (●) 20, (▲) 30, (▼) 40.
REFERENCES


CHAPTER V

LIQUID CHROMATOGRAPHY AT THE CRITICAL CONDITION USING

ENHANCED-FLUIDITY LIQUIDS

INTRODUCTION

Synthetic polymers are essentially copolymers that are “tailored” in order to improve the initial properties of the starting homopolymer or to achieve some other properties. The majority of commercially important polymers are synthetic copolymers. For example, the incorporation of 20 to 35 % of acrylonitrile to styrene produces a copolymer that has a better oil and grease resistance than the homopolymer of polystyrene. The cross-linking of polyisobutene is favored when the polymer is cationically polymerized with 1 to 3 mole percent of isoprene [1]. The flexibility of the polymers commercially available under the name of Spandex are achieved by the copolymerization of a stiff polymer such as polyurethane with a flexible polymer such as a polyester or a polyether [2].

Copolymers present chemical as well as molecular weight distribution functions. These distributions originate from several reasons. During the copolymerization process, the reactivities of the monomers may differ from those in a homopolymerization reaction. The most reactive copolymer is incorporated preferentially into the copolymer chain.
The difference in reactivities of the homopolymers leads also to a change in the composition of the feed mixture. This "composition drift" produces copolymers with significantly different chemical composition [3]. The molecular weight distribution is dependent both upon the reaction time (percentage of conversion) and the reaction mechanism of the polymerization process [4], [5]. The molecular weight distribution broadens and shift to higher molecular weight as the percentage of conversion of the polymerization reaction increases. The polydispersity obtained from most copolymerization reactions ranges between 1.05 to 5. Therefore, there is a need to control both the chemical and molecular weight distributions of copolymers because the polymer properties are usually linked to these distributions. The physical and mechanical properties such as the glass transition, the solution viscosity and the tensile strength are functions of the molecular weight and of the chemical composition of the polymers [6], [7]. Copolymers have distinctive properties depending on the proportion and the distribution of each monomer along the chain. In a random, alternating or short length block copolymer (one of the constituent is present to less than 5%), the properties are the average of those of the constituting n-mers. For the block copolymers that have a large block of repeating n-mers, the properties of the copolymer will vary significantly from its homopolymers.

Numerous analytical methods have been used to provide insight into the chemical and molecular weight distribution of the polymers. Mass spectrometry has been successfully applied to the characterization of macromolecular species through the improved ionization techniques. Fourier transform mass spectrometry coupled with laser
desorption or electrospray enables a direct and accurate analysis of the chemical structure and molecular weight analysis of complex macromolecules [8]. Spectroscopic techniques such as $^{13}$C NMR, IR Fourier transform spectroscopy, Raman and UV-visible absorption spectroscopy also give information on the structure of the polymer [9]. IR, Raman and $^{13}$C NMR provide a fingerprint of the polymer. Information on the molecular structure (tacticity) and on the average copolymer composition are also available. UV-visible spectroscopy has a limited application concerning the chemical identification of the polymer. The broad absorption bands and the application of this method to chromophore containing polymers limit its application. Chromatographic methods are also used to provide insights on the molecular weight and/or chemical distribution functions. Gradient elution and thin layer chromatography are frequently used to characterize polymers according to their chemical distribution. Thin layer chromatography is free from any molecular weight dependence if the molecular weight of the polymer is above 50,000 [10]. In the case of gradient elution chromatography, the mobile phase composition is changed in order to induce selective precipitation or adsorption of the polymer. Nevertheless, the use of nonisocratic conditions is not always the best choice. As a matter of fact, problems such as variation in the retention time and/or in the background (especially when using a UV-visible detector) may occur. Moreover, the fractionation by solubility depends upon the molecular weight as well as the chemical composition of the molecule. In the case of pronounced chemical heterogeneity, the fractionation is only governed by the chemical composition of the polymer. The separation of copolymers by gradient elution chromatography may also be hindered if the effects of copolymer solubility as well as the
polarity of the mobile phase do not support each other [11]. Moreover, little information is gained concerning the molecular weight distribution corresponding to each species.

The chromatography of polymers can be divided in two additional areas, each one addressing a specific kind of separation. Size exclusion chromatography is used to fractionate copolymers according to their hydrodynamic volume. Nevertheless, size exclusion chromatography of a copolymer may also be influenced to a certain extent by its chemical distribution function. Chemically inhomogeneous copolymers may have the same molecular weight but not the same retention volume. There might be a preferential solvation of chemical groups in the polymer and hence a difference in their hydrodynamic volume. As a matter of fact, the hydrodynamic volume of a copolymer is proportional to the product of its specific viscosity by its molecular weight. The specific viscosity, \([\eta]\), depends on the chemical heterogeneity of the copolymer according to the following equation [12]:

\[
[\eta] = KM^a
\]  

(1)

where \(M\) is the molecular weight and \(K\) and \(a\) are the Kuhn-Mark-Houwink parameters, respectively. The hydrodynamic volume is then a function of the chemical composition of the copolymer [13]. Unfortunately, there is no linear dependence of the Kuhn-Mark-Houwink parameters with the composition of the copolymer. Size exclusion chromatography will yield fractions that are more or less broad in term of chemical distribution. Information regarding both the compositional heterogeneity and weight distribution of a copolymer was gained through the use of size exclusion chromatography
coupled with selective detectors (UV, FT-IR, refractive index) [14], [15] or by orthogonal chromatography (OC) [16].

In OC the chemical distribution function is achieved by using liquid adsorption chromatography with either normal or reversed phase [17]; the molecular weight distribution corresponding to each fraction is then analyzed either by supercritical fluid chromatography or by traditional size exclusion chromatography [18]. This method can be time consuming.

Liquid chromatography at the critical condition thereafter denoted LCCC is an alternative chromatographic technique where part of the macromolecules controls the separation [19], [20]. The type of separation that will predominate is governed by the magnitude of the change in free energy when the macromolecule enters the pore of the stationary phase. The distribution coefficient of the macromolecule, $K_D$ is expressed as:

$$K_D = \exp\left(-\frac{\Delta F}{kT}\right) = \exp\left(-\frac{\Delta H}{kT} + \frac{\Delta S}{k}\right)$$  \hspace{1cm} (2)

where $\Delta F$ is the change in free energy, $k$ is the Boltzmann constant, $\Delta H$ and $\Delta S$ are the changes in enthalpy and entropy of the macromolecule when it enters the pore. A value of $\Delta F > 0$ corresponds to a size exclusion type process whereas a value of $\Delta F < 0$ allows a separation based upon the adsorption or precipitation/redissolution process.

Liquid chromatography at the critical condition occurs for $\Delta F = 0$. At the critical point, both the size exclusion (the entropy term) and the adsorption partition effects (the enthalpy term) cancel each other. The magnitude of the term "TAS" is always
negative and the magnitude of $\Delta F$ depends on the enthalpy term. The change in enthalpy is related to the energy of interaction of the macromolecular segment with the stationary phase.

In liquid chromatography at the critical condition, the retention volume of the polymer does not depend on its molecular weight, since the value of $K_D$ is equal to unity. LCCC is a method that allows the separation based upon the chemical heterogeneities of the polymer and is most often conducted in isocratic mode. For some given experimental conditions (mobile phase composition, temperature and nature of the stationary phase) part or the entire molecule could become "invisible" to the gel. At the critical condition, only a portion of the polymer controls the separation. For telechelic polymers, the backbone polymer would be made chromatographically invisible and then the observed separation would be achieved according to the number and nature of the functional groups on the backbone [21], [22]. For block copolymers, the separation is governed by some part of the molecule. Separations of copolymers that are quite close in terms of chemical nature have been successfully achieved. Pasch and coworkers reported the separation of a block copolymer of propylene oxide block ethylene oxide as well as the separation of a copolymer of decyl and methyl methacrylate [23], [24]. In the case of copolymer $A_nB_m$, the block $B$ may become invisible if we operate at its critical adsorption point and the block $A$ controls the separation and vice versa.

Finding the exact experimental critical conditions for the adsorption chromatography is a matter of trial and error. Usually, a nonsolvent of the polymer is added gradually to the mobile phase composition. The solvent strength of the mobile
phase is then reduced and the interactions between the macromolecule and the stationary phase begin to increase. The change in the mobile phase composition is the most widely used method to get to the critical point. The change in the temperature may be another alternative to alter the environment of the polymer [25]. Nevertheless this is not the preferred method because the temperature may affect the mechanism of interaction of the polymer with the stationary phase.

The use of liquid chromatography at the critical condition presents one drawback, the thermodynamic domain in which it happens is very narrow. Usually, a variation as small as 0.1 % in the mobile phase composition is enough to be outside the critical adsorption domain.

This study reports on the determination of the experimental conditions for the LCCC of polystyrene polymers using enhanced-fluidity liquid mixtures of tetrahydrofuran (THF) and carbon dioxide (CO₂). It also describes the benefits that may be achieved in term of chromatographic properties using enhanced-fluidity liquids.

**EXPERIMENTAL SECTION**

Chromatographic system. An ISCO LC-260D syringe pump (ISCO, Lincoln, NE) was used to deliver the mobile phase under high pressure. A Valco-W-series high pressure injection valve (Valco Instruments Co, Inc., Houston, TX) was used. The injection volume was 200 nL. The column used was a Jordi-Gel (divinylbenzene polymer) 100 mm x 4.5 mm, 500 Å, packed with 5 µm particles diameter (Keystone Scientific, Bellefonte, PA). The absorbance detector was a Spectra-Physics UV-2000.
An HP-SFC chromatographic oven was used (Hewlett-Packard, Novi, MI). The UV-visible flow cell was a piece of fused silica whose the polyimide coating has been removed (250 μm id) (Polymicro Technologies, Phoenix, AZ). The detection was done at 254 nm for the polymers containing the polystyrene moieties. It was done at 240 nm for the poly(methyl methacrylate) standards. A stainless steel tube of 0.004 in. id was the connection from the injector to the column. A pressure transducer was used to calibrate the pressure reading of the syringe pump (Setra System Inc., MA). Its precision and accuracy were of ± 0.37 atm.

Materials. All reagents have been used as received. The polystyrene samples as well as the copolymers of poly(styrene-maleic anhydride) and poly(styrene-methyl methacrylate) were purchased from Polysciences, Inc., Warrington, PA. The molecular weight (MW) of the polystyrene polymers covered a large range of molecular weight (MW = 4 x 10³, 9 x 10³, 1.6 x 10⁴, 2 x 10⁴, 4.0 x 10⁴, 6.3 x 10⁵, 9.0 x 10⁵, 1 x 10⁶). Polystyrene standards with molecular weights of 687, 2727 and 12,860 were obtained from Aldrich Chemical Milwaukee, WI. The poly(methyl methacrylate) standards were purchased from Polymer Laboratories, Amherst, MA. The copolymers of poly(methyl acrylate) were provided by Dr. K. Matyjaszewski from The Carnegie Mellon University. The copolymers of poly(styrene-butyl methacrylate) were donated by The BF Goodrich Company, Breckville, OH. Some of the properties of the copolymers are given in Table 11. Tetrahydrofuran (99.9 % purity) and methanol (100 % purity) (Baker Analyzed grade) were purchased from Mallinckrodt Chemical, Inc. Paris, KY. Acetonitrile (HPLC grade, 99.9% purity) and dichloromethane (99.9 % purity) were purchased from Fischer
Table 11. Characteristics of the copolymers.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Molecular weight$^1$</th>
<th>Ratio$^2$</th>
<th>M*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(styrene-maleic anhydride)(s)</td>
<td>1700</td>
<td>67</td>
<td>561</td>
</tr>
<tr>
<td>Poly(butyl methacrylate-g-styrene) #416$^3$</td>
<td>51000</td>
<td>39</td>
<td>31100</td>
</tr>
<tr>
<td>Poly(butyl methacrylate-g-styrene) #431$^3$</td>
<td>34500</td>
<td>68</td>
<td>11040</td>
</tr>
<tr>
<td>Poly(styrene-methyl acrylate)(#0526-2)</td>
<td>16000</td>
<td>62</td>
<td>6080</td>
</tr>
<tr>
<td>Poly(styrene-methyl acrylate)(#0626-12)</td>
<td>12000</td>
<td>45</td>
<td>6600</td>
</tr>
<tr>
<td>Poly(styrene-methyl acrylate)(#0622-8)</td>
<td>5000</td>
<td>44</td>
<td>2800</td>
</tr>
<tr>
<td>Poly(styrene-methyl acrylate)(#0519-2)</td>
<td>22000</td>
<td>45</td>
<td>12100</td>
</tr>
</tbody>
</table>

$^1$ The molecular weights are approximate values for the copolymers of poly(styrene-butyl methacrylate)

$^2$ Weight ratio of the polystyrene copolymer

$^3$ BF Goodrich designation for this copolymer sample

(s) statistical

(g) graft

M* "apparent" molecular weight calculated without the polystyrene part
Scientific, Pittsburgh, PA. All polymer samples were dissolved in tetrahydrofuran (1-4 mg/ml) or in the corresponding solvent mixtures of the mobile phase.

**Procedure.** All of the experiments were performed at room temperature unless otherwise specified. The flow rate was 0.29 ml/min. The flow rate variation was ± 0.004 ml/min. Measurements were made in triplicate.

**Solvatochromic study.** The solvent strength of the THF/CO₂ mixture was determined by monitoring the solvatochromic shift of various dyes. N, N-Dimethyl-p-nitroaniline was purchased from Eastman Kodak Company, Rochester, NY. 2-nitroanisole (99% purity) and 4-nitroaniline (99+% purity) were purchased from Aldrich Chemical Company, Inc. Milwaukee, WI. All reagents were used as received. A DMS-100 UV-visible spectrophotometer (Varian, Sunnyvale, CA) was used to measure the spectra of the dyes. The absorption spectrum of each dye 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 determined 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 the dyes used in these studies was approximatively 10⁻⁴ M.

**RESULTS AND DISCUSSION**

THF was chosen as the main eluent for several reasons. It is a good solvent for polystyrene as demonstrated by its Kuhn Mark-Houwink constant, “a” which is equal to 0.73. Its miscibility with both polar (methanol) and non polar (dichloromethane) solvents
allows its use in mixed solvents SEC experiments. CO₂ will act as a non solvent for the polymer. The choice of the mixture was also dictated by our ability to change its solvent strength by changing the mole percent of CO₂.

The solvent strength of the THF/CO₂ mobile phase was determined experimentally by solvatochromic studies. Kamlet-Taft parameters such as the polarity-polarizability value, π*, and the hydrogen acceptor ability, β, were calculated over the entire THF/CO₂ mobile phase composition. Moreover the use of THF/CO₂ allows for a smaller pressure drop across the column than with pure THF. This is due to the lower viscosity of the fluid mixture as demonstrated in Table 12.

Solvatochromic studies.

Numerous studies were undertaken to characterize the necessary solvent strength for a given SEC separation. Altelt and Moore showed that for a solvent to be compatible with the gel, the Hildebrand solubility of the solvent and the gel should be similar [26]. Dawkins also proposed guidelines for choosing solvents in SEC in terms of solubility parameters that minimize nonexclusion retention mechanism [27]. From these guidelines, the solvent strength should be carefully evaluated relative to that of the solutes and the gel to achieve optimum SEC performance. From these studies as long as a solvent strength similar to that of THF is maintained in the THF/CO₂ mixtures, the nonexclusion mechanisms should be minimized.

Kamlet-Taft solvatochromic parameters π* and β were chosen to characterize the solvent strength of the THF/CO₂ mixtures. The Kamlet-Taft parameters are unique in
Table 12  Pressure drop across the column as a function of the mobile phase composition. Flow rate is 0.29 ml/min, 136 atm.

<table>
<thead>
<tr>
<th>Mole % CO₂ in THF</th>
<th>ΔP (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.2±1.3</td>
</tr>
<tr>
<td>53.7</td>
<td>4.5±0.93</td>
</tr>
<tr>
<td>54.0</td>
<td>4.0±0.25</td>
</tr>
</tbody>
</table>
that they provide information on the specific types of molecular-level interactions present.
The $\pi^*$ is a measure of dipolar and polarizability interactions, and $\beta$ is a measure of the hydrogen bond Lewis basicity of a solution [28]. The Kamlet-Taft $\alpha$ parameter which measures the hydrogen bond acidity was not measured because both solvents have no hydrogen bond acidity.

The solvatochromic shift of the 4-nitroanisole absorption peak maximum was used to measure the $\pi^*$ of the THF/CO$_2$ mixtures using the following equation [29]:

$$\pi^* = \left( \frac{\nu_{\text{max}} - \nu_0}{s} \right)$$

where $\nu_{\text{max}}$ is the frequency of the absorbance maximum of 4-nitroanisole, when dissolved in the solvent of interest, $\nu_0$ 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 solvent. The dye 4-nitroanisole was chosen because it is neither an H-bond donor nor an H-bond acceptor. The literature value of $s$ for 4-nitroanisole, $-2.428 \pm 0.195$ kK was used to calculate $\pi^*$ in these experiments. Cyclohexane was used as the reference solvent. The absorption maximum for 4-nitroanisole in cyclohexane was 47.1 kK.

Figure 61 shows the variation of $\pi^*$ parameter with THF/CO$_2$ composition at 26 °C and 136 atm. The value of $\pi^*$ decreases as the mole fraction of CO$_2$ increases in the mixture. Two negative slopes are observed on the curve. The slope changes at approximately 0.70 mole fraction of CO$_2$ in THF. For mole fractions of CO$_2$ greater
Figure 61. Polarity-polarizability, $\pi^*$, of the THF/CO$_2$ mixture at 136 atm.
than 0.70, \( \pi^* \) decreases drastically with added CO\(_2\).

The values of the \( \beta \) parameter for the THF/CO\(_2\) mixtures were determined by measuring solvatochromic shift of 4-nitroaniline (1) relative to that of N,N-Dimethyl-p-nitroaniline (2) \[30\]. Figure 62 is a plot of \( \nu_{\text{max}} \) for N, N-Dimethyl-p-nitroaniline versus the \( \nu_{\text{max}} \) of 4-nitroaniline in solvents that vary in polarity but lack hydrogen bonding capabilities. The wavelengths of maximum absorption are reported in Table 13. The regression equation for the linear plot was then determined according to equation (4):

\[
\nu_{\text{max}}(1) = 1.0534 \nu_{\text{max}}(2) - 6.45 \ kK \quad r^2 = 0.997
\]  

(4)

The presence of hydrogen bonding in solvents causes deviation from this line. The extent of deviation is used to calculate the \( \beta \) parameter. The term, \( \beta \), is calculated using equations (5) and (6):

\[
\beta = \frac{\Delta \Delta \nu_{\text{max}(1\rightarrow2)}}{2.80}
\]  

(5)

\[
\Delta \Delta \nu_{\text{max}(1\rightarrow2)} = \nu_{\text{max}}(1\text{cal}) - \nu_{\text{max}}(1\text{exp})
\]  

(6)

where \( \nu_{\text{max}}(1\text{cal}) \) is the frequency of 4-nitroaniline calculated from equation (4) and \( \nu_{\text{max}}(1\text{exp}) \) is the frequency maximum in the solvent of interest (THF/CO\(_2\) mixtures) \[31\]. Figure 63 shows the variation of the \( \beta \) parameter with THF/CO\(_2\) composition at 26 °C and 136 atm. The \( \beta \) parameter, like \( \pi^* \), decreases with increasing proportions of CO\(_2\) and a
Figure 62. Frequency of N, N-Dimethyl-p-nitroaniline versus the frequency of 4-nitroaniline.
Table 13  Wavelength of maximum absorbance of para-nitroaniline (1) and N,N-Dimethyl p-nitroaniline (2).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{1, \text{max}}$ (nm)</th>
<th>$\lambda_{2, \text{max}}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>350.3</td>
<td>390.4</td>
</tr>
<tr>
<td>Toluene</td>
<td>340.9</td>
<td>379.0</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>350.3</td>
<td>390.4</td>
</tr>
<tr>
<td>Tetrachloromethane</td>
<td>328.9</td>
<td>364.3</td>
</tr>
<tr>
<td>Pentane</td>
<td>320.0</td>
<td>350.3</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>322.2</td>
<td>355.0</td>
</tr>
</tbody>
</table>

The precision of the spectrophotometer was 0.1 nm.
Figure 63. Hydrogen bond basicity, $\beta$, of the THF/CO$_2$ mixture at 136 atm.
slope change occurs at approximately 0.50 mole fraction of CO₂ in THF.

In summary, a substantial decrease in solvent strength of the THF/CO₂ mixtures occurs when more than 50.0 mole percent of CO₂ is present in the mixture. This is also the condition where the retention mechanisms are expected to change and where interactions of the polymer with the stationary phase should be favored.

Effect of the mobile phase composition.

The chromatography of polystyrene polymers is affected markedly by the mole fraction of CO₂ in THF. Figure 64 is a plot of the calibration curves of the polystyrene standards as function of the mole fraction of CO₂ in the mixture. As more CO₂ is added to the mobile phase, its solvent strength decreases favoring the interaction of the polymer with the stationary phase. With the introduction of a nonsolvent in the mobile phase, the calibration curve of the polystyrene polymers shifts from a pure size exclusion mechanism to a mixed mode that includes both size exclusion effect as well as adsorption or partition of the polymer. The retention volume observed can be expressed as the following equation [32]:

\[ V_R = V_0 + K_{sec} V_i + K_{ic} V_s \]  \hspace{1cm} (7)

where \( V_0 \) and \( V_i \) are the intracolumn volumes of mobile phase contained outside (o) or within (i) the pores of the packing. \( K_{sec}, K_{ic} \) are the size exclusion and the reversed phase retention distribution constants, respectively. \( V_s \) is the volume of stationary phase within the column.
Figure 64. Calibration curve of polystyrene polymers at 136 atm in THF/CO$_2$ mixtures. Mole percent of CO$_2$: (■) 0, (◇) 50.0, (▼) 53.0, (□) 53.7, (+) 54.0, (●) 55.0.
At a mole fraction greater than 54.0 mole %, the polystyrene polymers are separated either by adsorption or by dissolution/precipitation. At a precise mole fraction of 54.0 mole % of \( \text{CO}_2 \) in THF at 136 atm, all of the polymers that eluted, did so at the same retention volume (there is a slight discrepancy for the oligomer polystyrene of 687 molecular weight). Figure 65 is a chromatogram of some polystyrene polymers obtained at their critical condition. At the critical condition, the polystyrene polymers whose molecular weights were \( \leq 100,000 \) eluted. The polystyrene whose the molecular weight were higher did not elute (the PS with MW of 200,000 eluted but its peak shape was very bad).

To check to see whether the elution limitation (from MW 687 to 1000,000) is a characteristic of the THF/\( \text{CO}_2 \) mixture or a more universal problem, chromatographic experiments in mixtures of THF/methanol (MeOH) (methanol is a non solvent for the polystyrene), as well as, in mixtures of THF/acetonitrile (ACN) and THF/dichloromethane (CH\(_2\)Cl\(_2\)) (dichloromethane is a solvent for the polymer) were undertaken [25]. The polystyrene polymers were soluble in the THF/ACN over the composition chosen (except for the molecular weight 900,000 and 1000,000). For the THF/methanol mixtures, the polystyrene polymers up to MW \( \leq 200,000 \) were soluble in the mixtures even when 45 mole % of methanol was present. The polymers with MW > 200,000 were only slightly miscible with the THF/methanol mixtures. The poor solubility of the polystyrene polymers that have a molecular weight greater than 200,000 prevented their test with the THF/MeOH mixtures.
Figure 65. Chromatogram of polystyrene polymers,

54.0 mole % CO₂ in THF at 136 atm.

Polystyrene (MW): 2727, 4000, 12860, 20000, 100000.

(*) PS 12860 impurity.
The choice of methanol, acetonitrile and dichloromethane as cosolvents for tetrahydrofuran was dictated by their solubility parameter. Mori noticed that it is possible to shift from the adsorption/partition mode to the size exclusion mode by changing the Hildebrand solubility parameter of the mobile phase [33]. The solubility parameter of the stationary phase is $\delta \approx 9.1$ (cal cm$^{-3}$)$^{0.5}$ [12], the solubility parameters of THF, MeOH, ACN, CH$_2$Cl$_2$ and CO$_2$ are reported in Table 14. If the Hildebrand solubility parameter of the mobile phase is equal to that of the gel, there is preferential interaction between the solvent and the gel. This prevents the solutes from adsorbing to the gel and favors the size exclusion effect. Figures 66, 67 and 68 represent the calibration curve for the polystyrene in each solvent mixture at 136 atm. The addition of dichloromethane to tetrahydrofuran does not lead to any shift in the calibration curve of the polystyrene because both THF and dichloromethane are good solvents for the polymers and they have similar values of solubility parameters. Regarding the mixtures of THF/ACN, THF/MeOH and THF/CO$_2$ the addition of the cosolvent leads to a change in the type of interaction of the polystyrene with the stationary phase. The magnitude of the solute adsorption to the gel is given by the following equation [34]:

$$\Delta E = \Delta E_{\text{solute}} - (\Delta E_{\text{solv}} + \Delta E^*_{\text{solv}})
$$

where $\Delta E_{\text{solute}}$ and $\Delta E_{\text{solv}}$ are the magnitude of the partial molar energy of mixing of the solute and the solvent into the polystyrene gel, respectively. $\Delta E^*_{\text{solv}}$ is the partial molar energy of mixing of the solute into the solvent. These parameters are related to the solubility parameter according to these equations:
Table 14. Hildebrand solubility parameter of the solvent.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\delta$ (cal$^{1/2}$ cm$^{-3/2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>14.5</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>11.9</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>9.68</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>9.09</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>7.43$^1$</td>
</tr>
</tbody>
</table>

$^1$ calculated according to the formula [35]:

$$\delta = 1.25 \sqrt{P_c} \left( \frac{\rho}{\rho_{\text{liq}}} \right)$$ \hspace{1cm} (9)

where $P_c$, $\rho$ and $\rho_{\text{liq}}$ are the critical pressure in atm, the reduced density and the reduced density in the liquid state, respectively.
Figure 66. Calibration curve of polystyrene polymers at 136 atm in THF/ACN mixtures. Volume percent of THF:

(■) 100, (○) 70.0, (▼) 60.0, (□) 47.5, (+) 47.2, (○) 46.0.
Figure 67. Calibration curve of polystyrene polymers at 136 atm in THF/MeOH mixtures. Volume percent of THF:

■ 100, ◇ 80.0, ▼ 60.0, □ 57.0, (+) 55.0.
Figure 68. Calibration curve of polystyrene polymers at 136 atm in THF/CH₂Cl₂ mixtures. Volume percent of THF:

- ■ 100,
- ◊ 75.0,
- ▼ 60.0,
- □ 10.0.
\[ \Delta E_{sol}^* = (\delta_{solvent} - \delta_{solute})^2 \]  \hspace{1cm} (10)

\[ \Delta E_{solv} = (\delta_{gel} - \delta_{solvent})^2 \]  \hspace{1cm} (11)

\[ \Delta E_{solv} = (\delta_{gel} - \delta_{solute})^2 \]  \hspace{1cm} (12)

Since the polystyrene has about the same solubility parameter as the gel, \( \Delta E_{sol}^* \approx \text{zero}. \)

The solubility parameter of the binary fluid mixture has been calculated according to the following equation [36]:

\[ \delta = \frac{\Phi_i \delta_i + \Phi_j \delta_j}{\Phi_i + \Phi_j} \]  \hspace{1cm} (13)

where \( \delta \) and \( \Phi \) are the Hildebrand solubility parameter and the volume fraction of each component in the mixture respectively. By adding methanol or acetonitrile to tetrahydrofuran, the solubility parameter of the mobile phase increases significantly (Figure 69). The addition of \( \text{CO}_2 \), on the other hand, decreases the solubility parameter.

As the value of the solubility parameter of the mixed solvent \( \delta_{solvent} \) departs from the one of the gel, \( \delta_{gel} \), the solvent layer around the gel becomes weak and adsorption/partition of the polymer dominates. The addition of dichloromethane does not change the solubility parameter of the mobile phase significantly. The size exclusion mechanism is favored over the entire composition of THF/CH\(_2\)Cl\(_2\). We noticed that for the mixtures of
Figure 69. Hildebrand solubility parameter, $\delta$ of the fluid mixtures.

(■) THF/MeOH, (○) THF/ACN, (▼) THF/CH$_2$Cl$_2$. 
THF/ACN and THF/MeOH, the critical point occurs at a different solubility parameter value. It seems that the solubility parameter of the mobile phase is not the only parameter governing the mechanism of separation in the case of a polymer. Moreover at the critical point of each mixture (57/43 vol.% THF/MeOH and 47.2/52.8 vol.% THF/ACN), all of the polystyrene polymers did not elute. For the THF/MeOH mixtures, if the volume percent of THF drops below 57.0, there is adsorption of the polystyrene polymers whose molecular weights are greater than 20,000. It is similar with the THF/ACN mixtures, but the adsorption of the polystyrene polymers occurs if the volume percent of THF drops to 46.0. In the THF/ACN mixtures, the molecular weight range of the polymer that can be eluted is the same as in the THF/MeOH mixtures.

The molecular weight limitation is not totally unexpected since most of the polymers separations at the critical point published in the literature have an upper limit of log MW = 4 - 4.5 [24]. As mentioned by Entelis et al., in the case of high molecular weight polymers (MW > 10^6), the adsorption process takes place resulting in difficulties in the critical chromatography [37]. Figure 70 illustrates the peak shape degradation of the high molecular weight polystyrene at the critical point of adsorption. As the molecular weight of the polymer increases past 100,000, the adsorption process dominates. The calibration curves obtained either with THF/ACN or THF/MeOH demonstrate the very narrow range of mobile phase composition suitable for the elution of the high molecular weight polymer. A change in the mobile phase composition as small as 1.2 volume percent is sufficient to go from completely unretained to totally adsorbed. Studies of reversed phase liquid chromatography of polystyrene polymers have also shown this
Figure 70. Effect of the molecular weight of the polystyrene polymers on the peak shape at the critical adsorption condition (54 mole % CO₂ in THF, 136 atm).

Top: PS MW 12,860. Bottom: PS MW 100,000.
trend. In reversed phase chromatography, the polystyrene whose molecular weights are \( \geq 50,000 \) are totally adsorbed when the mobile phase composition is changed from 85/15 (v/v) of THF/H\(_2\)O to 83/17 (v/v) of THF/H\(_2\)O [32]. The difficulty with gradually increasing the retention volume of the high molecular polymers for a minute change in the mobile phase composition is explained by the multi-site attachment of the polymer. A polymer can be transported in the mobile phase only if all of its segments are not retained by the stationary phase.

The capacity factor of a homopolymer composed of \( P \) units, \( k_{\text{total}} \), can be expressed as a function of the capacity factor of its monomer unit, \( k_u \) [11]:

\[
k_{\text{total}} = (k_u + 1)^n - 1
\]  

(14)

The value of \( k_{\text{total}} \) can become very large for high molecular weight polymer. The dependence of the capacity factor of the high molecular weight polymer on the change in the mobile phase composition is expressed as the following equation [38]:

\[
k_{\text{total}} = \exp[AM(X-X_c)]
\]  

(15)

where \( A \) is a negative constant dependent on solvent-solvent and solvent-polymer interchange energy, \( M \), is the molecular weight of the polymer, \( X \) and \( X_c \) are the volume fraction of the good solvent in the mobile phase and its volume fraction in the critical mobile phase composition. Equation (15) illustrates that \( |\partial k'/\partial X| \) is larger for the high molecular weight polymer than for the oligomers and explains why the chromatography of high molecular weight is more challenging. This implies that the critical condition can
be achieved only for a limited polymer molecular weight range despite the theory that does not predict any molecular weight limitation. This limitation comes from the fact that the theory of critical adsorption chromatography assumes that the adsorption interaction per molecular subunit is constant regardless of the polymer structure [39]. This is not true in the case of the high molecular weight polymers. This explains why in our case, the polystyrene polymers that have a molecular weight greater than $2 \times 10^5$ did not elute and this also could explain the poor chromatographic efficiency obtained for the high molecular weight polymer that did elute as demonstrated in Figure 70. Nevertheless, the THF/CO$_2$ mixtures allowed a largest range of polystyrene polymers to elute at the critical condition than the other liquid mixtures.

**Effect of pressure near the critical adsorption point.**

As mentioned earlier, the critical point area is a very narrow region where a change in the mobile phase composition as small as 0.1 mole % will be enough to cause a deviation from the critical point. An alternative way to reach the critical condition without having to change the mobile phase was studied. This method derives from the unique properties of enhanced-fluidity mixtures. The solvating effect of the mobile phase can be varied by changing its density. The use of compressible mobile phase is then particularly advantageous. The addition of the low viscosity fluid to an organic solvent leads to a highly compressible fluid mixture as demonstrated by previous work [40]. The isothermal compressibility of these kinds of mixture is intermediate between those of a liquid and those of a supercritical fluid. It is then possible to change the solvent strength
of any enhanced-fluidity liquid mixtures by changing the pressure of the system. The thought was therefore that the critical condition could be found by varying the pressure. Figure 71 demonstrates the effect of pressure on the calibration curve for polystyrene at 54.0 mole percent CO₂ in THF (the critical composition at 136 atm). The pressure was changed from 136 atm to 238 atm. As the pressure increases, the calibration curve shifts to the size exclusion mode. The main advantage of using an enhanced-fluidity liquid as a mobile phase for critical size exclusion chromatography on a "near critical" mobile phase composition, namely 53.7 mole % CO₂ in THF is that the critical condition of the polymer is reached by changing the pressure. Smaller increments of pressure change were investigated in the case of a "near-critical" mixture (53.7 mole % CO₂ in THF at 136 atm) in order to see how much of a change in the pressure is needed to get to the critical point (see Figure 72). At this composition, the critical condition point is reached at 92.3 atm. Fortunately, the pressure change seems to affect much more the high molecular weight polystyrene polymers than it does with the lowest molecular weight polystyrene. In order to demonstrate that this effect is due to the unique properties of these enhanced-fluidity liquid mixtures, the same chromatographic experiment was performed in THF. Figure 73 shows the effect of pressure on the calibration curve of polystyrene in THF. The pressure was varied from 102 atm to 238 atm. Negligible change in retention occurs at these pressures because of the lower isothermal compressibility of the mobile phase. Figure 74 is the chromatogram that illustrates the pressure effect on the polystyrene polymers as well as on one of its copolymer, the poly(styrene-butyl methacrylate). The chromatograms have been obtained at 136 and
Figure 71. Pressure effect on the calibration curve of polystyrene,

54.0 mole % CO₂ in THF.

(■) 238 atm, (○) 203 atm, (▼) 169 atm, (□) 136 atm.
Figure 72. Pressure effect on the calibration curve of polystyrene,

53.7 mole % CO₂ in THF.

(■) 69.3 atm, (◇) 92.3 atm, (▼) 116 atm, (□) 136 atm.
Figure 73. Pressure effect on the calibration curve of polystyrene, 100 mole %THF.

(■) 102 atm, (◇) 136 atm, (▼) 170 atm, (□) 238 atm.
Figure 74. Pressure effect on the chromatograms of polystyrene polymers, 53.7 mole % CO₂ in THF.

Left: (a) PS-PBMA #416 and 431, (b) PS 200000, (c) PS 12860, 4075, 2727, (d) PS 687.

Right: (a) PS-PBMA #416 and 431, (b) PS 200000, 12860, 4075, 2727, 687.
116 atm for a 53.7 mole % of CO₂ in THF. The pressure effect seems to affect only the polystyrene homopolymers. The first eluting peak is the copolymer of poly(styrene-butyl methacrylate). Its molecular weight is too high to see any effect of the pressure on its retention volume (see explanation later in text). It seems that the critical condition is broader in term of pressure tuning than it is in term of mobile phase composition. The plots done at 116 and 92.3 atm are very much alike in terms of the slope. Figure 71 and Figure 72 indicate that both the polarity and the solvation properties (clustering of solvent molecules around the solute) of the mobile phase control the retention volume of the polystyrene polymers. The contribution of each of these effects depends upon the pressure. At high pressure, i. e., in the 136-238 atm range, the isothermal compressibility of the enhanced-fluidity liquid is low. The clustering of the solvent molecules around the polymer is not significant. The polarity of the mobile phase governs the retention volume of the polystyrene polymers. In the low pressure region, i. e., in the 69.3-238 atm range, the isothermal compressibility of the mixture is quite large. There is some significant solvent clustering around the polymer. The relative contribution of the polarity and the clustering phenomenon on the retention volume can not be predicted without further investigations. The effect of the pressure on the solvation in the THF/CO₂ mixture could be investigated by the determination of the partial molar volume of the polystyrene polymers in the THF/CO₂ mixture. Another parameter that needs to be accounted for is the effect of the pore size. It was not investigated in this study.

Nevertheless, for a given mobile phase composition, the pressure at which the critical adsorption point is reached should be dependent on the mobile phase composition,
the pore size and the nature of the stationary phase.

Effect of the temperature.

Pressure and temperature are the two parameters that lead to a significant density change of the mobile phase at constant composition in the case of a compressible mobile phase. At constant pressure, the increase in the temperature leads to a decrease in the density of the mixture. This would decrease the solvent strength of the mobile phase; the interactions of the polymer with the stationary phase would be expected to increase with the temperature increase. The effect of temperature on the calibration curve was investigated in this study. The increase in the temperature may lead to the swelling of the gel, the expansion of the mobile phase and a change in the hydrodynamic volume of the polymer [41], [34]. The increase in the viscosity of the mobile phase was counterbalanced by running the experiments at a constant flow rate.

The temperature was raised from 26 °C to 50 °C in 100 mole % THF where a pure size exclusion mechanism exists and from 26 °C to 60 °C near the critical condition (53.7 mole % CO₂ in THF). The pressure was maintained at 136 atm. The experimental results are in good agreement with our prediction concerning the shift in the calibration curve for the THF/CO₂ toward the adsorption type mode as we increase the temperature. Figures 75 and 76 are the calibration curves of polystyrene as a function of temperature in the near critical region of adsorption (53.7 mole % CO₂ in THF, 136 atm) and in pure THF, respectively. The high molecular weight polystyrene polymers (MW > 50,000) were
Figure 75. Effect of temperature on the calibration of polystyrene polymers, 53.7 mole % CO$_2$ in THF.

(■) 26°C, (○) 30°C, (▼) 40°C, (□) 50°C, (+) 60°C.
Figure 76. Effect of temperature on the calibration of polystyrene polymers, 100 mole % THF. (■) 26 °C, (○) 30 °C, (▼) 40 °C, (□) 50 °C.
noticeably adsorbed even at temperatures as low as 40 °C. Poor peak shape resulted and for all the polystyrene polymers especially for the molecular weight range of 687 to 20000, significant tailing occurred.

One of the drawbacks in using temperature variation to reach the critical condition is that temperature change seems to cause different results depending on the molecular weight and the nature of the polymer [42], [43]. Previous investigations studied the effect of temperature on the retention volume of polystyrene (MW from 1800,000 to 900) and polyisobutene (MW from 1050,000 to 4300) on various stationary phase (porous glass beads and polystyrene gel). For a temperature range of 25 °C to 150 °C, a temperature inversion was noted only for the polystyrene polymers. For polyisobutene, an increase in the temperature leads to an increase in the retention volume of the polymer over the entire molecular weight range. The same effect is observed for the polystyrene polymers except for the polymers with a molecular weight below 100,000. In this case, the retention volume decreases with an increase in the temperature. In the present study, the "temperature inversion" occurs for polystyrene polymers whose MW < 10,000. The previous studied described the temperature inversion as a change in the pore volume accessible to the polymer. As the gel expands, the inside pore volume increases and the interstitial volume decreases. According to the linear expansion coefficient of polystyrene, the swelling of the polystyrene gel was considered to be negligible (even for a temperature increase to 150 °C). In this study, the temperature increases is small. According to the Figure 76, the increase in temperature does not leads to any significant change in the retention volume of the polymer: the swelling of the gel is then negligible.
The change in the hydrodynamic volume of the polymer could be another factor responsible for the increase of the retention volume of the high polystyrene polymers. Nevertheless, a change in the hydrodynamic volume is very improbable (at least over this temperature range). Cantow et al. did not find any detectable change in the coil size of the polystyrene polymers upon a larger temperature change [43]. For a temperature increases from 15 to 45 °C, the change in the hydrodynamic volume of polystyrene polymers in the system polystyrene gel/THF was also negligible [34]. In the case of the lower molecular weight (below 10,000 MW), the net result of increasing the temperature is not clear and it appears that the solubility effect overcomes the adsorption effect. The increase in temperature (from 40 °C to 60 °C) decreases the retention time of the lower polystyrene polymer. The solubility of the polymer in the mobile phase increases as the temperature increases. This is typical of a solubility effect. If the polymer was adsorbed, an increase in temperature would have increased its retention time.

Investigation of the chromatography of copolymers of polystyrene.

The concept of invisibility of part of the copolymers was investigated using the following copolymers: poly(styrene-maleic anhydride) (PS/PMAH), poly(styrene-butyl methacrylate) (PS/PBMA) and poly(styrene-methyl acrylate) (PS/PMA). The chemical composition of these copolymers is given in Table 11. The experimental conditions needed to reach the critical condition for polystyrene polymers are different from that required for the others polymers namely poly(methyl acrylate) and poly(butyl methacrylate). The reason is due to the difference in polarity of the styrene with the
others polymers. Previous analysis of blends of poly(styrene) and poly(methyl methacrylate) using LCCC revealed that they have distinct critical adsorption domains [44]. It is then possible to achieve the critical condition of one part of the block copolymer without affecting the chromatography of the other part. Regarding the similar chemical nature of the butyl methacrylate and methyl methacrylate group, the critical conditions for those blocks should be similar.

The experimental conditions for the copolymers of poly(styrene-maleic anhydride) and poly(styrene-butyl methacrylate) were chosen in order to be at the critical condition of the polystyrene (116 atm for 53.7 mole % of CO₂ in THF). The experimental conditions for the chromatography of the poly(styrene-methyl acrylate) were also at the critical condition namely 136 atm for 54 mole % CO₂ in THF.

No significant change in the retention volume of the poly(styrene-butyl methacrylate) was noticed at the critical point whereas a significant change in the retention volume was noticed for the copolymer of poly(styrene-maleic anhydride) (PMAH) as demonstrated in Figure 77. The retention volume of the copolymer PS/PMAH (two peaks seen) shifted from \( V_r = 0.72 \ (0.94) \) ml in THF (size exclusion mode) to 0.90 (0.95) ml at the critical condition of the polystyrene backbone. The polystyrene part of this copolymer is 67 weight %, the observed shift in retention volume corresponds to a polymer having a molecular weight less than 687 in the size exclusion mode (the retention volume of PS 687 in THF is about 0.85 ml). This is true only when the polystyrene backbone becomes invisible. The exact shift in retention volume of the PS/PMAH at the critical condition cannot be predicted, even knowing its apparent molecular weight.
Figure 77. Chromatograms of poly(styrene-maleic anhydride) in different chromatographic modes, 116 atm

Top: 100 mole % THF. Bottom: 53.7 mole % CO$_2$ in THF.
This is because maleic anhydride does not homopolymerize and the calibration curve related to this part of the copolymer could not be obtained. The copolymer of poly(butyl-methacrylate) eluted at the same retention volume as the high molecular weight polystyrene (MW > 50,000) under size exclusion condition (in 100 mole % THF). In the case of the copolymer of poly(styrene-butyl methacrylate), the effect is not obvious since the size exclusion limit of the stationary phase used is approximatively 10,000. For this copolymer, even if the polystyrene part is invisible, the “apparent” molecular weight of the butyl methacrylate portion is still past the exclusion limit of the SEC particle, namely ~ 31100 for PS/PBMA (#431). This explains why there is no significant change in the retention volume of the copolymer of poly(styrene-butyl methacrylate) as the chromatography is changed from a size exclusion process to the chromatography at the critical point of the polystyrene polymer.

The chromatograms at the critical condition of polystyrene for the copolymers of poly(styrene-methyl acrylate) are in Figure 78 through Figure 81. A net increase in the retention volume of the copolymers of poly(styrene-methyl acrylate) was noticed at the critical condition. Table 11 lists the apparent molecular weights that the copolymers of poly(styrene methyl acrylate) would have if the polystyrene polymer part was invisible. This corresponds to an apparent decrease in the molecular weight of the copolymer as predicted according to the theory. In order to ensure that the methyl acrylate part of the copolymer is not affected at the critical adsorption point, calibration curves of homopolymers of poly(methyl methacrylate) were measured both in 100 mole % of THF and at 54 mole % of CO₂ in THF (see Figure 82). The behavior of the homopolymers of
Figure 78. Chromatograms of poly(styrene-methyl acrylate) #0626-12 in different chromatographic modes, 136 atm.

(Top) 100 mole % THF. (Bottom) 54.0 mole % CO$_2$ in THF.
Figure 79. Chromatograms of poly(styrene-methyl acrylate) #0622-8 in different chromatographic modes, 136 atm.

(Top) 100 mole % THF. (Bottom) 54.0 mole % CO₂ in THF.
Figure 80. Chromatograms of poly(styrene-methyl acrylate) #0562-2 in different chromatographic modes, 136 atm.

(Top) 100 mole % THF. (Bottom) 54.0 mole % CO₂ in THF.
Figure 81. Chromatograms of poly(styrene-methyl acrylate) #0519-2 in different chromatographic modes, 136 atm.

(Top) 100 mole % THF. (Bottom) 54.0 mole % CO₂ in THF.
Figure 82. Calibration curve of poly(methyl methacrylate) standards at 136 atm.

(◊) 100 mole % THF, (■) 54.0 mole % CO₂ in THF.
poly(methyl methacrylate) were used as representative models of the methyl acrylate part since their structure differ only in one methyl group. The methyl methacrylate homopolymers behave in a size exclusion type mode both in 0.0 and 54.0 mole % of CO₂ in THF. At the critical adsorption point of the polystyrene, the retention volume of the copolymers should correspond to the retention volume of the methyl acrylate part of the copolymer. Good correlation was obtained at the critical adsorption point between the retention volume of homopolymers of methyl methacrylate and the retention volume of the copolymer as indicated in Tables 15 and 16. Each chromatogram is composed of three peaks. The first peak of the chromatograms correspond to the copolymer of poly(styrene-methyl acrylate) with the molecular weight specified in the Table 11. The other two peaks, eluting at the later retention volumes, correspond to copolymers with higher weight percent of polystyrene than the one specified in Table 11. The retention times of these peaks are >3 min. At the critical adsorption point, the “apparent” molecular weight is less than the molecular weight of the copolymer. From Figure 82, the last two eluting peaks correspond to a copolymer that would have a weight of the methyl methacrylate part below 600 MW (the homopolymer of methyl acrylate with a MWₙ of 603 elutes at 3.2 min at the critical adsorption point). The detection for the copolymers was done at 254 nm, a wavelength where only the polystyrene part absorbs. The intensity of each peak is proportional to the concentration of the species as well as its molar extinction coefficient. In Figures 78 and 79 (gradient copolymers), the last two eluting peaks have a higher intensity than the first one. It reflects the highest weight percent of the polystyrene in the last two eluting peaks. In Figure 80 and 81 (the block and random copolymers), the last
Table 15. Retention time of homopolymers of poly(methyl methacrylate) at the
critical adsorption point (54.0 mole % CO$_2$ in THF, 136 atm).

<table>
<thead>
<tr>
<th>$M_n$</th>
<th>$t_R$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2769</td>
<td>2.7</td>
</tr>
<tr>
<td>5790</td>
<td>2.5</td>
</tr>
<tr>
<td>10965</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 16. Retention time of copolymers of poly(styrene-methyl acrylate) at the critical adsorption point (54.0 mole % CO₂ in THF, 136 atm).

<table>
<thead>
<tr>
<th>Copolymer #</th>
<th>tᵣ (min)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0519-2</td>
<td>2.5</td>
<td>random</td>
</tr>
<tr>
<td>0526-2</td>
<td>2.5</td>
<td>block</td>
</tr>
<tr>
<td>0626-12</td>
<td>2.5</td>
<td>gradient</td>
</tr>
<tr>
<td>0622-8</td>
<td>2.7</td>
<td>gradient</td>
</tr>
</tbody>
</table>
two eluting peaks have a lower intensity than the first one. This may be due to the highest purity of the copolymers.

The effect of invisibility concept is then clearly demonstrated. Resolution of the chemical distribution of the methyl acrylate in the copolymers could not be achieved in this case because of the size exclusion behavior. A stationary phase that provides more interactions with the polar part of the copolymer (normal-phase such as silica) would be advisable and would lead to the separation of the methyl acrylate part according to the adsorption effect. Toluene could be used as a mobile phase in normal phase chromatography because it induces the selective adsorption of poly(methyl methacrylate) polymers but it is a size exclusion solvent for the poly(styrene) polymers [45].

**Adsorption and solubility effect.**

Further investigations on the mechanisms governing the chromatography of polystyrene outside the domain of size exclusion in the THF/MeOH, THF/ACN and THF/CO$_2$ solvent mixtures were also undertaken. Adsorption and/or precipitation-dissolution are mechanisms frequently encountered in the chromatography of polymers [46]. The identification of the nature of these processes can be done either by the study of the sample size, the temperature or the gradient elution effects on the retention volume [47]. The effect of temperature on the retention volume depends on the interaction mechanisms of the polymer with the stationary phase. An adsorption phenomenon is predicted if an increase in temperature leads to an increase in the retention volume of the polymer. The adsorption mechanism implies displacement of the solvent.
molecules followed by the adsorption of the polymer. Adsorption of the polymer leads to a negative change in its entropy whereas there is a gain in the entropy of the solvent upon its displacement from the stationary phase. The overall change in entropy is positive resulting in increase of adsorption by an increase in the temperature. In the case of a dissolution-precipitation mechanism, an increase in temperature leads to a decrease in the retention volume due to the increase in the solubility.

The nature of the mobile phase, as well as, the characteristics of the stationary phase influence which mechanism will dominate. Glöckner et al. demonstrated that adsorption of polystyrene polymers occurs on a reversed phenyl bonded phase whereas the retention mechanism was governed by the solubility of the polymers in the case of a C18 bonded phase [17].

A study of the temperature effect was done in the THF/CO₂ system. Figure 75 demonstrates that the retention volume of the polystyrene increases with temperature (at least for PS whose MW ≥ 10,000). This is the trend expected if adsorption is the controlling mechanism. The adsorption mechanism is also favored by the ability of the stationary phase used in this study to interact with the polystyrene polymers by π-π interaction.

Gradient elution chromatography was not performed in our study. Nevertheless from the calibration curves obtained in the THF/ACN and THF/MeOH (Figure 66 and Figure 67, respectively), the effect of a gradient elution could be predicted. The solubility of polystyrenes in a mixture of THF/MeOH increases with the percentage of THF in the mixture [23]. The same behavior could be expected in the THF/ACN mixture since THF
is the good solvent. If the retention mechanism is governed by a precipitation/dissolution process, the retention volume of the polystyrene should increase with increasing molecular weight when the volume percent of methanol in THF is increased. Moreover, the increased polarity of the mobile phase during this gradient elution should favor an increase of retention volume with an increase in the molecular weight of the polymers. As shown in the graphs, the effect is inverse. A mechanism of precipitation/dissolution is ruled out and the mechanism of adsorption seems more probable in these mixtures also.
REFERENCES


44. Pasch, H. *Polymer* 1993, 34, 19.


APPENDIX

UV-VISIBLE ABSORPTION SPECTRA OF ANTHRACENE AND NAPHTHALENE AND FLUORESCENCE OF NAPHTHALENE IN MeOH/CO₂ MIXTURES

This appendix deals with the preliminary results concerning the application of the spectroscopic ruler to the enhanced-fluidity liquid mixtures of MeOH/CO₂. The data concerning the UV-visible absorption spectra of anthracene and naphthalene as well as the fluorescence spectrum of naphthalene in the MeOH/CO₂ mixtures are presented. The mole percent of methanol in the mixtures was varied from 10 to 40 and the pressure and temperature were remained constant at 136 atm and 25 °C, respectively. These data allow the calculation of the efficiency of the energy donor absorption, P, as defined in Chapter II, as a function of the excitation wavelength and the mixture composition.
EXPERIMENTAL SECTION

UV-visible absorption spectrum of anthracene and naphthalene in MeOH/CO₂ mixtures.

Material. Naphthalene (≥98% purity, Mallinckrodt, Inc., Paris, KT), anthracene (Baker grade from J. T Baker Chemical Co., Philipsburg, NJ) and research SFC/SFE grade CO₂ (99.999% pure, Matheson, Newark, CA) were used without further purification. Methanol was HPLC-grade (99.99% pure with < 0.01% H₂O) from J.T. Baker (Phillipsburg, NJ). To remove the dissolved air from the methanol, the methanol was sonicated before use.

Mixture preparation. The MeOH/CO₂ mixtures were prepared according to the procedure described in Chapter III.

Absorption spectra. The absorption spectra of anthracene and naphthalene in MeOH/CO₂ mixtures were obtained according to the experimental procedure similar to the one described in Chapter II. The concentration of anthracene and naphthalene in the high pressure cell were 4.76 x 10⁻³ M and 4.49 x 10⁻³ M, respectively.

Fluorescence measurements.

Fluorescence spectra. The fluorescence spectrum of naphthalene in the MeOH/CO₂ mixtures were performed in steady-state fluorescence according to the procedure described in Chapter II. The concentration of naphthalene in the sapphire tube (V=0.3384 ml) was 2.8 x 10⁻³ M. The fluorescence spectrum of naphthalene were taken at 270, 272 and 275 nm.
RESULT AND DISCUSSION

Bathochromic shift of naphthalene and anthracene in MeOH/CO₂ mixtures.

According to the discussion presented in Chapter II, a wavelength shift is expected to occur for the UV-visible absorption maximum of the solutes as the mole percent of methanol is increased in the mixture. A bathochromic shift is the result of a larger stabilization of the excited state by the solvent than the ground state. As the polarity of the mixture is increased, there is both a slight increase in the absorbance of the solute and a bathochromic shift in the wavelength. Figure 83 shows the bathochromic shift observed for anthracene in the 10 and 40 mole percent of methanol mixtures. Figures 84 and 85 represent the absorption spectrum of anthracene in the 20 and 30 mole percent of methanol mixtures, respectively.

The bathochromic shifts of anthracene and naphthalene are evidenced by Figure 86 through Figure 89. The increase in the mole percent of methanol in the mixture provides with a larger wavelength shift for anthracene than for naphthalene. This may be due to the larger change in the polarizability of anthracene than naphthalene (see Chapter II). A the mole percent of methanol changes from 10 to 40, there are an average of 2 nm and 1 nm bathochromic shift for anthracene and naphthalene, respectively.

The wavelength shifts observed for anthracene and naphthalene are smaller than the one observed for the solvation of polar compounds in MeOH/CO₂ mixtures. Even in the region of the low compressibility of the MeOH/CO₂ mixtures (35°C, 300 atm), there is a 3.8 nm shift in the wavelength of phenol blue when the mole percent of methanol increases from 1 to 2. The magnitude of the wavelength shift observed for anthracene
Figure 83. Quantitative UV-visible absorption spectrum of anthracene in MeOH/CO$_2$ mixture, 136 atm, 25 °C

Mole percent of methanol: (+) 10, (∆) 40.
Figure 84. Quantitative UV-visible absorption spectrum of anthracene in 20/80 MeOH/CO₂ mixture, 136 atm, 25 °C.
Figure 85. Quantitative UV-visible absorption spectrum of anthracene in 30/70 MeOH/CO₂ mixture, 136 atm, 25 °C.
Figure 86. Wavelength shifts of the peak of maximum absorption for anthracene in MeOH/CO$_2$ mixture, 136 atm, 25° C.

The error bars not shown on the graph are within the size of the dots.
Figure 87. Wavelength shifts of the peak of maximum absorption for anthracene in MeOH/CO₂ mixture, 136 atm, 25° C.

The errors bar not shown on the graph are within the size of the dots.
Figure 88. Wavelength shifts of the peak of maximum absorption for naphthalene in 
MeOH/CO₂ mixture, 136 atm, 25° C.

The error bars not shown on the graph are within the size of the dots.
Figure 89. Wavelength shifts of the peak of maximum absorption for naphthalene in MeOH/CO$_2$ mixture, 136 atm, 25$^\circ$ C.

The error bars not shown on the graph are within the size of the dots.
and naphthalene in this study are smaller than those found in supercritical CO$_2$ at reduced densities greater than 1.2 (see Figure 24 of Chapter II). The extent of methanol clustering around the solute is then smaller in the enhanced-fluidity mixture than in supercritical liquid.

**Calculation of the efficiency of the energy donor absorption.**

The determination of the efficiency of the energy donor absorption, $P$, requires the choice of the wavenlengths defined as $\lambda_1$ and $\lambda_2$, according to Chapter II.

Naphthalene absorbs mainly between 250 nm and 285 nm. Figure 90 shows the effect of the excitation wavelength, $\lambda_1$, on the absorbance of naphthalene in the MeOH/CO$_2$ mixtures. For all of the mixtures, the highest absorbance of naphthalene occurs for $270 \text{ nm} \leq \lambda_1 \leq 275 \text{ nm}$. The excitation wavelength to be chosen depends also on the absorption of the anthracene in this region. The calculation of the efficiency of the energy donor absorption in the wavelength region from 270 nm to 282 nm was done according to the equation (11) of the Chapter II. Figure 91 is the effect of the excitation wavelength on the $P$ term. As the excitation wavelength is increased from 275 nm to 282 nm, the magnitude of the $P$ term levels off and reach its highest value. The MeOH/CO$_2$ mixtures with the highest mole fraction of methanol have the lowest $P$ term value. This may be due to the larger increase in the absorbance of anthracene in the 270 nm - 275 nm region compared to the increase in the absorbance of naphthalene. From a comparison of Figures 90 and 91, the excitation wavelength defined as $\lambda_1$ should
Figure 90. Effect of the wavelength on the absorbance of naphthalene in MeOH/CO₂ mixtures, 136 atm, 25°C.

Mole percent of methanol: (□) 10, (+) 20, (▲) 30, (▼) 40.

The error bars not shown on the graph are within the size of the dots.
Figure 91  Effect of the excitation wavelength on the efficiency of the energy donor absorption, $P$, in MeOH/CO$_2$ mixtures, 136 atm, 25$^\circ$ C.

Mole percent of methanol: (●) 10, (◇) 20, (+) 30, (□) 40.
be set at 275 nm. This wavelength provides the highest value of $P$ as well as the highest absorbance for the energy donor.

**Calculation of the overlap integral.**

The fluorescence spectrum of the naphthalene in the MeOH/CO$_2$ mixtures was monitored at different wavelengths. These wavelengths, namely, 270 nm, 272 nm and 275 nm were chosen because they yield the highest absorbance of the naphthalene. Figure 92 through Figure 95 are the normalized fluorescence spectrum of naphthalene in the MeOH/CO$_2$ mixtures. Except for the excitation of 272 nm, the fluorescence spectrum does not depend on the excitation wavelength. The overlap integral, $J$, was calculated according to the equations (9) and (10) of Chapter II and according to the procedure described in the same chapter. Figure 96 is the magnitude of the overlap integral, $J$, versus the composition of the MeOH/CO$_2$ mixtures.
Figure 92. Normalized fluorescence spectrum of naphthalene in 10/90 mole % MeOH/CO₂ mixture. 136 atm, 25 °C.

Excitation wavelength (nm): (○) 270, (□) 272, (◇) 275.
Figure 93. Normalized fluorescence spectrum of naphthalene in 20/80 mole % MeOH/CO₂ mixture. 136 atm, 25 °C.

Excitation wavelength (nm): (○) 270, (□) 272, (◇) 275.
Figure 94. Normalized fluorescence spectrum of naphthalene in 30/70 mole % MeOH/CO₂ mixture. 136 atm, 25 °C.

Excitation wavelength (nm): (○) 270, (□) 272, (✧) 275.
Figure 95. Normalized fluorescence spectrum of naphthalene in 40/60 mole % MeOH/CO₂ mixture. 136 atm, 25 °C.

Excitation wavelength (nm): (○) 270, (□) 272, (◇) 275.
Figure 96. Overlap integral, $J$, in MeOH/CO$_2$ mixtures, 136 atm, 25$^\circ$ C.
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