EXTRACTION OF POLAR POLLUTANTS USING
SUPERCRITICAL FLUIDS AND ENHANCED-FLUIDITY LIQUIDS

DISSERTATION

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By

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ABSTRACT

Supercritical fluid extraction (SFE) is developing as a viable method for the removal of pollutants from environmental matrices. However, CO₂, the most commonly used extraction fluid, is limited in its ability to solvate high molecular weight and/or polar analytes. The addition of 1-10% organic modifier often helps considerably, although less than quantitative results are frequently reported. Enhanced-fluidity liquid extraction (EFLE) is investigated herein as a means of extending the range of SFE for polar pollutants. An enhanced-fluidity liquid is a mixture consisting of a large proportion (10-50%) of a common organic solvent and a high fluidity, low viscosity liquid such as CO₂. The mixture is used in the single phase liquid region of the respective cosolvent/CO₂ phase diagram.

Phase transitions were determined for extraction fluids of interest using a high pressure, variable volume view cell. The single phase liquid or supercritical fluid regions were mapped across the 0-1 mole fraction CO₂ range for methanol/CO₂, acetonitrile/CO₂, methanol/H₂O/CO₂, and acetonitrile/H₂O/CO₂.

Phenolic and nitroaromatic pollutants were first extracted from an octadecyl polysiloxane sorbent to compare the mass transport properties of enhanced-fluidity
liquids relative to supercritical fluids and conventional liquids. The enhanced-fluidity liquids behaved similarly to the supercritical fluids tested.

House dust was then evaluated as an extraction matrix. Pollutants that are "tracked in" to homes and other buildings may adsorb to house dust, a matrix with a high H₂O and organic content, and persist for extended periods of time when natural degradation pathways such as sunlight, weather, and microorganisms are reduced or eliminated. The phenolics and nitroaromatics were most efficiently extracted with enhanced-fluidity liquid methanol/H₂O/CO₂ mixtures. Phenoxyacid herbicides were also extracted from house dust using CO₂ and methanol/CO₂ mixtures. The use of 20/80 mole % methanol/CO₂ at supercritical conditions yielded the highest overall recoveries.

Finally, a literature review of other alternative extraction techniques developed over the past five years is presented. Like EFLE, these techniques attempt to bridge the gap between the conventional liquid-solid and liquid-liquid techniques and SFE.
To Mom, Dad, and Boo

with love
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A very special thank you to Gene Ohler for providing a stable and constant source in my life during the often trying days of graduate school. From advice on organic chemistry to the many walks through the town, I truly appreciate his friendship and concern. Ohio would have been a much worse place without him in it.

Last, but by no means least, I thank the Lord for the strength, courage, and perseverance needed to make it through the past five years that brought this dissertation from a dream to a reality one day at a time.
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FIELDS OF STUDY

Major Field: Chemistry

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CHAPTER 1
INTRODUCTION

Steps in Extraction Processes

The process of identification and quantitation of environmental pollutants present in solid samples typically begins with an extraction step to isolate the pollutants from the bulk matrix material and render the pollutants in an appropriate form for analysis. Three factors affecting the extraction of pollutants, or analytes, from solid matrices are shown in Figure 1.1. First, the mass transport properties of the extraction fluid affect the extraction rate. Fast mass transport via rapid diffusion and low fluid viscosity is desirable for short extractions. Diffusion of the extraction fluid is dependent on the state of the fluid such as gas or liquid but also on matrix properties such as tortuosity and pore size distribution. For a given fluid, the diffusion of an analyte is inversely proportional to the square root of its molecular weight. Second, analyte solubility in the bulk extraction fluid is necessary for preferential analyte partitioning from the matrix to the fluid. The effects of extraction fluid density and analyte vapor pressure on solubility will be discussed in more detail below. Third, chemical interactions between the matrix and analytes must be efficiently interrupted.
Figure 1.1. Factors affecting extraction processes.
by the extraction fluid. Possible interactions between the organic and inorganic matrix components and the analytes include van der Waals attraction, hydrophobic bonding, hydrogen bonding, bonding by charge transfer, ligand exchange and ion bonding, ion-dipole interactions, dipole-dipole interactions, and chemisorption.\textsuperscript{3} In addition, an adsorbed H\textsubscript{2}O layer on the surface of the matrix must also be considered because the analytes may be trapped in the pore structure beneath the H\textsubscript{2}O or may be soluble in and even residing in the H\textsubscript{2}O layer. Thus, partitioning of analytes between the extraction fluid and the H\textsubscript{2}O layer influences extraction as well.\textsuperscript{4} While analyte solubility is necessary, it is often not sufficient for extraction when matrix-analyte interactions are strong. As depicted in Figure 1.1, the three factors are interrelated and must be considered together in order to optimize extraction rates and analyte recoveries. The judicious choice of an extraction fluid is therefore extremely important.

**Liquid-Solid Extraction Techniques**

Soxhlet extraction, sonication, and wrist shaking techniques routinely use organic liquids as extraction fluids. Standard procedures describing solvent choice and length of extraction are available.\textsuperscript{5} Minimal operator supervision is required once the extraction is in progress. The choice of an extraction fluid with a polarity similar to that of the analytes ensures analyte solubility and promotes the interruption of matrix-analyte interactions.
However, extended periods of time on the order of 2-24 h are often required because the mass transport properties of conventional liquids are poor compared to other fluid states. Some of these properties are shown in Table 1.1. Liquids are roughly 100 times more viscous and have self-diffusion coefficients over four orders of magnitude slower than gases.

The extraction often also serves as a concentration step if the analytes are present in the matrix at trace levels. In a typical liquid-solid technique, a 1-10 g sample is extracted with 20-500 ml of liquid solvent. The resulting solution is concentrated to a few milliliters for analysis. Since the extraction is usually exhaustive, additional clean-up steps to remove coextracted matrix material may be required. The extraction, concentration, and clean-up steps generate an often substantial volume of organic solvent waste. Environmental pollution from extraction fluids such as methylene chloride, benzene, and chloroform is of great concern. The rather severe disadvantages of long extraction times and the consumption of large quantities of solvents resulted in the search for alternative techniques.

**Supercritical Fluid Extraction (SFE)**

A pressure-temperature phase diagram illustrating the supercritical fluid region is shown in Figure 1.2. At the critical point, the densities of the gas and liquid states merge, forming the supercritical fluid. Unlike the phase transitions marked by solid lines in Figure 1.2, transitions from the liquid and gas states to the supercritical regime
Table 1.1. Properties of gases, supercritical fluids, and liquids from references 1 and 6.

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<th>Viscosity (cP)</th>
<th>Self-Diffusion Coefficient (cm³/s)</th>
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<tr>
<td>Gas</td>
<td>$10^{-3}$</td>
<td>$10^{-2}$</td>
<td>0.1</td>
</tr>
<tr>
<td>Supercritical Fluid</td>
<td>0.2-0.9</td>
<td>$10^{-2}$-10^{-1}</td>
<td>$10^{-3}$-10^{-4}</td>
</tr>
<tr>
<td>Liquid</td>
<td>1</td>
<td>1</td>
<td>$&lt;10^{-5}$</td>
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Figure 1.2. Pressure-temperature phase diagram.
at temperatures and pressures above the critical point are not distinct. Therefore, liquids at temperatures near $T_c$ and gases at pressures near $P_c$ are expected to behave in a manner similar to supercritical fluids.

Properties of supercritical fluids, shown in Table 1.1, fall between those of liquids and gases as well. The density of a supercritical fluid is closer to that of a liquid than a gas; tunable with pressure and temperature, the density gives the supercritical fluid its solvating power. Although the viscosities and self-diffusion coefficients of gases are optimal, gases are not suitable as extraction fluids because they lack sufficient density for solvating power. Supercritical fluids have viscosities that are similar to gases and self-diffusion coefficients that are at least an order of magnitude higher than liquids.

The use of supercritical fluid extraction (SFE) for the recovery of pollutants from environmental matrices was the subject of several reviews over the past five years. Extraction times are typically shorter (< 2 h) than with liquid extraction techniques as a result of the favorable mass transport properties. Organic solvent usage is greatly reduced and SFE instrumentation is commercially available from a number of vendors.

Carbon dioxide is the most commonly used supercritical fluid due to its inert and nontoxic nature and its easily accessible critical temperature and pressure of 31.06 °C and 72.86 atm. The polarity of CO$_2$ is comparable to liquid pentane or hexane. CO$_2$ lacks a permanent dipole moment but has a relatively large
quadrupole moment, allowing some interaction with polar components. However, CO₂ is limited in its ability to solvate high molecular weight and/or polar compounds. For example, the CO₂ solubility of docosane, a nonpolar alkane, is ~30 times higher than the polar analyte, dibenzothiophene, and ~100 times greater than pyrene, a high molecular weight polycyclic aromatic hydrocarbon (PAH), at 400 atm and 50 °C. Analyte solubility increases with increasing fluid density and solvating power or conversely with increasing temperature due to enhanced analyte volatility. The solubility of anthracene increased ~48 times when the temperature was elevated from 50 °C to 200 °C at 395 atm, although the CO₂ density decreased by ~50% at the higher temperature.

The solubility of H₂O in CO₂ is only ~0.4 mole % at 25 °C and increases to ~2.9 mole % at 100 °C with little dependence on pressure over the range of 20-500 atm. Penetration of a thin H₂O layer on the matrix is difficult with CO₂, potentially trapping the analytes in the pore structure below. Thus, although the extraction fluid mass transport properties are favorable, analyte solubility and the ability of CO₂ to interrupt matrix-analyte interactions are often problematic.

The addition of small proportions (1-10 mole %) of polar modifiers such as methanol, either directly to the sample or via incorporation with CO₂, enhances the solubility of the analytes and H₂O in the extraction fluid and aids in the interruption of matrix-analyte interactions by interacting with the analytes and competing for the active sites on the matrix. When modifier is added directly to the
sample, a static (non-flowing) extraction step is generally employed first to maximize interactions between the modifier and sample before the modifier is purged from the vessel during the dynamic (flowing) extraction step. When modifier is incorporated with CO₂, a constant modifier composition is supplied throughout the entire dynamic extraction. However, the critical point of the modifier/CO₂ mixture is elevated relative to CO₂ alone and phase diagram information for the modifier/CO₂ mixture is required.

The positive effects of modifiers are clearly depicted in numerous applications. The solubility of 2-aminobenzoic acid was enhanced by ~620% with the addition of 3.5% methanol in CO₂. Diuron, a phenylurea herbicide, was not recovered at all with CO₂ but quantitatively upon the addition of methanol as a static modifier. Recoveries of phenylureas from sediment were also increased from < 20% with CO₂ to ~100% with 20/80 mole % acetone/CO₂. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was recovered at ~50% with CO₂ and at > 90% with 2% methanol in CO₂.

Furthermore, modified SFE results are generally comparable to or better than the competing liquid-solid techniques. Recoveries of organophosphate pesticides (OPPs) and organochlorine pesticides (OCPs) from topsoil averaged 92-95% with SFE, sonication, and Soxhlet extraction, and TCDD was detected at only ~65% by Soxhlet but at > 90% by SFE.

Many samples are still not efficiently extracted with modified SFE however. For example, diazinon (an OPP) was not recovered from spiked sand using either CO₂ or 10% methanol in CO₂. Although the recoveries of 16 PAHs from marine
sediment were greatly improved with 10% methanol in CO₂ relative to CO₂ alone, recoveries of 12 of the analytes were still ≤ 75%. The extraction of six OCPs and six OPPs from topsoil ~24 h after spiking was easily accomplished with an average yield of 92% using 3% methanol in CO₂. However, when the sample was aged for eight months, recoveries dropped to 64% using the same extraction conditions. Alternative approaches are needed for the samples and analytes for which SFE fails to produce quantitative results.

**Enhanced-Fluidity Liquid Extraction (EFLE)**

Enhanced-fluidity liquid extraction (EFLE) is a possible means of augmenting SFE for difficult samples. An enhanced-fluidity liquid is prepared by combining a large proportion of a common organic solvent such as methanol with a high fluidity, low viscosity liquid such as CO₂. Enhanced-fluidity liquids are known to have diffusional properties that are similar to supercritical fluids. These liquids are expected to also provide high analyte solubility and the capacity to interrupt matrix-analyte interactions, comparable to liquid-solid extraction techniques. Thus, each apex of the extraction triangle is considered and possibly optimized.

Properties of enhanced-fluidity liquid methanol/CO₂ mixtures were previously studied. Average viscosities and diffusion coefficients for methanol/CO₂ mixtures at 170 atm and 25 °C are shown in Figure 1.3. The viscosity is lowest with CO₂ (0.09 cP) and less than half that of pure methanol (0.57 cP) until ~60/40 mole %
Figure 1.3. Experimentally determined (●) average viscosities of methanol/CO$_2$ mixtures and (+) diffusion coefficients of benzene in methanol/CO$_2$ mixtures at 170 atm and 25 °C from reference 32.
methanol/CO₂. Similarly, the diffusion coefficient of benzene in CO₂ is more than five times that in pure methanol and remains at twice that in methanol until > 40 mole % methanol is added to CO₂. Therefore, mass transport properties are expected to be favorable with mixtures containing up to ~40 mole % methanol in CO₂.

The densities of liquid methanol/CO₂ mixtures were measured at 26 °C and pressures of 85-215 atm. Variations in density were observed at pressures < 170 atm. The 10/90 mole % methanol/CO₂ mixture was of lower density than the 20-40 mole % methanol mixtures (which were of comparable magnitude). The densities increased with increasing pressure. At pressures ≥ 170 atm, the densities were constant and nearly identical (~0.9 g/ml) for mixtures containing 10-40 mole % methanol in CO₂. The mixture densities are greater than those of the pure components, implying an increased solvent strength.

Finally, the Kamlet-Taft solvatochromic parameters, describing solvent strength at the molecular level, have been measured for methanol/CO₂ and are shown in Figure 1.4. The π* parameter (Figure 1.4A), measuring dipolarity/polarizability interactions of the solvent, increases almost linearly from that of pure CO₂ to that of pure methanol. By ~40/60 mole % methanol/CO₂, π* is roughly half that of methanol. The α parameter (Figure 1.4B), defining the hydrogen bond donating capability or acidity of the solvent, is ~80% that of pure methanol at only 20/80 mole % methanol/CO₂ and is > 90% that of methanol by 40/60 mole % methanol/CO₂.

Describing the hydrogen bond accepting properties or basicity of the solvent, β is
Figure 1.4. Experimentally determined Kamlet-Taft solvatochromic parameters (A) $\pi^*$ and (B) $\alpha$ (△) and $\beta$ (●) for methanol/CO$_2$ mixtures at 170 atm and 25 °C from reference 32.
Figure 1.4 (continued).
−70% that of pure methanol at 20/80 mole % methanol/CO₂ and is > 90% by −50/50 mole % methanol/CO₂ (Figure 1.4B). Therefore, when a polar extraction fluid is needed, mixtures containing increasing proportions of methanol in CO₂ are expected to be beneficial with properties intermediate between those of supercritical fluids and conventional liquids.

EFLE was previously demonstrated as a viable method for the extraction of PAHs from sea sand³⁵-³⁶ and house dust.³⁷ The optimum extraction fluids were found to be 40/60 mole % methanol/CO₂ for sea sand and 30/70 mole % methanol/CO₂ for house dust. Extraction yields of some of the native PAHs from house dust were ≥ 200% relative to the Soxhlet extraction results.³⁷

**Goals of this Research**

Phase diagram studies for potential extraction fluids are described in Chapter 2. Liquid-liquid, liquid-vapor, and supercritical fluid-vapor phase transitions are located for methanol/CO₂, acetonitrile/CO₂, methanol/H₂O/CO₂, and acetonitrile/H₂O/CO₂ mixtures at temperatures of 25-100 °C and pressures ≤ 306 atm. The single phase liquid and supercritical fluid regions appropriate for SFE and EFLE are clearly defined. This work is directly applicable to supercritical fluid chromatography (SFC) and enhanced-fluidity high performance liquid chromatography (HPLC) as well.

In Chapters 3-5, the controlling factors in the extraction process, shown in Figure 1.1, are considered. A comparison of extraction fluid mass transport properties
for supercritical and enhanced-fluidity liquid methanol/CO₂ mixtures and conventional liquids is reported in Chapter 3. Phenolic and nitroaromatic pollutants are extracted from an octadecyl polysiloxane sorbent material. The analytes are quite soluble in CO₂ and the sorbent acts as a “model” surface, providing uniform, well defined matrix interaction sites.

In Chapter 4, the extraction of the phenolics and nitroaromatics from house dust is considered. The house dust is an adsorptive environmental matrix with a high H₂O and organic content. Matrix-analyte interactions and extraction fluid mass transport properties are the relevant parameters investigated. Both methanol/CO₂ and methanol/H₂O/CO₂ enhanced-fluidity liquid mixtures are tested and important properties of the cosolvents are considered.

The extraction of phenoxyacid herbicides from house dust is studied in Chapter 5. The polar and acidic phenoxyacids have limited solubilities in CO₂ such that all three factors are important. Binary methanol/CO₂ mixtures are compared to static modifiers. Results for Soxhlet extractions, the common competing liquid-solid extraction technique, are included in each extraction chapter as well.

With the limitations of SFE and modified SFE being rapidly realized, EFLE is only one of a myriad of alternative approaches tested over the past five years. A literature review of these alternatives is presented in Chapter 6. The approaches taken are categorized based on whether changes were made to the extraction fluid, the
analytes, the matrix, or the experiment/instrumentation. Highlights and pitfalls of each technique are discussed.

Finally in Chapter 7, results reported herein are summarized, the current status of SFE and EFLE is assessed, and areas of interest for further study are considered.
REFERENCES


37. Monserrat, M. *M.S. Thesis*, The Ohio State University, Columbus, OH, 1996.
CHAPTER 2
PHASE DIAGRAM DETERMINATION FOR
METHANOL/CO₂, ACETONITRILE/CO₂, METHANOL/H₂O/CO₂,
AND ACETONITRILE/H₂O/CO₂ MIXTURES

INTRODUCTION

Available phase diagram information describing liquid-liquid, liquid-vapor, and supercritical fluid-vapor phase transitions is important in the choice of an extraction fluid for SFE and EFLE. In the subsequent chapters, all extractions using pure CO₂ and binary or ternary cosolvent(s)/CO₂ mixtures were performed at pressure and temperature conditions well within a single liquid or supercritical regime of the respective phase diagram. By so doing, homogeneous, reproducible extraction fluids were tested. Because the syringe pump containing the extraction fluid was operated at room temperature and the temperature of the extractor was varied from 25-150 °C, phase diagram information across a similar temperature range was needed. This information is relevant to supercritical fluid chromatography (SFC) and enhanced-fluidity high performance liquid chromatography (HPLC) as well.
Previous Phase Diagram Studies with Methanol, Acetonitrile, H$_2$O, and CO$_2$

The liquid-vapor phase transition line for carbon dioxide is well established in the literature. The line terminates at a critical pressure, $P_c$, of 72.86 atm and critical temperature, $T_c$, of 31.06 °C, with the supercritical region defined at pressures and temperatures exceeding $P_c$ and $T_c$.\(^1\)

Methanol and acetonitrile are commonly used as polar cosolvents in CO$_2$ for SFE and SFC due to the large dielectric constants (at 20 °C) of 32.7 and 37.5, respectively.\(^2\) Methanol/CO$_2$ phase behavior was previously reported by a number of researchers.\(^3,4,5,6,7,8,9,10,11,12,13,14,15\) However, acetonitrile/CO$_2$ phase diagram information is limited. Byun et al.\(^16\) showed acetonitrile/CO$_2$ isotherms at 35, 55, and 75 °C from 0-1 mole fraction CO$_2$, Page\(^17\) reported interpolated data for the 35 °C acetonitrile/CO$_2$ isotherm from 0.74-0.99 mole fraction CO$_2$, and Ziegler et al.\(^15\) estimated 21 points along the mixture critical curve using a solvent peak-shape method performed with chromatographic equipment.

H$_2$O is a more polar solvent than methanol or acetonitrile with a dielectric constant of 80.1 at 20 °C.\(^2\) Phase diagram information for H$_2$O/CO$_2$ is available.\(^18,19,20,21,22,23,24,25\) The solubility of H$_2$O in CO$_2$ is ≤ 1.3 mole % at 25-75 °C and 40-500 atm. At 100 °C, ~2.5-3 mole % H$_2$O is soluble and at 150 °C, up to ~6 mole % H$_2$O is soluble across the same pressure range.\(^26\) Therefore, the preparation of H$_2$O/CO$_2$ mixtures in a syringe pump operating at 25 °C is not very feasible, although the use of H$_2$O/CO$_2$ mixtures at elevated temperatures may be advantageous.
H$_2$O is often added to methanol or acetonitrile to increase the polarity of the mobile phase in HPLC. Ternary mixtures of methanol/H$_2$O/CO$_2$ and acetonitrile/H$_2$O/CO$_2$ are also anticipated to extend the mobile phase or extraction fluid polarity in enhanced-fluidity HPLC and liquid extraction. Limited phase diagram information exists for methanol/H$_2$O/CO$_2$. Francis$^{27}$ graphically reported the methanol/H$_2$O/CO$_2$ system at -65 atm and -25 °C. Chang and Rousseau$^6$ showed liquid-vapor phase transitions for methanol/H$_2$O/CO$_2$ mixtures at -30, -15, 0, and 25 °C for a constant H$_2$O to methanol ratio of 0.2 and 0-0.65 mole fraction CO$_2$ added. Page$^{17}$ studied six methanol/H$_2$O/CO$_2$ compositions over the temperature range of 25-100 °C for H$_2$O to methanol mole ratios of 0.08-0.30 with 0.54-0.92 mole fraction CO$_2$ added. Yoon et al.$^{28}$ recently reported phase behavior for methanol/H$_2$O/CO$_2$ at 69.1, 98.7, and 118.5 atm and 40 °C. Eight data points were also included for phase transitions at 32, 35, and 38 °C in the pressure range of 71.1-80.0 atm. With the exception of the six isopleths reported by Page,$^{17}$ little experimental methanol/H$_2$O/CO$_2$ phase diagram information is available at temperatures other than 25 °C and 40 °C. Furthermore, acetonitrile/H$_2$O/CO$_2$ phase diagram information was not found in the literature at this time.

**Goals of this Study**

Methods for determining phase behavior are broadly classified as analytical or synthetic.$^{29}$ In analytical methods, coexisting phases are sampled and their
compositions are determined, while in synthetic methods, a known composition is prepared and phase transitions are observed for fixed temperatures or pressures. The phase diagrams in this study were determined using a synthetic method via visual observation in a high pressure, variable volume view cell. View cells similar to the one used herein were previously utilized by Page et al.,12 Roškar et al.,13 Byun et al.,16 Page,17 Occhiogrosso et al.,30 and Warzinski et al.31 No sampling of the coexisting phases was necessary, and each visual observation yielded one point on the pressure-temperature-mole fraction (P-T-x) envelope.

Methanol/CO₂ and acetonitrile/CO₂ phase transitions were determined at temperatures of 25-100 °C over the 0-1 mole fraction CO₂ range. The methanol/CO₂ experiment was used mainly to calibrate the accuracy and precision of the variable volume view cell relative to the literature available. A comprehensive acetonitrile/CO₂ phase diagram is reported for the first time. Ternary phase behavior for methanol/H₂O/CO₂ and acetonitrile/H₂O/CO₂ mixtures was then studied. Six H₂O to methanol mole ratios of 0.1197-0.5633 and two H₂O to acetonitrile mole ratios of 0.3206 and 0.7316 were examined at temperatures from 25-100 °C with varying mole fractions of CO₂ added. In all experiments, the single phase liquid or supercritical regions appropriate for use in extraction and chromatography were established.
EXPERIMENTAL

A schematic diagram of the instrumentation used for determining liquid-liquid, liquid-vapor, and supercritical fluid-vapor phase transitions is shown in Figure 2.1. Briefly, the view cell was equipped with a temperature controller and a pressure transducer to directly monitor the cell contents. A syringe pump was used to move the pneumatic assembly, varying the view cell volume. Each component of the experimental setup will now be addressed in more detail.

View Cell

A high pressure, variable volume view cell was purchased from Temco, Inc. (Tulsa, OK). A detailed diagram of the view cell is given in Figure 2.2. The 0-35 ml cell is rated to 585 atm and a maximum temperature of 176.7 °C. The stainless steel cylindrical sample chamber (3.89 cm i.d. x 2.95 cm) is enclosed on one end by a threaded brass cap containing a stainless steel volume displacer and an attached pneumatic assembly that drives the volume displacer, allowing the cell volume to be varied. The pneumatic portion and sample volume are isolated from one another by two sets of high pressure seals on either side of a region at atmospheric pressure. A borosilicate glass window (2.50 cm diameter x 2.15 cm thick) was originally mounted within the pneumatic assembly. This window was replaced by a mixer assembly described in detail in the Sample Mixing section below. The other end of the sample chamber consists of a larger window (3.75 cm diameter x 1.88 cm thick) for visual
Figure 2.1. Schematic diagram of the instrumentation used to observe liquid-liquid, liquid-vapor, and supercritical fluid-vapor phase transitions: (A) view cell sample chamber, (B) view cell pneumatic region, (C) thermocouple, (D) temperature controller, (E) Powerstat power supply, (F) connection to band heaters, (G) three-way valve for sample input, (H) pressure transducer, (I) two-way valve, and (J) syringe pump for varying the view cell volume.
Figure 2.2. Diagram of the high pressure, variable volume view cell used in this study: (A) borosilicate glass viewing window, (B) sample chamber, (C) volume displacer of the pneumatic assembly, (D) stainless steel mixer assembly, (E) Teflon o-rings, (F) Teflon spacers, (G) thermocouple port, (H) port to the pressure transducer and sample input, (I) port to the syringe pump used to move the volume displacer, (J) extra port, (K) region at atmospheric pressure, isolating the sample chamber and the pneumatic assembly, (L) Buna-N o-rings, (M) Teflon coated octagonal spinbar®, and (N) external ring magnets.
observation of the cell contents. The glass window is held in place by another threaded brass cap.

Buna-N, ethylene propylene, viton, and Teflon encapsulated silicone o-rings were tested and found unacceptable as seals in contact with the cell contents. The fluids and conditions used caused extraction of the Buna-N, ethylene propylene, and viton o-rings forming a yellow solution and contaminating the cell contents. The Teflon portion of the Teflon encapsulated silicone o-rings was thin and cracked under pressure, causing these seals to fail at elevated pressures and allowing the interior silicone portion to leach into the cell contents forming an orange solution. In addition, graphite-filled Teflon spacers that were supplied with the cell were positioned on either side of the o-rings to cover the entire thickness of the windows. These spacers extracted and formed a yellow-green solution when immersed in methanol. Teflon o-rings, sizes 120 and 325, obtained from McMaster-Carr Supply Co. (Chicago, IL), were found acceptable as high pressure seals. The Teflon o-rings were positioned around each window and around the volume displacer. Teflon spacers, made in-house, were installed on either side of the o-rings. All materials in contact with the cell contents were either stainless steel, borosilicate glass, or Teflon.

The pneumatic assembly was isolated from the cell contents, and sealed with Buna-N o-rings of sizes 216 and 223 (McMaster-Carr Supply Co., Chicago, IL). The volume displacer was moved by applying or releasing pressure via an ISCO model 260D syringe pump (Lincoln, NE) filled with H₂O. The syringe pump was operated
in the constant flow and refill modes in order to pressurize and depressurize the view cell contents.

**Temperature Measurement and Control**

The view cell is equipped with 1/8" NPT pipe fittings near the viewing window of the sample chamber. An Omegaclad type J thermocouple, enclosed in a 1/16" stainless steel sheath, was purchased from Omega Engineering, Inc. (Stamford, CT) and installed through one port via a Parker A-Lok thermocouple connector (Forberg Scientific of Ohio, Inc., Columbus, OH). The thermocouple extended into the center of the sample portion of the view cell and monitored the temperature of the cell contents directly. A CN9000 series temperature controller (Omega Engineering, Inc., Stamford, CT) was used to monitor and control the cell temperature. The temperature controller reads and controls to ±0.1 °C with a maximum of 200 °C. The exterior of the stainless steel cell was surrounded by three mica band heaters (2-11.25 cm i.d. x 3.75 cm wide, 240 V and 400 W each, and 1-11.25 cm i.d. x 5.00 cm wide, 240 V and 500 W), obtained from Watlow (St. Louis, MO), that were wired in parallel to a Powerstat power supply (Hughes-Peters, Columbus, OH). The Powerstat dial was generally set to 20 to provide a gentle rate of heating and to maintain the set point temperature when reached. The entire view cell was wrapped in an insulating jacket prepared from Flexweave 1000 tape (0.3 cm thick x 10.0 cm wide), obtained from The
Carborundum Co. (Niagara Falls, NY), to minimize heat loss and temperature fluctuations.

Prior to installation, the thermocouple was calibrated in a Techne TU-16D immersion circulator H₂O bath (Techne, Inc., Princeton, NJ) relative to the Techne Tempunit® thermoregulator and a Fisher thermometer (-20-110 °C, Pittsburgh, PA) from 30 to 70 °C. Thermometer temperatures were estimated to 0.1 °C and used for the calibration curve. The correlation coefficient, r², of the resulting curve was 0.99999.

**Pressure Measurement**

To the other sample chamber port, a three-way valve (Scientific Systems, Inc., State College, PA) was attached with 1/16" stainless steel tubing. A Setra model 204 pressure transducer (Setra Systems, Inc., Acton, MA) and the cell contents were connected via the two open sides of the valve. The transducer was factory calibrated relative to NIST standards before use. The output of the pressure transducer is 0.000 to 5.000 V for 0 to 5000 psi (340.2 atm) with an accuracy of ± 0.11% full scale (5.5 psi, 0.4 atm) at constant temperature. The output was read with a Fluke 77 multimeter (John Fluke Mfg Co., Inc., Everett, WA) operated on the volt DC setting and readable to ± 1 psi (0.1 atm) from 0 to 3200 psi (217.8 atm) and to ± 10 psi (0.7 atm) above 3200 psi.
Sample Mixing

The thickness of the stainless steel cell (3.0 cm) and the movement of the volume displacer made the use of a magnetic stir bar inside for mixing the cell contents impossible. The cell was modified in-house to replace the small rear window with a mixer assembly (see Figure 2.2). A long, cylindrical stainless steel chamber, open to the cell contents, was sealed with the Teflon o-rings and spacers previously used with the small window. The chamber protruded beyond the cell dimensions by ~9 cm. A circular stainless steel paddle was mounted on a stainless steel shaft (21.5 cm long x 1 cm wide x 0.3 cm thick). A 5/16" x 1 1/2" Teflon coated octagonal spinbar® (VWR Scientific, West Chester, PA) was fitted into a hole cut in the stainless steel near the end of the shaft. The shaft was inserted into the mixer chamber until the paddle touched the volume displacer so that the end of the shaft fitted with the spinbar® was near the closed end of the mixer chamber. The spinbar® was coupled with six ceramic ring magnets (2.70 cm o.d. x 1.49 cm i.d. x 0.63 cm thick, McMaster-Carr Supply Co., Chicago, IL) on the exterior of the mixer chamber. The magnets were moved by hand to push and pull the shaft containing the spinbar® inside the cell, moving the circular paddle back and forth through the entire sample chamber. The stainless steel paddle (3.5 cm diameter x 0.15 cm thick) contains 24 small holes (0.5 cm diameter) so that the fluid was effectively circulated throughout the cylindrical cell. The mixer assembly adds 12 ml to the overall cell volume and requires no
permanent cell modification. Visual observation of the cell contents was still possible by illuminating the large window at the other end of the view cell.

**Mixture Preparation**

HPLC grade methanol and acetonitrile were obtained from J.T. Baker (Phillipsburg, NJ) and were specified at 100.0% purity with a H$_2$O content of < 0.01% and 0.002% for methanol and acetonitrile, respectively. H$_2$O was OSU distilled and deionized using a NANOpure II system (SYBRON/Barnstead, Boston, MA).

SFE/SFC grade CO$_2$ without a helium pad was obtained from Air Products and Chemicals (Allentown, PA) and was specified at > 99.9999% purity. Impurities listed were H$_2$O (< 250 ppb), total hydrocarbons, C$_1$-C$_{20}$ (< 50 ppb), and total halocarbons (< 1.0 ppb).

H$_2$O/methanol and H$_2$O/acetonitrile stock solutions were prepared in 500 ml amber glass bottles. The solutions were prepared at approximate volume ratios, with the components carefully weighed before combining in the glass bottles. The number of moles of methanol or acetonitrile and H$_2$O was then calculated. The approximate H$_2$O/methanol volume ratios studied were 5/95, 7.5/92.5, 10/90, 13/87, 16/84, and 20/80. The H$_2$O/methanol mole ratios, R, for the above volume ratios were $0.1197 \pm 0.0008$, $0.1810 \pm 0.0009$, $0.2490 \pm 0.0005$, $0.3318 \pm 0.0007$, $0.4298 \pm 0.0006$, and $0.5633 \pm 0.0006$, respectively. The approximate H$_2$O/acetonitrile volume ratios studied were 10/90 and 20/80. The H$_2$O/acetonitrile
mole ratios, $R$, for the above volume ratios were $0.3206 \pm 0.0006$ and $0.7316 \pm 0.0008$, respectively. The $H_2O$/methanol and $H_2O$/acetonitrile stock solutions were sealed and stored at $4^\circ C$ when not in use.

Prior to preparing a mixture in the view cell, the sample chamber was charged with CO$_2$ to ~21 atm through the three-way valve and was checked for leaks. The contents were then emptied so that only CO$_2$ remained inside the cell at atmospheric pressure.

All mixtures were prepared on a mole fraction basis calculated from the masses of materials added to the view cell. An aliquot of methanol, acetonitrile, $H_2O$/methanol, or $H_2O$/acetonitrile was degassed by sonication. A weighed quantity of the pure solvent or mixture was then added through the three-way valve from a 10 ml or 30 ml glass syringe equipped with the appropriate valve fitting and ferrule. The syringe was weighed to ± 0.0001 g before and after transfer.

The number of moles of methanol or acetonitrile was calculated directly from the mass of solvent added. The number of moles of $H_2O$/methanol or $H_2O$/acetonitrile was calculated by solving equation 1 for $m$, the total moles of mixture introduced into the view cell:

$$\frac{M}{G} = \frac{m}{g}$$  

where $M$ is the total moles of $H_2O$/methanol or $H_2O$/acetonitrile mixture in the stock solution, $G$ is the total mass of the stock solution, and $g$ is the mass of the mixture.
added to the view cell. The number of moles of H₂O was then calculated:

\[ m_{H_2O} = m \left(\frac{R}{1 + R}\right) \]  \hspace{1cm} (2)

where R is the mole ratio of H₂O/methanol or H₂O/acetonitrile. The number of moles of methanol or acetonitrile was determined by subtracting the moles of H₂O from the moles of mixture, m, added to the view cell.

CO₂ was maintained in an ISCO model 260D syringe pump operated in constant pressure mode at 123 atm. A 40 ml stainless steel vessel (Part # 304L-HDF4-50, Whitey Co., Highland Heights, OH), equipped with a two-way valve and rated to an operating pressure of 123 atm, was used as a transfer vessel. A known volume of CO₂ was added to the empty transfer vessel. After weighing to ± 0.05 g, the vessel was connected to the three-way valve of the view cell. The valves were opened, allowing the cell to fill with CO₂ until equal pressures were reached. Because the cell and transfer vessel were of approximately equal volumes, no more than half of the vessel contents could be transferred at ambient temperatures when methanol/CO₂, methanol/H₂O/CO₂, and acetonitrile/H₂O/CO₂ mixtures were prepared. Cooling the cell briefly with dry ice prior to CO₂ transfer was unsuccessful because the Teflon o-rings shrunk and the cell leaked. Successful transfers of more than half of the vessel contents were facilitated by gently heating the stainless steel transfer vessel with a heat gun. This was only done with the transfer vessel valve and three-way valve of the view cell open so that the maximum operating pressure of the transfer vessel was not
exceeded. More than half of the 40 ml vessel contents could be transferred at room temperature when acetonitrile/CO₂ mixtures were prepared. This implies that the change in volume upon mixing is more negative for acetonitrile/CO₂ relative to methanol/CO₂. The transfer vessel was then reweighed in order to calculate the number of moles of CO₂ delivered into the mixture. The ideal gas law (PV=nRT) was used to approximate the amount of residual CO₂ in the cell prior to loading the cell with the mixture materials. This correction factor was added to the total moles of CO₂ delivered from the stainless steel transfer vessel.

**Experimental Procedure**

After transferring the methanol, acetonitrile, H₂O/methanol, or H₂O/acetonitrile and CO₂, the view cell was pressurized by operating the syringe pump in the constant flow mode, delivering H₂O to the pneumatic assembly and forcing the volume displacer inward, decreasing the volume of the sample chamber. This was continued, with mixing, until the two phase liquid-vapor or liquid-liquid mixture or three phase liquid-liquid-vapor mixture originally in the cell became a single liquid phase. The temperature controller was then set to the desired temperature (initially 25 °C). Upon temperature stabilization, the cell contents were depressurized by operating the syringe pump in the refill mode, transferring H₂O from the pneumatic assembly back into the syringe pump. The higher pressure in the sample chamber, relative to the pneumatic assembly, then forced the volume displacer outward, increasing the volume of the
sample chamber. The cell was depressurized until a phase transition from one phase to two phases was observed. The cell was depressurized at rates not exceeding 6 atm/min because higher rates made visual observation of the phase transition and recording accurate temperature and pressure readings difficult. The cell was then pressurized until a single phase mixture was again formed. This procedure was repeated at least four times at each temperature for all reported observations.

Below the critical point of the mixture, phase transitions were noted by a bubble of vapor at the top of the cell (bubble point). Near and above the critical point, a fog or mist, and pools of liquid at the bottom of the cell (dew point) were observed as the liquid separated from the supercritical fluid. Efforts were not made to locate the critical point exactly but visual observation of bubble or dew points indicated when the critical temperature was surpassed. In this fashion, the mixture critical properties observed below 100 °C were bracketed within a pair of temperature measurements. This basic procedure was used to map the single liquid or supercritical fluid phase region for all compositions in this study.

RESULTS AND DISCUSSION

Carbon Dioxide

The liquid-vapor equilibrium line for CO₂ was followed from 23-32 °C to check the accuracy of the pressure and temperature measurements. CO₂ was transferred from the syringe pump directly to the view cell, filling it approximately
half full with liquid. This experiment could have been performed by simply measuring the vapor pressure with changing temperature. However, bubble point measurements were taken by varying the cell volume in anticipation of utilizing this method with the binary and ternary mixtures. Results from this study are compared with literature values in Figure 2.3. The agreement between experimental results from this study and a smooth fit line drawn through the literature values was within an average relative error of 0.9%. The critical point of CO₂ was observed with color changes from clear to opaque, yellow, and dark brown. The critical point was measured at 31.0-31.1 °C and 72.6 atm, compared to the literature value of 31.06 °C and 72.86 atm.

**Methanol/CO₂**

Eight methanol/CO₂ compositions were studied across the 0-1 mole fraction CO₂ range with nine isotherms determined at 25, 30, 40, 50, 60, 70, 80, 90, and 100 °C. Table 2.1 lists the experimental P-T-x values for the average of four measurements with the associated standard deviation (SD) in the phase transition pressure (tables are located at the end of the chapter). The average standard deviation in the set point temperature, or the temperature fluctuation during the replicate measurements, was ± 0.3 °C for all of the data. A comparison of the experimental data from this study with the literature available is shown in Figures 2.4-2.6. Excellent agreement between data from this study and the literature values was found.
Figure 2.3. Comparison of the liquid-vapor line for CO$_2$ (●) from this study (▲) with literature values from reference 1.
Figure 2.4. Comparison of methanol/CO$_2$ liquid-vapor equilibrium isotherms from this study at (▲) 25 °C, (●) 50 °C, and (♦) 80 °C with literature values at (△) 25 °C from references 3, 4, 6, and 9, (○) 50 °C from references 5, 9, and 13, and (◇) 80 °C from reference 12.
Figure 2.5. Comparison of methanol/CO$_2$ liquid-vapor equilibrium isotherms from this study at (▲) 30 °C, (●) 60 °C, and (◆) 90 °C with literature values at (○) 60 °C and (◇) 90 °C from reference 12.
Figure 2.6. Comparison of methanol/CO₂ liquid-vapor equilibrium isotherms from this study at (△) 40 °C, (●) 70 °C, and (◆) 100 °C with literature values at (△) 40 °C from references 4, 11, and 14, (○) 70 °C from reference 12, and (◇) 100 °C from references 5, 9, and 12.
Liquid-vapor phase transitions were not observed for 0.9117 mole fraction CO₂ at 90 °C and 100 °C. A steep, vertical transition along a closed vapor-liquid loop was previously reported for ~0.90 mole fraction CO₂ at 100 °C.5,9 Therefore, decreasing the pressure for 0.9117 mole fraction CO₂ at 90 °C and 100 °C does not intersect the liquid-vapor isotherm loops and only a single phase is detected.

Figure 2.7 shows a three dimensional phase diagram illustrating the single phase region for methanol/CO₂ mixtures at 25-100 °C. Each intersection on the gridded surface represents one P-T-χ measurement. The nine isotherms run from left to right, and the eight isopleths run from front to back on the diagram. The region above the surface is either a single phase liquid or supercritical fluid, while below, a two phase liquid-vapor region and a single phase vapor region at very low pressures are observed. In general, a pressure in excess of ~154 atm produces a one phase mixture for temperatures ≤ 100 °C over the entire composition range. More specifically, a pressure > 63 atm should be maintained to ensure a single liquid phase at 25 °C, such as in a syringe pump or transfer lines between the pump and heated components of a chromatographic or extraction system.

According to the classification scheme developed by van Konynenburg and Scott33 for binary mixtures, methanol/CO₂ displays Type I phase behavior. In Type I mixtures, the two components are usually of similar chemical type or have critical properties (T_c, P_c, and V_c) of approximately equal magnitude.34 Methanol has critical values of 240 °C, 78.5 atm, and 118 ml/mol, respectively.2,35 The critical pressure and
Figure 2.7. Phase equilibrium diagram of methanol/CO₂ as a function of P-T-\(\chi\) from 25-100 °C. The region above the surface is single phase.
volume compare closely with those of pure CO₂ (72.86 atm and 94 ml/mol). Type I mixtures are further characterized by a continuous mixture critical curve connecting the critical points of the pure components. Above this curve, a single supercritical fluid phase occurs. Below this curve and bounded on either side by the liquid-vapor lines of the pure components, one phase liquid, one phase vapor, and liquid-vapor phases are possible.³³,³⁴

Liquid-vapor critical loci (defining the mixture critical curve) were previously calculated for binary mixtures containing CO₂ using methods originally designed for simple hydrocarbon mixtures.³⁶,³⁷,³⁸,³⁹ These methods were applied to methanol/CO₂ for use as mobile phases in SFC.¹²,⁴⁰,⁴¹ Rather large errors in the accuracy of the methanol/CO₂ critical loci by these methods were noted.¹² Experimental determinations of the critical loci were also reported for methanol/CO₂.⁸,⁹,¹⁴,¹⁵ Brunner first visually observed the disappearance of two phases coexisting in equilibrium by the addition of a small amount of one component or a minute change in temperature.⁸ Brunner et al. later reported composition measurements and the critical pressure for critical temperatures of 50, 100, 150, and 200 °C.⁹ While 50, 100, and 150 °C were determined experimentally, the composition at 200 °C was obtained by graphical interpolation. Yoon reported the mixture critical point at 40 °C,¹⁴ and Ziegler et al. plotted 20 points along the mixture critical curve using a chromatographic peak-shape method.¹⁵ Because composition data were not determined with these measurements,¹⁵
a pressure above the maximum of the curve should be used at all temperatures to ensure supercritical conditions.

In this study, the mixture critical point for methanol/CO$_2$ was bracketed between two P-T-χ measurements at temperatures below 100 °C for 0.9117, 0.8208, and 0.7100 mole fraction CO$_2$. The transition from liquid to supercritical fluid was apparent by the observation of a bubble point (bp) below and a dew point (dp) above the mixture critical point. These transitions are marked in Table 2.1 with the designations bp and dp. Two compositions, 0.8404 and 0.6735 mole fraction CO$_2$, reported by Brunner et al.$^9$ are directly comparable to and show good agreement with data from this study. Critical values of 50 °C and 94.3 atm for 0.8404 mole fraction CO$_2$ match well with the critical ranges of 50-60 °C and 95.1-110.4 atm for 0.8208 mole fraction CO$_2$ obtained here. At 100 °C and 0.6735 mole fraction CO$_2$, Brunner et al.$^9$ reported a critical pressure of 152.2 atm which compares favorably with the critical ranges of 90-100 °C and 152.0-153.5 atm for 0.7100 mole fraction CO$_2$ determined in this study. Furthermore, a temperature and pressure above 50 °C and 95 atm should ensure a supercritical fluid for 0.9117 mole fraction CO$_2$, a composition very close to the 0.10/0.90 mole fraction methanol/CO$_2$ mixture commonly used in SFC and SFE.
Acetonitrile/CO₂

Ten acetonitrile/CO₂ compositions were studied over the 0-1 mole fraction CO₂ range with six isotherms determined at 25, 35, 50, 70, 80, and 100 °C. Table 2.2 lists the experimental P-T-x values for the average of four measurements with the associated standard deviation (SD) in the phase transition pressure. The average standard deviation in the set point temperature, or the temperature fluctuation during the replicate measurements, was ±0.3 °C for all of the data. Figure 2.8 shows good agreement for data at −35 °C from this study with the literature data available at 35 °C.¹⁶,¹⁷

A three dimensional phase diagram for acetonitrile/CO₂ mixtures from 25-100 °C is shown in Figure 2.9. Each intersection of the gridded surface again represents one P-T-x measurement. The single phase region of acetonitrile/CO₂ mixtures (above the surface) is reached at lower pressures than the complementary methanol/CO₂ mixtures at the same temperatures. A pressure above ~140 atm ensures a one phase liquid or supercritical fluid over the entire composition range at temperatures ≤ 100 °C. Only one mixture critical point at temperatures below 100 °C was detected for acetonitrile/CO₂. A transition from bubble point to dew point was noted for 0.9037 mole fraction CO₂ between 49.7- 69.6 °C and 90.18-114.8 atm, indicating that the critical point of the mixture is between these two values (see Table 2.2). Byun et al.¹⁶ reported a mixture critical point for 0.888 mole fraction CO₂ at 75 °C and 117.5 atm.
Figure 2.8. Comparison of acetonitrile/CO₂ liquid-vapor equilibrium isotherms at 35 °C from (●) this study with (○) literature values from references 16 and 17.
Figure 2.9. Phase equilibrium diagram of acetonitrile/CO₂ as a function of P-T-χ from 25-100 °C. The region above the surface is single phase.
The liquid-vapor critical loci for acetonitrile/CO\textsubscript{2} were previously estimated\textsuperscript{16,41} and experimentally determined.\textsuperscript{15} Acetonitrile/CO\textsubscript{2} appears to also display Type I phase behavior. This statement is supported by the lack of a liquid-liquid-vapor region in this study and the presence of a continuous mixture critical curve connecting the critical points of the pure components.\textsuperscript{15} All other binary mixture classifications described by van Konynenburg and Scott\textsuperscript{33} exhibit a liquid-liquid-vapor region. Pure acetonitrile has a critical temperature, pressure, and volume of 274.7 °C, 47.7 atm, and 173 ml/mol, respectively.\textsuperscript{2,35} Although Type I mixtures are characterized by similar chemical type or similar critical properties, none of the critical properties are particularly close for pure acetonitrile and CO\textsubscript{2} (31.06 °C, 72.86 atm, and 94 ml/mol). The formation of a continuous critical mixture curve between the critical points of the pure components accounts for the acetonitrile/CO\textsubscript{2} mixture critical curve forming at higher temperatures, relative to methanol/CO\textsubscript{2}, due to the higher critical temperature of acetonitrile. The result is a larger single liquid phase region, relative to methanol/CO\textsubscript{2}, due to the onset of liquid-vapor phase transitions at lower pressures and a mixture critical curve extended over a greater temperature range.

**Comparison of Methanol/CO\textsubscript{2} and Acetonitrile/CO\textsubscript{2} Phase Behavior**

Inspection of Figure 2.7 and the methanol/CO\textsubscript{2} data listed in Table 2.1 shows that at the higher temperatures, a pressure maximum occurs between 0.50-0.70 mole fraction CO\textsubscript{2}, with a decrease in transition pressures at 0.80-0.90 mole fraction CO\textsubscript{2}. 

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Figure 2.9 and Table 2.2 for acetonitrile/CO₂ show a gradual increase in transition pressures with increasing temperature and mole fraction CO₂ over the entire composition range.

Between 0.90-1.00 mole fraction CO₂ at high temperatures, the solubility of methanol in CO₂ is very high. This was noted by the closed liquid-vapor loop at ≥ 0.90 mole fraction CO₂ reported by Brunner et al.⁹ and Semenova et al.⁵ at 100 °C, and by the lack of phase transitions observed for 0.9117 mole fraction CO₂ at 90 °C and 100 °C in this study.

Both methanol and acetonitrile self associate in the liquid state.⁴²,⁴³

Aggregation of methanol and acetonitrile in CO₂ is expected, to a lesser degree, as well.⁴⁴ Methanol can hydrogen bond with other methanol molecules in CO₂, leading to enhanced aggregate stability⁴⁴ and a reduced dipole moment and polarity relative to pure liquid methanol.⁴⁵ Fulton et al.⁴⁵ reported extensive aggregate formation at ≤ 0.90 mole fraction CO₂. Methanol aggregation, resulting in reduced polarity, may contribute to the solubility of methanol and CO₂, particularly over the range of 0.50-0.70 mole fraction CO₂, where high pressures were required to form and maintain a single phase. In contrast, acetonitrile is unable to intermolecularly hydrogen bond. The solubility of acetonitrile in increasing proportions of CO₂ decreases, requiring increasingly higher pressures to form and maintain a single phase.
**Methanol/H₂O/CO₂**

Nine isotherms were determined at 25, 30, 40, 50, 60, 70, 80, 90, and 100 °C. The single liquid phase regions were first thoroughly studied for H₂O/methanol mole ratios, R, of 0.5633, 0.4298, and 0.2490.

For R = 0.5633 (20/80 v/v H₂O/methanol), four methanol/H₂O/CO₂ compositions were tested across the 0-0.33 mole fraction CO₂ range. However, with 0.3290 mole fraction CO₂, a single liquid phase could not be formed at 25 °C and pressures < 306 atm, within the constraints of the pressure transducer. Table 2.3 lists the experimental P-T-χ values for the average of four measurements with the associated standard deviation (SD) in the recorded phase transition pressure. A three dimensional phase diagram for methanol/H₂O/CO₂ with R = 0.5633 from 25-100 °C is shown in Figure 2.10. Each intersection of the gridded surface again represents one P-T-χ measurement. The region above and to the left of the surface is a single phase liquid region.

Five methanol/H₂O/CO₂ compositions were studied over the 0-0.43 mole fraction CO₂ range with R = 0.4298 (16/84 v/v H₂O/methanol). Compositions with > 0.43 mole fraction CO₂ were not tested because the initial pressure necessary to form a single phase at 25 °C for 0.4334 mole fraction CO₂ was near the limit of the pressure transducer. The experimental P-T-χ values are given in Table 2.4 and a three dimensional phase diagram is shown in Figure 2.11.
Figure 2.10. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.5633 as a function of P-T-χ from 25-100 °C. The region above and to the left of the surface is single phase.
Figure 2.11. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.4298 as a function of P-T-χ from 25-100 °C. The region above and to the left of the surface is single phase.
For $R = 0.2490$ (10/90 v/v H$_2$O/methanol), eleven methanol/H$_2$O/CO$_2$ compositions were tested across the 0-0.87 mole fraction CO$_2$ range. However, mixtures prepared with 0.8063 and 0.8690 mole fraction CO$_2$ could not be compressed into a single liquid phase at 25 °C and pressures < 306 atm. The last mixture tested that formed a single phase at 25 °C contained 0.7317 mole fraction CO$_2$. The experimental P-T-x values and a three dimensional phase diagram are shown in Table 2.5 and Figure 2.12, respectively.

At this point, a trend in the shape and location of the three dimensional phase diagrams was detected by comparing, for example, Figure 2.12 for methanol/H$_2$O/CO$_2$ with $R = 0.2490$ to Figure 2.7 depicting methanol/CO$_2$. The methanol/H$_2$O/CO$_2$ phase diagram closely followed that of methanol/CO$_2$ at low CO$_2$ mole fractions. At some increased CO$_2$ mole fraction, an inflection occurred in the shape of the isotherms from concave down to concave up. Beyond the inflection, phase transitions were detected at considerably higher pressures, and a limit in the amount of CO$_2$ that could be added with single phase formation was approached. The remaining H$_2$O/methanol compositions studied were tested only from the inflection to the CO$_2$ limit, on the concave up side of the isotherms. At low CO$_2$ mole fractions before the inflection, a three dimensional surface nearly identical to that of methanol/CO$_2$ and bracketed by the methanol/H$_2$O/CO$_2$ mixtures already described was anticipated.

The 25 °C isotherms for the methanol/CO$_2$ and methanol/H$_2$O/CO$_2$ mixtures tested are shown in Figure 2.13. Literature values at $R = 0.2$ are included for
Figure 2.12. Phase equilibrium diagram of methanol/H$_2$O/CO$_2$ at $R = 0.2490$ as a function of P-T-x from 25-100 °C. The region above and to the left of the surface is single phase.
Figure 2.13. Methanol/H$_2$O/CO$_2$ isotherms as a function of P-χ at 25 °C from this study for (●) R = 0 (methanol/CO$_2$), (○) R = 0.5633, (▼) R = 0.4298, (●) R = 0.3318, (▼) R = 0.2490, (●) R = 0.1810, and (▲) R = 0.1197 with (●) literature values for R = 0.2 from reference 6. The line at 63.5 atm represents the vapor pressure of CO$_2$ at 25 °C.
comparison. Figure 2.13 further clarifies the above discussion on the inflection in the shape of the isotherms and the way that the methanol/H₂O/CO₂ phase transitions follow those for methanol/CO₂ at low CO₂ mole fractions before the point of inflection. Figure 2.13 will be discussed in more detail below.

Three H₂O/methanol ratios were then studied within the boundaries already imposed by the methanol/CO₂ and methanol/H₂O/CO₂ compositions. For R = 0.3318 (13/87 v/v H₂O/methanol), three compositions were tested across the 0.40-0.55 mole fraction CO₂ range. A three dimensional plot is given in Figure 2.14 and the data are presented in Table 2.6. At ≤ 0.40 mole fraction CO₂, the surface falls between those already defined by R = 0.4298 and R = 0.2490. At ≤ 0.40 mole fraction CO₂, liquid-vapor phase transitions are anticipated to occur at pressures bracketed by these H₂O/methanol ratios as well (see Figure 2.13).

Four compositions were studied from 0.71-0.91 mole fraction CO₂ for R = 0.1810 (7.5/92.5 v/v H₂O/methanol) with the results shown in Figure 2.15 and Table 2.7. At ≤ 0.71 mole fraction CO₂, phase transitions are expected to fall between those for R = 0.2490 from this study or R = 0.2 from a previous study and methanol/CO₂ (see also Figure 2.13).

Finally, for R = 0.1197 (5/95 v/v H₂O/methanol), three compositions were tested across the range of 0.76-0.90 mole fraction CO₂. The three dimensional surface and the respective data are reported in Figure 2.16 and Table 2.8. In this case, the
Figure 2.14. Phase equilibrium diagram of methanol/H₂O/CO₂ at $R = 0.3318$ as a function of $P-T-x$ from 25-100 °C. The region above and to the left of the surface is single phase.
Figure 2.15. Phase equilibrium diagram of methanol/H₂O/CO₂ at $R = 0.1810$ as a function of P-T-$\chi$ from 25-100 °C. The region above and to the left of the surface is single phase.
Figure 2.16. Phase equilibrium diagram of methanol/H₂O/CO₂ at R = 0.1197 as a function of P-T-x from 25-100 °C. The region above the surface is single phase.
methanol/H₂O/CO₂ phase transitions closely followed the methanol/CO₂ transitions and no inflection point was detected.

By comparing the three dimensional methanol/H₂O/CO₂ and methanol/CO₂ surfaces, the following general trends were noted. First, the proportion of CO₂ that was soluble decreased with increasing mole fraction H₂O (indicated by higher R values), and the pressure necessary to form a single phase increased with increasing mole fraction CO₂ for a given H₂O/methanol ratio. This behavior is attributable to the limited solubility of H₂O in CO₂. Second, liquid miscibility was observed for all three components at low H₂O and/or CO₂ mole fractions such that the phase diagram closely followed that of methanol/CO₂. In this region, higher pressures were required to maintain a single liquid phase with increasing temperatures.

As observed in Figure 2.13, the inflection point in the 25 °C methanol/H₂O/CO₂ isotherms occurs at ~63.5 atm. This corresponds well with the vapor pressure of CO₂ at 25 °C. When the phase transition occurred at pressures < 63.5 atm at 25 °C, a liquid-vapor to single phase liquid transition was observed upon pressurizing the cell. The shape of the resulting isotherm at pressures < 63.5 atm was concave down. When the mole fraction of CO₂ or H₂O was sufficiently high such that the pressure required to form a single liquid phase exceeded the vapor pressure of CO₂ (63.5 atm), liquid-liquid phase separation was observed prior to formation of a single liquid phase. At 25 °C and pressures > 63.5 atm, a transition from liquid-liquid-vapor to liquid-liquid to single liquid phase was observed upon pressurizing the cell. The
shape of the isotherm was concave up, and the liquid-liquid to single liquid phase transition occurred at increasingly higher pressures with increasing mole fraction CO₂.

An inflection point can be observed in the other isotherms (running from left to right in the three dimensional diagrams) in Figures 2.11, 2.12, and 2.15 in particular. While the inflection is very distinct at low temperatures, it becomes less pronounced with increasing temperature. This trend was observed for all methanol/H₂O/CO₂ mixtures with R ≥ 0.1810. An inflection point was not observed with R = 0.1197; transitions were observed from liquid-vapor to single liquid phase when pressurizing the cell contents and none of the transitions occurred at pressures ≥ 63.5 atm at 25 °C. Because the isotherms for R = 0.1197 follow the methanol/CO₂ data so closely, methanol/H₂O/CO₂ mixtures with R values between 0.1197 and 0.1810 will probably exhibit at least a small inflection and liquid-liquid to single liquid phase transitions (see Figure 2.13).

A comparison of isopleths (running from front to back in the three dimensional plots) also yields information about the methanol/H₂O/CO₂ system. For high mole fractions of CO₂ with R ≥ 0.1810, upper critical solution temperature (UCST) lines were observed. The UCST is the temperature at which two liquids critically merge to form a single liquid phase as the temperature is elevated. The UCST line starts at a pressure above the critical pressures of all of the components. The line drops in pressure with increasing temperature and terminates at the upper critical end point (UCEP). The UCEP is the point at which a liquid and vapor critically merge to form a
single liquid phase in the presence of another noncritical liquid phase as the temperature increases.\textsuperscript{34} Therefore, a liquid-liquid-vapor line exists at temperatures below the UCEP, and a liquid-vapor line is present at temperatures above the UCEP. The region to the left of the UCST and above the liquid-liquid-vapor line is a two phase liquid-liquid region. To the right of the UCST and above the liquid-vapor line, a single phase liquid region is formed. The UCEP, UCST, and phase transition lines are depicted in Figure 2.17. Thus, by increasing the temperature at fixed pressure, transitions from liquid-liquid to single phase liquid to liquid-vapor are possible. Isopleths exhibiting a UCST are shown in Figures 2.10, 2.11, 2.12, 2.14, and 2.15 at the high CO\textsubscript{2} mole fractions. The onset of a UCST line occurred at lower CO\textsubscript{2} mole fractions as the R value increased.

Two isopleths from this study and two isopleths from Page\textsuperscript{17} are shown in Figure 2.18 to further illustrate the UCST lines for methanol/H\textsubscript{2}O/CO\textsubscript{2} mixtures. The possible phase transitions with increasing temperature at fixed pressure are marked by the dashed lines. For example, at 170 atm using a 0.3416/0.1133/0.5451 mole fraction methanol/H\textsubscript{2}O/CO\textsubscript{2} mixture, a single phase liquid is obtained at temperatures above \(-30\) °C and below \(-61\) °C. A two phase liquid-liquid region exists at temperatures \(<30\) °C and a two phase liquid-vapor region is present at temperatures in excess of 61 °C. Therefore, only the region from 30-61 °C at 170 atm is appropriate for use as an enhanced-fluidity liquid extraction fluid or mobile phase.
Figure 2.17. P-T diagram illustrating the UCST line, UCEP, and phase equilibrium lines.
Figure 2.18. Comparison of isopleths from this study for methanol/H₂O/CO₂ mole fractions of (♦) 0.3416/0.1133/0.5451 and (●) 0.2148/0.0535/0.7317 with literature values from reference 17 for methanol/H₂O/CO₂ mole fractions of (▲) 0.275/0.078/0.657 and (✦) 0.210/0.062/0.728.
Ternary mixtures such as the methanol/H₂O/CO₂ system are often depicted by triangular phase diagrams. Each apex of the triangle represents one of the components. Triangular phase diagram information is perhaps most useful for determining how to separate one component from the other two. Experimental triangular phase diagrams are generally formed via analytical methods (i.e., by sampling the liquid and vapor phases of a mixture to determine the compositions at a fixed pressure and temperature). Using the variable volume view cell, pressure and temperature data defining phase transitions were obtained for fixed compositions. Therefore, data in this study must be extrapolated from the determined isotherms for triangular phase diagram construction.

Triangular phase diagrams for methanol/H₂O/CO₂ were previously reported by Yoon et al.²⁸ at 69.10 atm and at 118.5 atm and 40 °C. Good agreement between results from this study and those of Yoon et al.²⁸ is observed in Figure 2.19. The miscibility of methanol with H₂O and methanol with CO₂, and the low solubility of H₂O with CO₂ are evident. Figure 2.19 also shows that the single phase liquid region for methanol/H₂O/CO₂ increases with increasing pressure, such as from 69.10 atm (Figure 2.19A) to 118.5 atm (Figure 2.19B), at a constant temperature.

**Acetonitrile/H₂O/CO₂**

Six isotherms were determined at 25, 35, 50, 70, 80, and 100 °C. For R = 0.3206 (10/90 v/v H₂O/acetonitrile), five compositions were tested from 0.11-0.28
Figure 2.19. Triangular phase diagram comparing methanol/H₂O/CO₂ equilibrium data at 40 °C and (A) 69.1 atm and (B) 118.5 atm from (▲) literature values from reference 28 with (●) extrapolated data from this study.
Figure 2.19 (continued).
mole fraction CO₂. However, mixtures with 0.272 and 0.278 mole fraction CO₂ could not be compressed into a single liquid phase at pressures < 306 atm. A three dimensional phase diagram is shown in Figure 2.20 and the data are listed in Table 2.9. The sharp spike in Figure 2.20 shows a UCST line for 0.217 mole fraction CO₂. A relatively high pressure of 120.9 atm was required to form a single liquid phase at 25 °C. Phase transitions were observed at much lower pressures (24.3-54.6 atm) at 35-100 °C. The formation of a UCST line for acetonitrile/H₂O/CO₂ occurred at a lower mole fraction CO₂ and a lower mole fraction H₂O than in the methanol/H₂O/CO₂ system.

Two compositions were tested across the range of 0.097-0.19 mole fraction CO₂ using R = 0.7316 (20/80 v/v H₂O/acetonitrile). However, a single liquid phase could not be formed with this mixture at 25 °C within the constraints of the pressure transducer. The compositions tested are given in Table 2.10.

Comparison of Methanol/H₂O/CO₂ and Acetonitrile/H₂O/CO₂ Phase Behavior

Methanol and H₂O are capable of hydrogen bonding with one another and strongly associating in the liquid state. Methanol and CO₂ were reported to associate in the supercritical⁴⁴,⁴⁶ and liquid⁴⁷ states. On the other hand, H₂O and CO₂ exhibit very low solubility in one another.¹⁸,¹⁹,²⁰,²¹,²²,²³,²⁴,²⁵ Since much higher proportions of H₂O may be added in the methanol/H₂O/CO₂ mixtures than in H₂O/CO₂ mixtures, methanol may be described as a homogenizing agent between the two nearly
Figure 2.20. Phase equilibrium diagram of acetonitrile/H₂O/CO₂ at R = 0.3206 as a function of P-T-χ from 25-100 °C. The region above and to the left of the surface is single phase.
immiscible components. This can be attributed to the capacity of methanol to
associate with both CO₂ and H₂O.

Acetonitrile cannot donate hydrogen bonds and therefore does not strongly
associate with H₂O. Furthermore, self association of acetonitrile in supercritical CO₂
occurs but to a lesser extent than methanol.⁴ It is proposed that the lower degree of
acetonitrile/H₂O and acetonitrile/CO₂ association may account for the smaller
proportions of H₂O and CO₂ soluble in acetonitrile/H₂O/CO₂ mixtures relative to
methanol/H₂O/CO₂.

**SUMMARY**

Single phase liquid or supercritical regions were defined for methanol/CO₂,
acetonitrile/CO₂, methanol/H₂O/CO₂, and acetonitrile/H₂O/CO₂ mixtures at
temperatures of 25-100 °C. Methanol and acetonitrile were found to be soluble in
CO₂ across the entire composition range. Overall, pressures in excess of ~154 atm and
~140 atm ensure a single liquid phase for all methanol/CO₂ and acetonitrile/CO₂
mixtures, respectively. More specifically, pressures above 62.3 atm for methanol/CO₂
and 57.9 atm for acetonitrile/CO₂ are required to form a single phase liquid at 25 °C,
such as in a syringe pump and in transfer lines between various components of a
chromatographic or extraction system.

Methanol/H₂O/CO₂ phase behavior is considerably more complex than
methanol/CO₂. The single phase liquid regions and required pressures at 25 °C are
shown in Figure 2.13. With increasing proportions of H₂O, lower CO₂ mole fractions could be added. Furthermore, careful temperature control is necessary because liquid-liquid and liquid-vapor separation are possible with small variations in temperature at constant pressure (see Figure 2.18). Acetonitrile/H₂O/CO₂ will less useful for chromatography and extraction because of the limited amounts of H₂O and CO₂ that can be added. These factors illustrate the importance of phase diagram information in separation science.
Table 2.1. Experimental P-T-χ single phase transition data for the methanol(1)-
CO₂(2) system. SD signifies one standard deviation in the four replicate pressure
measurements. Transitions from bubble point (bp) to dew point (dp) are indicated.

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\[
\begin{align*}
\chi_1 &= 0.1792, \chi_2 = 0.8208 \\
\chi_1 &= 0.0883, \chi_2 = 0.9117
\end{align*}
\]

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74
Table 2.2. Experimental P-T-\(\chi\) single phase transition data for the acetonitrile(1) - CO\(_2\)(2) system. SD signifies one standard deviation in the four replicate pressure measurements. A transition from bubble point (bp) to dew point (dp) is indicated.

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<th>Temp. Press.</th>
<th>SD</th>
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<td>(\chi_1 = 0.830, \chi_2 = 0.170)</td>
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<tr>
<td>(\chi_1 = 0.671, \chi_2 = 0.329)</td>
<td></td>
<td>(\chi_1 = 0.622, \chi_2 = 0.378)</td>
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<tr>
<td>(\chi_1 = 0.516, \chi_2 = 0.484)</td>
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<td>(\chi_1 = 0.4202, \chi_2 = 0.5798)</td>
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<td></td>
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<tr>
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<td>50.8 17.3 0.1</td>
<td>50.5 36.8 0.1</td>
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<tr>
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<td>69.5 23.1 0.2</td>
<td>69.6 48.9 0.05</td>
<td>69.4 56.9 0.1</td>
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<tr>
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<tr>
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<td>50.4 66.9 0.3</td>
<td>49.8 71.48 0.07</td>
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<tr>
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Table 2.3. Experimental P-T-χ single phase transition data for the methanol(1) - H₂O(2) - CO₂(3) system at χ₂/χ₁ = 0.5633. SD signifies one standard deviation in the four replicate pressure measurements.

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χ₁ = 0.504, χ₂ = 0.284, χ₃ = 0.212

χ₁ = 0.4651, χ₂ = 0.2620, χ₃ = 0.2729

χ₁ = 0.4292, χ₂ = 0.2418, χ₃ = 0.3290

χ₁ = 0.4292, χ₂ = 0.2418, χ₃ = 0.3290

25.0 > 306
Table 2.4. Experimental P-T-χ single phase transition data for the methanol(1) -
H₂O(2) - CO₂(3) system at χ₁/χ₁ = 0.4298. SD signifies one standard deviation in the
four replicate pressure measurements.

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χ₁ = 0.624, χ₂ = 0.268, χ₃ = 0.108

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χ₁ = 0.557, χ₂ = 0.240, χ₃ = 0.203

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</tr>
</tbody>
</table>

χ₁ = 0.5061, χ₂ = 0.2175, χ₃ = 0.2763

<table>
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</tr>
<tr>
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χ₁ = 0.4341, χ₂ = 0.1866, χ₃ = 0.3794

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<tr>
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<td>0.1</td>
</tr>
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χ₁ = 0.3963, χ₂ = 0.1703, χ₃ = 0.4334

78
Table 2.5. Experimental P-T-x single phase transition data for the methanol\((1)\) - H\(_2\)O\((2)\) - CO\(_2\)(3) system at x\(_2\)/x\(_1\) = 0.2490. SD signifies one standard deviation in the four replicate pressure measurements.

<table>
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<th>Temp. Press.</th>
<th>SD</th>
</tr>
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<td>((^\circ)C)</td>
<td>(atm)</td>
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<td>x(_1) = 0.669, x(_2) = 0.167, x(_3) = 0.164</td>
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<td>40.4</td>
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<td>38.0</td>
<td>0.4</td>
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<td>41.8</td>
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<td>79.5</td>
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<td>89.6</td>
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<td>48.8</td>
<td>0.2</td>
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<td>x(_1) = 0.5471, x(_2) = 0.1362, x(_3) = 0.3166</td>
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<td>40.3</td>
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<td>0.09</td>
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<td>105.1</td>
<td>0.6</td>
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<td>121.5</td>
<td>0.2</td>
<td>100.0</td>
</tr>
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<td>x(_1) = 0.4144, x(_2) = 0.1032, x(_3) = 0.4824</td>
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<td>25.3</td>
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<td>150.9</td>
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<tr>
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<td>99.4</td>
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Table 2.5 (continued).

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<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
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<td>189.9</td>
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</tbody>
</table>

\( \chi_1 = 0.3485, \chi_2 = 0.0868, \chi_3 = 0.5647 \)

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<tr>
<th>Temp. (°C)</th>
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<th>SD</th>
<th>Temp. (°C)</th>
<th>Press. (atm)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.0</td>
<td>&gt; 306.0</td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

\( \chi_1 = 0.2438, \chi_2 = 0.0607, \chi_3 = 0.6955 \)

\( \chi_1 = 0.2148, \chi_2 = 0.0535, \chi_3 = 0.7317 \)

\( \chi_1 = 0.1551, \chi_2 = 0.0386, \chi_3 = 0.8063 \)

\( \chi_1 = 0.1049, \chi_2 = 0.0261, \chi_3 = 0.8690 \)

\( 25.0 > 306.0 \)
Table 2.6. Experimental P-T-χ single phase transition data for the methanol(1) - H$_2$O(2) - CO$_2$(3) system at $\chi_2/\chi_1 = 0.3318$. SD signifies one standard deviation in the four replicate pressure measurements.

<table>
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<tr>
<th>Temp. Press.</th>
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<td>(°C) (atm)</td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi_1 = 0.4480, \chi_2 = 0.1486, \chi_3 = 0.4034$

- 25.0 65.1 0.4
- 30.7 74.1 0.45
- 40.8 94.3 0.41
- 49.7 111.6 0.2
- 60.8 127.5 0.4
- 69.9 140.7 0.3
- 79.4 153.1 0.5
- 89.5 164.0 1.4
- 98.9 172.5 0.9

$\chi_1 = 0.4090, \chi_2 = 0.1357, \chi_3 = 0.4553$

- 25.7 101.1 0.4
- 30.1 101.6 0.1
- 39.9 116.7 0.1
- 49.9 130.2 0.05
- 60.2 142.6 0.1
- 70.3 154.8 0.1
- 80.3 166.2 0.4
- 89.5 174.7 0.3
- 99.0 182.4 0.4

$\chi_1 = 0.3416, \chi_2 = 0.1133, \chi_3 = 0.5451$

- 25.1 179.4 1.4
- 30.5 171.4 0.5
- 40.7 162.5 0.05
- 50.7 164.0 0.1
- 60.9 169.8 0.1
- 70.0 177.4 0.1
- 80.1 184.7 0.2
- 90.1 191.7 0.2
- 100.7 197.8 0.4
Table 2.7. Experimental P-T-χ single phase transition data for the methanol(1)-H₂O(2)-CO₂(3) system at χ₂/χ₁ = 0.1810. SD signifies one standard deviation in the four replicate pressure measurements.

<table>
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<th>Temp. Press.</th>
<th>SD</th>
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</thead>
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<td>(°C) (atm)</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>25.1 103.7 0.1</td>
<td></td>
</tr>
<tr>
<td>30.4 79.83 0.12</td>
<td></td>
<td>30.1 106.2 0.05</td>
<td></td>
</tr>
<tr>
<td>40.0 101.0 0.1</td>
<td></td>
<td>39.9 116.6 0.1</td>
<td></td>
</tr>
<tr>
<td>50.0 121.2 0.4</td>
<td></td>
<td>49.6 129.7 0.4</td>
<td></td>
</tr>
<tr>
<td>59.6 137.2 0.1</td>
<td></td>
<td>59.8 143.1 0.3</td>
<td></td>
</tr>
<tr>
<td>69.5 151.5 0.3</td>
<td></td>
<td>69.6 154.7 0.3</td>
<td></td>
</tr>
<tr>
<td>79.2 163.0 0.4</td>
<td></td>
<td>79.8 164.4 0.3</td>
<td></td>
</tr>
<tr>
<td>89.2 172.0 0.3</td>
<td></td>
<td>89.5 170.9 0.1</td>
<td></td>
</tr>
<tr>
<td>99.4 179.0 0.1</td>
<td></td>
<td>99.3 175.0 0.05</td>
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<table>
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<th>Temp. Press.</th>
<th>SD</th>
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<td>(°C) (atm)</td>
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<td>25.1 193.4 0.7</td>
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<td>30.3 128.8 1.6</td>
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<td>30.1 155.3 1.7</td>
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<tr>
<td>40.1 123.9 0.5</td>
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<td>40.1 127.6 1.0</td>
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<tr>
<td>49.9 130.3 0.2</td>
<td></td>
<td>50.5 130.1 0.1</td>
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<tr>
<td>59.8 139.7 0.1</td>
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<td>60.0 137.1 0.3</td>
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<tr>
<td>69.9 148.9 0.2</td>
<td></td>
<td>69.8 144.4 0.1</td>
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<tr>
<td>79.8 155.4 0.05</td>
<td></td>
<td>79.7 148.5 0.1</td>
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<tr>
<td>89.2 158.3 0.1</td>
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<td>89.3 147.1 0.5</td>
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<tr>
<td>99.3 156.1 0.2</td>
<td></td>
<td>98.6 136.7 0.9</td>
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</table>

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Table 2.8. Experimental P-T-χ single phase transition data for the methanol(1)-H₂O(2)-CO₂(3) system at χ₂/χ₁ = 0.1197. SD signifies one standard deviation in the four replicate pressure measurements.

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<tr>
<td>59.5</td>
<td>123.0</td>
<td>0.4</td>
</tr>
<tr>
<td>69.7</td>
<td>138.7</td>
<td>0.05</td>
</tr>
<tr>
<td>79.3</td>
<td>151.0</td>
<td>0.2</td>
</tr>
<tr>
<td>89.3</td>
<td>160.1</td>
<td>0.1</td>
</tr>
<tr>
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</tr>
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<td>106.9</td>
<td>0.3</td>
</tr>
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<td>123.4</td>
<td>0.1</td>
</tr>
<tr>
<td>69.4</td>
<td>139.2</td>
<td>0.2</td>
</tr>
<tr>
<td>79.1</td>
<td>150.3</td>
<td>0.7</td>
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<tr>
<td>89.3</td>
<td>159.0</td>
<td>0.6</td>
</tr>
<tr>
<td>99.0</td>
<td>164.0</td>
<td>0.05</td>
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</table>

<table>
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<tr>
<th>Temp. Press. (°C)</th>
<th>Press. SD (atm)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>30.4</td>
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<td>100.9</td>
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<td>69.6</td>
<td>122.9</td>
</tr>
<tr>
<td>79.2</td>
<td>123.7</td>
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</table>
### Table 2.9. Experimental P-T-\(\chi\) single phase transition data for the acetonitrile(1)-H\(_2\)O(2)-CO\(_2\)(3) system at \(\chi_2/\chi_1 = 0.3206\). SD signifies one standard deviation in the four replicate pressure measurements.

<table>
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<th>Press.</th>
<th>SD</th>
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<td>(atm)</td>
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<td>0.3</td>
<td>79.4</td>
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<td>99.0</td>
<td>53.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(\chi_1 = 0.672, \chi_2 = 0.223, \chi_3 = 0.105\)

\(\chi_1 = 0.595, \chi_2 = 0.197, \chi_3 = 0.208\)

### Table 2.10. Experimental P-T-\(\chi\) single phase transition data for the acetonitrile(1)-H\(_2\)O(2)-CO\(_2\)(3) system at \(\chi_2/\chi_1 = 0.7316\).

<table>
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<tr>
<th>Temp.</th>
<th>Press.</th>
<th>SD</th>
<th>Temp.</th>
<th>Press.</th>
<th>SD</th>
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<tr>
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<td>(atm)</td>
<td></td>
<td>(°C)</td>
<td>(atm)</td>
<td></td>
</tr>
<tr>
<td>25.1</td>
<td>120.9</td>
<td>7.9</td>
<td>25.0</td>
<td>&gt;306.0</td>
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</tr>
<tr>
<td>35.4</td>
<td>24.3</td>
<td>0.05</td>
<td>50.1</td>
<td>30.7</td>
<td>0.05</td>
</tr>
<tr>
<td>69.9</td>
<td>40.4</td>
<td>0.2</td>
<td>79.3</td>
<td>45.2</td>
<td>0.1</td>
</tr>
<tr>
<td>99.2</td>
<td>54.6</td>
<td>0.6</td>
<td>99.2</td>
<td>54.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

\(\chi_1 = 0.588, \chi_2 = 0.195, \chi_3 = 0.217\)

\(\chi_1 = 0.547, \chi_2 = 0.181, \chi_3 = 0.272\)

\(\chi_1 = 0.542, \chi_2 = 0.180, \chi_3 = 0.278\)

\(25.0 > 306.0\)

### Table 2.10. Experimental P-T-\(\chi\) single phase transition data for the acetonitrile(1)-H\(_2\)O(2)-CO\(_2\)(3) system at \(\chi_2/\chi_1 = 0.7316\).

<table>
<thead>
<tr>
<th>Temp.</th>
<th>Press.</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(°C)</td>
<td>(atm)</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>&gt;306.0</td>
<td></td>
</tr>
</tbody>
</table>

\(\chi_1 = 0.5218, \chi_2 = 0.3817, \chi_3 = 0.0965\)

\(\chi_1 = 0.466, \chi_2 = 0.341, \chi_3 = 0.193\)

\(25.0 > 204.0\)
REFERENCES


CHAPTER 3

EXTRACTION OF PHENOLIC AND NITROAROMATIC POLLUTANTS
FROM AN OCTADECYL POLYSILIOXANE SORBENT
USING CO₂ AND METHANOL/CO₂ MIXTURES

INTRODUCTION

As discussed in Chapter 1, SFE with CO₂ is a viable method for the rapid extraction of organic pollutants from solid matrices due to the improved viscosity and diffusion of supercritical fluids relative to conventional liquids.¹⁻³ However, analyte solubility and the interruption of matrix-analyte interactions, the other two controlling factors in the extraction, may be problematic for polar and/or high molecular weight analytes and wet and/or adsorptive matrices. SFE with small proportions (1-10 mole %) of added organic modifier extended the range of applications considerably although numerous alternative approaches are being developed for complex samples (a literature review of these alternatives is presented in Chapter 6).

One alternative, enhanced-fluidity liquid extraction (EFLE), is investigated herein for the recovery of phenolic and nitroaromatic pollutants from a sorbent material. The pollutants chosen were phenol, o-cresol, m-cresol, 2,4-dimethylphenol,
2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, nitrobenzene, 2,4-dinitrophenol, and 4,6-dinitro-o-cresol. Although polar, these analytes are sufficiently soluble in CO₂ due to their small size and the large quadrupole moment of CO₂. Chemical structures of the eleven analytes are shown in Figure 3.1. Analyte solubilities in liquid CO₂, as well as formula weights, melting and boiling points, and pKₐ values for the analytes are included in Table 3.1.

**Previous Extractions of Phenolic and Nitroaromatic Pollutants**

An octadecyl (ODS) polysiloxane sorbent was used previously for the solid phase extraction (SPE) of chlorophenols from H₂O. After loading, the analytes were eluted with acetone. Recoveries of > 85% were obtained for chlorophenols with 2-5 chlorine substituents. Recoveries of phenol and 2-chlorophenol were only 5% and 35%, respectively, indicating low break-through volumes. SPE followed by analyte elution with CO₂ was also reported. A 1.5 ml volume of acetone was added directly to the ODS disk as a static modifier prior to extraction. Recoveries of phenol, 2,4-dimethylphenol, and several chlorophenols were > 75% from a spiked sample but ≤ 60% from a waste H₂O sample.

Several groups investigated the extraction of phenolics from spiked environmental matrices using supercritical CO₂ and methanol/CO₂ mixtures. Phenolics were recovered equally from spiked soil by SFE with 2% methanol in CO₂ and Soxhlet extraction. Chlorophenolics were removed from spiked sediment at
Figure 3.1. Structures of (A) phenol, (B) o-cresol, (C) m-cresol, (D) 2,4-dimethylphenol, (E) 2-chlorophenol, (F) 2,4-dichlorophenol, (G) 2,4,6-trichlorophenol, (H) pentachlorophenol, (I) nitrobenzene, (J) 2,4-dinitrophenol, and (K) 4,6-dinitro-o-cresol.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>FW (g/mol)</th>
<th>mp (°C)</th>
<th>bp (°C)</th>
<th>pKₐ</th>
<th>Solubility (wt. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
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<td>40-42</td>
<td>182</td>
<td>9.994</td>
<td>3</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>128.56</td>
<td>8</td>
<td>175-176</td>
<td>8.555</td>
<td>Miscible</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>108.14</td>
<td>32-34</td>
<td>191</td>
<td>10.22</td>
<td>2</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>108.14</td>
<td>8-10</td>
<td>203</td>
<td>10.098</td>
<td>4</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>123.11</td>
<td>5-6</td>
<td>210-211</td>
<td>----</td>
<td>Miscible</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>122.17</td>
<td>----</td>
<td>212</td>
<td>10.595</td>
<td>----</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>163.00</td>
<td>42-43</td>
<td>209-210</td>
<td>7.892</td>
<td>14</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>197.45</td>
<td>64-66</td>
<td>246</td>
<td>6.23</td>
<td>----</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>184.11</td>
<td>106-108</td>
<td>----</td>
<td>4.073</td>
<td>----</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>198.13</td>
<td>83-85</td>
<td>----</td>
<td>4.70</td>
<td>----</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>266.34</td>
<td>188-191</td>
<td>310</td>
<td>4.5</td>
<td>----</td>
</tr>
</tbody>
</table>

Table 3.1. Properties of the phenolic and nitroaromatic pollutants from references 4-6.
≥ 90% yields using supercritical CO₂,¹¹ and were also extracted from soil, wood, and biological tissue using 5% methanol in CO₂.¹² Recoveries from a wood sample averaged 85-106%. Most relevant to this study, Lopez-Avila et al.¹³ extracted base/neutral/acidic compounds, including ten of the eleven analytes tested here, from spiked sand, Florisil, alumina, and silica gel. The effect of adding 200 μl methanol, toluene, or acetone directly to the sample followed by extraction with CO₂ was evaluated. The extraction conditions were 150 atm and 50 °C for 10 min static, then 200 atm and 60 °C for 10 min dynamic, and finally 250 atm and 70 °C for 10 min dynamic. Analytes were collected in 5 ml hexane. Recoveries from Florisil spiked at 100 μg/g were ≤ 22% with methanol, ≤ 37% with acetone, and ≤ 19% with toluene added. 2,4-Dinitrophenol and 4,6-dinitro-o-cresol were not recovered at all and pentachlorophenol was only detected with methanol at 2% yield. Recoveries from the other matrices, also fortified at 100 μg/g, were ≤ 56% from sand, ≤ 29% from alumina, and ≤ 27% from silica gel with 200 μl acetone added. When sand was fortified at 3.6-18 μg/g each analyte, yields ranged from 33-141% without modifier and from 27-129% with acetone added. Recoveries were < 75% for 2-chlorophenol, nitrobenzene, 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol.

The SFE experiments described above primarily involved the extraction of samples that were spiked just a few minutes to a few hours prior to extraction and often directly in the extraction vessel. Analytes are more readily removed from spiked samples than from native matrices. For example, Hawthorne et al.¹⁴,¹⁵ showed that
native naphthalene on urban air particulate extracted five times slower than naphthalene that was “spiked” onto the air particulate and allowed to age for 15 h. Times on the order of 1-14 days are generally necessary for organic analytes to equilibrate with solid matrices.\textsuperscript{16}

**Goals of this Study**

In this chapter, the phenolic and nitroaromatic analytes are recovered from an ODS sorbent, a clean, “model” surface with well defined adsorption sites. The mass transport properties of supercritical fluids, enhanced-fluidity liquids, and conventional liquids are compared while the other two factors (analyte solubility and matrix-analyte interactions) are controlled and minimized. After fortification, a four week mixing and equilibration period was used that simulates native adsorption as much as possible.\textsuperscript{16} Extraction fluids tested were CO\textsubscript{2} and mixtures consisting of 10/90 and 20/80 mole % methanol/C0\textsubscript{2} at supercritical and liquid conditions. All SFE and EFLE experiments were performed at 238 atm and 25, 45, or 65 °C. Soxhlet extractions with methylene chloride were performed for comparison. The relative contributions of fluid composition and temperature to extraction rates and overall yields are considered and the solvent strengths of the extraction fluids are compared. Characteristic physical and/or chemical properties of the matrix and analytes are also discussed in order to explain trends in extraction yields.
EXPERIMENTAL

Materials

2,4-Dinitrophenol, stabilized with 10-15% water, and 4,6-dinitro-o-cresol, stabilized with 11% water, were obtained from Chem Service, Inc. (West Chester, PA). Phenol, 2-chlorophenol, o-cresol, m-cresol, nitrobenzene, 2,4-dimethylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and pentachlorophenol were purchased from Aldrich (Milwaukee, WI). p-t-Butylphenol (internal standard) and biphenyl (GC time reference compound) were also obtained from Aldrich. All were specified at 98% or higher purity and were used as received.

Methanol (100.0%, J.T. Baker HPLC grade, Phillipsburg, NJ) and methylene chloride (99.9%, Fisher Optima grade, Fairlawn, NJ) were used as extraction fluid modifiers and solvents. SFE/SFC grade CO₂ without a helium pad was purchased from Air Products and Chemicals (> 99.9999%, Allentown, PA). Polygosil® octadecyl (ODS) polysiloxane sorbent was obtained from Keystone Scientific, Inc. (Bellefonte, PA). The ODS bonded porous silica had a 63-200 µm particle size distribution, an average pore size of 60 Å, and a carbon loading of 12.1%.

The ODS was washed with methanol and methylene chloride then dried with N₂ prior to use. After weighing into an amber glass bottle, the ODS was saturated with methylene chloride and spiked at the 15 µg/g level with a 2 mg/ml stock solution of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and at the 10 µg/g level with a 2 mg/ml stock solution of the other eight analytes. The higher spiking
level was used because these three analytes exhibit higher limits of detection than the others by the analysis method used. The spiked sample was stored at 4 °C and mixed daily, by swirling the bottle, for two weeks before slowly evaporating most of the solvent with N₂ (~0.3 atm). The nearly dry sample was then mixed for two additional weeks to allow uniform adsorption of analytes onto the ODS and diffusion into the inner pore sites.

Soxhlet Extractions

A micro Soxhlet extractor equipped with a 30 ml flask and H₂O-cooled condenser was purchased from Ace Glass Inc. (Vineland, NJ). A 1.00 g sample of spiked ODS was extracted with 15 ml methylene chloride (the solvent recommended in U.S. EPA Method 604 for the extraction of phenols¹⁷). A 10-15 min solvent recycle time was used. The extraction was allowed to proceed at the boiling point of the solvent for 4 h until the ODS changed from yellow to white in color. For two of the five replicates, the solvent was replaced after 4 h and 8 h and the extraction was continued to 24 h. No analytes were detected in the extractant for the 4-8 h or 8-24 h fractions. Therefore, three further replicate extractions were performed for 4 h each.

SFE and EFLE Experiments

All extractions were performed using an ISCO SFX™ 220 automated supercritical fluid extractor and model 260D syringe pump (Lincoln, NE), shown
schematically in Figure 3.2. Briefly, the syringe pump containing the extraction fluid was operated in constant pressure mode at the chosen extraction pressure. The extractor temperature is variable from 25-150 °C and was preset to the desired temperature at least 15 min prior to extraction. Although the extractor is capable of simultaneously extracting two samples, only one sample was extracted at a time so that the volume of extraction fluid passing through the sample was carefully controlled. Upon starting an extraction, fluid flowed from the syringe pump into the extractor, pressurizing the stainless steel extraction vessel containing the sample and the surrounding volume of the extraction chamber. High pressure seals are used on the chamber cap, holding the vessel in place, and are not needed on the vessel itself.

Static (non-flowing) and/or dynamic (flowing) extraction steps were then performed. In a static step, the sample is soaked in the extraction fluid for a period of time to promote leaching of the analytes. A static step is generally used when modifier is added directly to the sample to maximize interactions between the modifier and matrix. A dynamic step is then used to purge the analytes from the vessel. In a dynamic extraction, the extraction fluid in contact with the sample is continually replaced by fluid from the pump. Modifier added directly to the sample is also purged from the vessel so that the static modifier concentration diminishes with time. When cosolvents are incorporated directly with CO₂, the extraction fluid composition is constant throughout the entire dynamic extraction.
Figure 3.2. Schematic diagram of the extraction apparatus: (A) syringe pump containing CO₂ for mixture preparation, (B) syringe pump containing the extraction fluid, (C) extractor with two sample chambers, (D) fused silica restrictor, and (E) analyte collection vial.
The high pressure inside the extractor was maintained via a small internal diameter fused silica restrictor. The flow rate of extraction fluid out of the vessel was controlled by varying the restrictor length. The extracted analytes and any cosolvent used were then trapped for off-line analysis in a few milliliters of organic solvent in a small vial or test tube.

Extractions with CO$_2$ were performed by filling the extraction pump directly from the CO$_2$ gas cylinder. Binary fluids required two syringe pumps for mixture preparation. On a mole fraction basis, an appropriate volume of methanol was added to the empty extraction pump. The second pump, acting as a pure CO$_2$ reservoir, was operated in the constant pressure mode. An appropriate volume of CO$_2$ at a given density was then transferred to the extraction pump while maintaining constant pressure at the CO$_2$ pump. The methanol/CO$_2$ mixture was pressurized to 238 atm in the extraction pump and allowed to equilibrate for at least 12 h prior to use to ensure complete mixing. All extractions were performed well within the single phase liquid or supercritical region for methanol/CO$_2$ since pressures > 154 atm define a single phase across the entire composition range (see Chapter 2).

The homogeneity of the extraction fluid mixture was also monitored based on the volume of methanol collected from the extraction chamber following the extraction. At the conclusion of the dynamic step, the analyte valve (connecting the extraction vessel and restrictor) closed and the vent valve (connecting the extraction vessel to atmospheric pressure via a length of stainless steel tubing) opened, releasing
the remaining pressure inside the extraction chamber. Vented CO\textsubscript{2} went off into the atmosphere. Any methanol that vented through the tubing at this time was collected in a large test tube and measured in a graduated cylinder. Consistent volumes of methanol were collected for each composition tested.

The 2.5 ml stainless steel extraction vessel was equipped with a 2 \textmu m stainless steel frit above and a 0.5 \textmu m stainless steel frit below the 0.500 g ODS sample with the flow of extraction fluid down through the vertically oriented vessel. All extractions were performed at 238 atm and either 25, 45, or 65 °C. A 1 min hold time (static step) was used to allow pump stabilization after rapidly filling the extraction chamber. A dynamic extraction step of chosen volume followed. The flow rate was maintained at \(-0.5 \text{ ml/min, measured as liquid flow at the syringe pump, via 25-40 cm lengths of 30 \textmu m i.d. fused silica tubing (Polymicro Technologies, Inc., Phoenix, AZ).}

To evaluate the extraction rate, fraction collection experiments were performed in triplicate by replacing the collection vial after a selected volume of fluid had passed from the syringe pump. Vials were changed after 1, 2, 3, 4, 6, 8, and 12 ml for extractions with pure CO\textsubscript{2} and after 0.5, 1, 1.5, 2, 4, 8, and 12 ml for extractions with methanol/CO\textsubscript{2}. An appropriate extraction volume was then chosen to maximize recoveries and five replicate extractions were done with the extract collected in a single vial.

The collection solvent was 5 ml methylene chloride containing 10 \mu g \textit{p}-t-butylphenol (internal standard) and 10 \mu g biphenyl (GC time reference compound).
The collection vial was chilled in an ice bath for 3-5 min prior to the dynamic step, but was then removed and mounted on the side of the extractor. Minor restrictor plugging during the CO₂ extractions was alleviated by lifting the restrictor out of the cold solvent for a few seconds. Restrictor plugging was not encountered when methanol/CO₂ mixtures were used.

Extracts were concentrated to ~25-50 µl with N₂ (~0.2 atm) to minimize the amount of methanol present in the extract for GC analysis; the concentration step was done with all extracts so that analyte losses due to evaporation were constant. The extracts were transferred to 2 ml autosampler vials (National Scientific Co., Lawrenceville, GA) and refilled to 1 ml with methylene chloride. The autosampler vials were rinsed with acetone and dried at 150 °C overnight before use.

**Extract Analysis**

Analyses were performed on a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a split-splitless injection port and a flame ionization detector (FID). A double tapered, deactivated injection liner was installed. An HP 7673 autosampler provided a splitless injection of 1 µl. A 30 m x 0.25 mm i.d. (1 µm film thickness) SPB-5 fused silica capillary column (Supelco, Inc., Bellefonte, PA) was used. The initial oven temperature of 40 °C (1 min hold) was followed by a 30 °C/min ramp to 100 °C (2 min hold). A 10 °C/min ramp to 250 °C (1 min hold) then allowed baseline separation of all components. The electronic pressure control
feature provided pressure programming of the helium carrier gas from 14 to 21 psi after an initial pressure pulse upon injection. The splitless purge valve was turned on at 0.6 min. The injector and FID were 290 and 300 °C, respectively. Instrument control and data acquisition and analysis were accomplished via HP 3365 ChemStation® software installed on a 486 computer.

Quantitation was achieved by analyzing standards prepared by serial dilution from the phenolics and nitroaromatics stocks. At least four standards across the concentration range of 1.5-18 µg/ml 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and 1-12 µg/ml of the other eight analytes were prepared and analyzed with each batch of extracts. p-t-Butylphenol was used as the internal standard and biphenyl was added as a time reference compound to aid in the identification of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol near their limits of detection. Linear curve fits with correlation coefficients ($r^2$) > 0.99 were consistently found for 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and $r^2 > 0.999$ for the other eight components. Limits of detection were ~1.5 µg/ml for 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and ~1 µg/ml for the other eight analytes. A typical chromatogram is shown in Figure 3.3.
Figure 3.3. Chromatogram for the phenolics and nitroaromatics standard prepared at 10 µg/ml for the first eight analytes and 15 µg/ml for the last three. Peaks are identified as (1) phenol, (2) 2-chlorophenol, (3) o-cresol, (4) m-cresol, (5) nitrobenzene, (6) 2,4-dimethylphenol, (7) 2,4-dichlorophenol, (8) p-t-butylphenol (internal standard), (9) 2,4,6-trichlorophenol, (10) biphenyl (GC time reference compound), (11) 2,4-dinitrophenol, (12) 4,6-dinitro-o-cresol, and (13) pentachlorophenol.
RESULTS AND DISCUSSION

Analyte Adsorption to the Matrix

A clean, relatively simple matrix was desirable for the initial evaluation of EFLE. Sea sand was first fortified using the spiking procedure outlined for ODS. However, extraction yields with CO₂ and methanol/CO₂ mixtures were consistently < 50%. Analyte losses during the spiking procedure due to insufficient analyte adsorption by the matrix and/or evaporative loss during solvent removal were then considered. Three 5.00 g samples of sand were saturated with methylene chloride and fortified at 10 or 15 μg/g. After 18 h, two 1 ml aliquots of spiking solvent were removed via pipet, concentrated with N₂, and analyzed. The % of analytes adsorbed to the sand, relative to control solutions, are shown in Table 3.2 and averaged 0-15%. The same experiment was repeated with ODS. Results in Table 3.2 show that 15-100% of the analytes were adsorbed in this short time period. Greater adsorption and equilibration is expected after the 4 week period allowed prior to extractions.

Chemical and Surface Properties of ODS

The ODS represents a simplified “model” surface for describing native solids such as soils and sediments because both hydrophobic sites (C₁₈ chains) and hydrophilic sites (Si-OH) are present. The ODS is nearly ideal in that the pore size and particle size distribution are quite uniform. Therefore, unlike native matrices, variation in extraction yield among the samples should not result due to differences in
Table 3.2. Average % recoveries ± one standard deviation in the spiking solvent and average % of analytes adsorbed onto sea sand and ODS after 18 h (n=3).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sea Sand</th>
<th>ODS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% Recovered</td>
<td>% Adsorbed</td>
</tr>
<tr>
<td>Phenol</td>
<td>88 ± 4</td>
<td>12</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>85 ± 5</td>
<td>15</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>91 ± 4</td>
<td>9</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>95 ± 3</td>
<td>5</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>94 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>98 ± 2</td>
<td>2</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>95 ± 3</td>
<td>5</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
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</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>105 ± 7</td>
<td>0</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>110 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>113 ± 3</td>
<td>0</td>
</tr>
</tbody>
</table>
pore distribution. Also, the pore sizes (average of 60 Å) of the ODS were much larger than the hydrodynamic radii of the analytes. The interior pore structure was equally accessible to all analytes. Accordingly, analyte adsorption to the C_{18} chains and to any exposed silanols of the underlying silica should predominately control matrix-analyte interactions and should be uniform throughout the sample. Therefore, the effect of the matrix on the extraction process was controlled and well defined. This allowed a better understanding of the influence of the extraction fluids and analytes on the extraction mechanism.

Solubility Data Available for the Test Analytes

All of the analytes tested are soluble in liquid methanol and methylene chloride. The Hildebrand solubility parameter (δ) is often used to describe solvating power and is dependent on pressure and temperature. Phenol and methanol have the same Hildebrand solubility parameter (δ = 14.52 cal^{1/2} cm^{3/2} mol^{-1}) at atmospheric pressure and 25 °C. Liquid CO\textsubscript{2} has a Hildebrand solubility parameter of δ = 7.0-7.5 cal^{1/2} cm^{3/2} mol^{-1} at 238 atm and 25 °C. The solubility of phenol in liquid CO\textsubscript{2} at 25 °C was reported as 3% by weight (see Table 3.1). This corresponds to ~28 mg phenol per 1 ml CO\textsubscript{2} at 238 atm (the pressure used here for extractions) and 25 °C. For a 0.50 g ODS extraction, the maximum concentration of phenol present was 5 μg, well below the solubility limit. Two of the analytes studied, 2-chlorophenol and nitrobenzene, are miscible with liquid CO\textsubscript{2}. These compounds were consistently...
extracted within the first 2 ml during the fraction collection experiments, indicating weak adsorption to the ODS and high solubility in all of the extraction fluids tested.

*Ortho*-substitution on phenol, in general, increases solubility in liquid CO₂ at room temperature.²⁰ Methyl substitution in any position also improves the solubility of phenols. However, the addition of chloro and nitro groups in positions other than *ortho*-tends to lower solubility.²⁰ This results because the intramolecular hydrogen bonding present with *ortho*-substitution is no longer possible, and thus the polarity increases considerably. While nitrobenzene was readily extracted with CO₂, 2,4-dinitrophenol was not recovered without the addition of methanol. The methyl substituent in the *ortho*-position of 4,6-dinitro-ortho-cresol may enhance solubility such that the analyte can be recovered to some extent with CO₂ alone. Analyte solubilities are expected to increase substantially with the addition of methanol to CO₂.

Due to preferential clustering interactions of cosolvents such as methanol around polar analytes, the 10/90 and 20/80 mole % methanol/CO₂ solutions have solvent strengths markedly closer to that of pure methanol than that of CO₂. For 20/80 mole % methanol/CO₂, the Kamlet-Taft solvatochromic parameters, α and β (measuring hydrogen bond acidity and basicity, respectively), were measured at ~70-80% those of pure methanol and π* (measuring dipolarity and polarizability) was ~40% that of pure methanol.²¹ Therefore, for the trace levels of analytes extracted, solubility in CO₂ and methanol/CO₂ mixtures should not be a factor in the extraction process.
Soxhlet Extractions

To allow direct comparison of SFE and EFLE with the most common competing liquid extraction, the ODS matrix was also extracted by Soxhlet using methylene chloride as the solvent. Table 3.3 summarizes the % recoveries, relative to the spiking levels, for the 4 h micro Soxhlet extractions. Because none of the analytes was recovered from the fractions collected after 4 h, the extraction was considered exhaustive. Soxhlet results ranged from a low value of 66% for a more volatile analyte such as 2-chlorophenol, to high values of 84% and 86% for 2,4-dinitrophenol and 2,4,6-trichlorophenol, respectively. The % RSDs were quite low at 3-9%. These data will be compared later to the results of the SFE and EFLE experiments.

Collection Efficiency of Analytes

Effective trapping of analytes during SFE and EFLE and minimal evaporative loss during the concentration step are crucial for high recoveries. Losses during the extraction and evaporation steps were considered together because both steps were used with all extracts. Methylene chloride collection solvent was spiked with analytes such that the final concentration in 1 ml solvent was 10 µg (first eight analytes) or 15 µg (2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol). The collection vial was then chilled in an ice bath for 3-5 min. Cooling the collection vial lowers the viscosity of the liquid solvent leading to a decrease in the size of CO₂ gas bubbles.
Table 3.3. Average % recoveries ± one standard deviation for Soxhlet extractions and collection efficiency experiments (n=5).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Soxhlet Extraction % Recoveries</th>
<th>Collection Efficiency % Recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>67 ± 3</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>66 ± 5</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>68 ± 3</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>68 ± 2</td>
<td>89 ± 11</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>72 ± 4</td>
<td>80 ± 14</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>71 ± 2</td>
<td>87 ± 10</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>76 ± 3</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>86 ± 3</td>
<td>101 ± 8</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>84 ± 8</td>
<td>128 ± 22</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>72 ± 2</td>
<td>102 ± 5</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>70 ± 3</td>
<td>106 ± 11</td>
</tr>
</tbody>
</table>
formed during collection. Better transfer of the analytes from the eluent gas to the collection solvent results.  

The solvent was then purged with 3 ml of 20/80 mole % methanol/CO₂ at 238 atm and 65 °C. After concentrating with N₂ and refilling to 1 ml, the solution was analyzed by GC/FID. The peak areas for the analytes in the collection solvent were compared to the peak areas for control standards that were not purged or concentrated to obtain the % recoveries for the analyte collection process.

Table 3.3 shows that recoveries of 80-90% were common for the more volatile components. Hawthorne et al. reported a collection efficiency of 77% for phenol using supercritical CO₂ extraction fluid and 3 ml methylene chloride as the collection solvent. When the solvent temperature was controlled to a constant 5 °C, the collection efficiency improved to 98% for phenol. Cooling due to the expansion of CO₂ upon depressurization of the extraction fluid may be somewhat less for the enhanced-fluidity liquid mixtures relative to supercritical fluids and may account for the collection efficiencies of 80-90% obtained in this study.

Solid phase trapping, another common collection method, involves depositing the analytes on a sorbent material then eluting the analytes with a liquid solvent after the extraction is finished. Solid phase trapping is not viable here because the large volume of cosolvent in the enhanced-fluidity liquid mixtures would continually wash the analytes off of the trap. Additionally, relatively poor trapping and/or inefficient rinsing of the phenolics from a variety of sorbents after SFE with CO₂ was reported.
SFE and EFLE with CO₂ and Methanol/CO₂ Mixtures

A. Effect of Extraction Fluid Composition and Extraction Temperature. The critical temperatures for 10/90 and 20/80 mole % methanol/CO₂ mixtures are ~50 °C and ~70 °C, respectively (see Chapter 2). Therefore, at 25, 45, and 65 °C extraction temperatures, 10/90 mole % methanol/CO₂ was tested at liquid and supercritical conditions while 20/80 mole % methanol/CO₂ was used at liquid conditions only.

Plots of % recovery vs. extraction fluid volume were prepared for each analyte at the nine conditions tested (see Table 3.4). Figure 3.4 shows the extraction of 2,4-dimethylphenol and 2,4-dichlorophenol at 238 atm and 45 °C using CO₂, 10/90 mole % methanol/CO₂, and 20/80 mole % methanol/CO₂. Lines are drawn as a guide to the eye. An initial rapid rise was followed by a plateau where little or no additional recovery of analytes was detected. Similar curves were observed for the other analytes. The extraction rates in Figure 3.4 provide insight on the solvent strengths of the various extraction fluids tested. The solvent strength is dependent upon the fluid's ability to remove the analytes from the matrix (i.e., to interrupt matrix-analyte adsorptive interactions and to solubilize the analytes in the bulk extraction fluid). As expected, the methanol/CO₂ mixtures resulted in more rapid extractions than pure CO₂ due to their increased solvent strengths for the polar analytes.

From the concentration vs. volume plots for all components at each composition and temperature, an optimum extraction volume was chosen on the plateau of the curves. For example, the optimum volumes chosen for pure CO₂, 10/90
Table 3.4. Summary of the extraction conditions tested. All extractions were performed at 238 atm using the extraction fluid volumes listed (n=5). The average flow rate was calculated from the average extraction time (± one standard deviation) and the volume of extraction fluid used.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature (°C)</th>
<th>Fluid State</th>
<th>Extraction Volume (ml)</th>
<th>Average Time (min)</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td>25</td>
<td>Liquid</td>
<td>12</td>
<td>23.2 ± 0.3</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Supercritical</td>
<td>10</td>
<td>19.6 ± 0.4</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>Supercritical</td>
<td>10</td>
<td>22.1 ± 0.9</td>
<td>0.45</td>
</tr>
<tr>
<td>10/90 mole % MeOH/CO₂</td>
<td>25</td>
<td>Liquid</td>
<td>6</td>
<td>12.3 ± 0.7</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Liquid</td>
<td>4</td>
<td>7.9 ± 0.1</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>Supercritical</td>
<td>4</td>
<td>8.5 ± 0.7</td>
<td>0.47</td>
</tr>
<tr>
<td>20/80 mole % MeOH/CO₂</td>
<td>25</td>
<td>Liquid</td>
<td>3</td>
<td>6.4 ± 0.1</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Liquid</td>
<td>3</td>
<td>5.6 ± 0.2</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>Liquid</td>
<td>3</td>
<td>6.6 ± 0.2</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Figure 3.4. % Recovery, relative to the spiking level, vs. extraction volume for (A) 2,4-dimethylphenol and (B) 2,4-dichlorophenol at 238 atm and 45 °C using (+) CO₂, (▲) 10/90 mole % methanol/CO₂, and (●) 20/80 mole % methanol/CO₂.
Figure 3.4 (continued).
mole % methanol/CO₂, and 20/80 mole % methanol/CO₂ as extraction fluids at 25 °C were 12, 6, and 3 ml, respectively (see Table 3.4). A minimum extraction volume of 3 ml was used so that the 2.5 ml vessel was swept at least once during the dynamic step. The dynamic extraction time was monitored directly on the extractor. The average flow rate, shown in Table 3.4, was then calculated from the time and volume used.

Tables 3.5-3.7 show the average % recoveries for five replicates at 238 atm and 25, 45, and 65 °C using the optimum extraction volumes. For the first eight analytes, the recoveries improved by ~20% at all three temperatures when using 10/90 and 20/80 mole % methanol/CO₂ rather than CO₂ alone. At the same time, the volumes of extraction fluids used and the subsequent times necessary for extraction were reduced by at least a factor of two (see Table 3.4). Recoveries of the first eight analytes with 10/90 and 20/80 mole % methanol/CO₂ at all three temperatures averaged 80% and 78%, respectively. Since collection efficiencies of these analytes were 80-101% (see Table 3.3), the extraction yields were nearly quantitative. Recoveries with pure CO₂ averaged 61%, implying that not all of the analytes were extracted. The 4 h Soxhlet extractions using methylene chloride as the extraction solvent yielded an average of 72% for the first eight analytes (see Table 3.3). Therefore, methanol/CO₂ extractions resulted in higher overall recoveries and were complete at least 20 times faster.

Lower recoveries of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol relative to the other phenolics and nitroaromatics were previously
Table 3.5. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of five replicate extractions at 238 atm and 25 °C using the extraction volumes shown in Table 3.4.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO₂</th>
<th>10/90 mole % MeOH/CO₂</th>
<th>20/80 mole % MeOH/CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>54 ± 12</td>
<td>76 ± 6</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>54 ± 15</td>
<td>67 ± 10</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>56 ± 11</td>
<td>73 ± 5</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>53 ± 10</td>
<td>82 ± 6</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>60 ± 13</td>
<td>66 ± 7</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>57 ± 9</td>
<td>82 ± 6</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>60 ± 11</td>
<td>93 ± 7</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>68 ± 9</td>
<td>85 ± 10</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0</td>
<td>48 ± 11</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>59 ± 7</td>
<td>34 ± 5</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>31 ± 3</td>
<td>53 ± 12</td>
<td>53 ± 10</td>
</tr>
</tbody>
</table>
Table 3.6. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of five replicate extractions at 238 atm and 45 °C using the extraction volumes shown in Table 3.4.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO₂</th>
<th>10/90 mole % MeOH/CO₂</th>
<th>20/80 mole % MeOH/CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>59 ± 9</td>
<td>82 ± 7</td>
<td>82 ± 3</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>59 ± 12</td>
<td>98 ± 10</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>61 ± 9</td>
<td>80 ± 7</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>59 ± 8</td>
<td>77 ± 6</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>65 ± 10</td>
<td>78 ± 8</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>62 ± 7</td>
<td>78 ± 7</td>
<td>79 ± 3</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>65 ± 8</td>
<td>89 ± 6</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>71 ± 7</td>
<td>103 ± 6</td>
<td>89 ± 12</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0</td>
<td>24 ± 17</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>54 ± 8</td>
<td>51 ± 15</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>32 ± 3</td>
<td>67 ± 16</td>
<td>42 ± 4</td>
</tr>
</tbody>
</table>
Table 3.7. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of five replicate extractions at 238 atm and 65 °C using the extraction volumes shown in Table 3.4.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO₂</th>
<th>10/90 mole % MeOH/CO₂</th>
<th>20/80 mole % MeOH/CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>58 ± 4</td>
<td>75 ± 2</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>56 ± 5</td>
<td>69 ± 4</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>59 ± 4</td>
<td>71 ± 3</td>
<td>72 ± 6</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>60 ± 4</td>
<td>75 ± 3</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>59 ± 4</td>
<td>78 ± 2</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>62 ± 4</td>
<td>77 ± 3</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>64 ± 4</td>
<td>77 ± 3</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>68 ± 4</td>
<td>87 ± 3</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0</td>
<td>36 ± 3</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>44 ± 5</td>
<td>58 ± 14</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>22 ± 1</td>
<td>88 ± 17</td>
<td>70 ± 10</td>
</tr>
</tbody>
</table>
reported by Lopez-Avila. From spiked Florisil, 2,4-dinitrophenol and 4,6-dinitro-o-cresol were not recovered at all and only 2% of pentachlorophenol was extracted with the addition of 200 μl methanol directly to the sample. In this study, 2,4-dinitrophenol was not detected with pure CO₂ but the yields improved considerably with methanol/CO₂ mixtures (24-53%). Recoveries of 4,6-dinitro-o-cresol and pentachlorophenol ranged from 33-59% and 22-88%, respectively. However, higher recoveries were obtained by Soxhlet extraction, with yields of 84%, 72%, and 70% for 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol, respectively (see Table 3.3).

The pH of the washed ODS in aqueous solution was determined to be 8.9. The pKₐ values of the analytes are listed in Table 3.1. The acidity of the hydroxyl hydrogen of the phenolics increases with the addition of electron withdrawing nitro and chloro substituents in the ortho- and para- positions due to resonance. Stronger adsorption to Si-OH sites on the ODS is expected for pentachlorophenol, 2,4-dinitrophenol, and 4,6-dinitro-o-cresol in particular based on their low pKₐ values. As discussed above, these analytes were the most difficult to extract from the basic ODS matrix.

The effect of extraction fluid composition and temperature on the recoveries was studied using a two-way analysis of variance (ANOVA) performed with Systat for Windows, version 5.0 (Systat, Inc., Evanston, IL). Extraction fluid composition and extraction temperature were used as factors with each analyte considered individually.
For all eleven analytes, the methanol/CO₂ compositions had significant effect (95% confidence level) on the measured extraction yield. No difference was indicated between the yields with CO₂ and 20/80 mole % methanol/CO₂ for 2,4,6-trichlorophenol. Higher recoveries were obtained for all other analytes using the methanol/CO₂ mixtures over CO₂ alone. Nitrobenzene, 2,4,6-trichlorophenol, 4,6-dinitro-o-cresol and pentachlorophenol did, however, show different means between 10/90 and 20/80 mole % methanol/CO₂. The 10/90 mole % methanol/CO₂ mixtures produced results that were on average 19% higher for these four analytes.

From Tables 3.5-3.7, average recoveries for the first eight analytes with 10/90 and 20/80 mole % methanol/CO₂ were 79% at 25 °C, 85% at 45 °C, and 79% at 65 °C. Recoveries with CO₂ were 58%, 63%, and 61%, respectively. Yields of 2,4-dinitrophenol were highest at 25 °C, while 4,6-dinitro-o-cresol and pentachlorophenol were generally improved with increasing temperature. The effect of temperature (over the limited range studied) on the extraction yields clearly was modest when compared to composition. Six of the eleven analytes, including m-cresol depicted in Figure 3.5A, showed no temperature dependence by ANOVA calculations (95% confidence level). The variation of temperature did affect the extraction of phenol, 2-chlorophenol, 2,4,6-trichlorophenol, 2,4-dinitrophenol, and pentachlorophenol. For most of these five compounds, 45 °C was an apparent optimum temperature (among 25, 45, and 65 °C). However, as Figure 3.5B shows, greater extraction yields were observed for pentachlorophenol with increasing temperature using the methanol/CO₂
Figure 3.5. % Recovery, relative to the spiking level, vs. extraction volume for (A) \(m\)-cresol using 20/80 mole % methanol/CO\(_2\) and (B) pentachlorophenol using 10/90 mole % methanol/CO\(_2\) at 238 atm and (♦) 25 °C, (▲) 45 °C, and (●) 65 °C.
Figure 3.5 (continued).
mixtures. The rates of extraction in Figure 3.5B with 10/90 mole % methanol/CO₂ were not compromised when using an enhanced-fluidity liquid at 25 and 45 °C compared to a supercritical fluid at 65 °C. Furthermore, the extraction rates with 20/80 mole % methanol/CO₂ in Figure 3.5A at liquid conditions exceeded those with 10/90 mole % methanol/CO₂ in Figure 3.5B at liquid and supercritical conditions. Therefore, the mass transport properties of the extraction fluid, evaluated by the rate of extraction in Figure 3.5, is virtually unaffected by the fluid state (liquid or supercritical).

B. Effective Diffusion of Analytes. The spherical or hot-ball model for heat conduction was successfully applied to extraction kinetics by Clifford and Bartle.¹²⁵ This model allows the calculation of the effective diffusion coefficient of the analyte out of the matrix particles into the bulk extraction fluid. The effective diffusion coefficient does not describe diffusion in the bulk extraction fluid. Data from the fraction collection experiments were plotted as ln m/m₀ vs. time, where m₀ is the initial mass of solute in the matrix (the spiking level) and m is the mass remaining at a given point in the extraction. The negative reciprocal of the slope of the linear portion of the resulting curve is defined as the characteristic time, tₑ:

\[
  t_e = \frac{r^2}{\pi^2 D_{eff}}
\]

where r is the radius of the sphere and D_{eff} is the effective diffusion coefficient. Using
an average ODS particle radius of 62.5 μm (quoted by the manufacturer), values of 
$D_{\text{eff}}$ were determined at those conditions for which three or more data points were
available (i.e., recovery of analytes in four or more fractions). Figure 3.6 shows a plot
of ln $m/m_0$ vs. time for o-cresol using CO$_2$ at 25, 45, and 65 °C and 10/90 mole %
methanol/CO$_2$ at 25 °C and 45 °C and Table 3.8 lists values of $t_c$ and $D_{\text{eff}}$. The
characteristic time, $t_c$, decreased with increasing temperature and/or with the addition
of methanol in CO$_2$. With CO$_2$, $D_{\text{eff}}$ increased 1.8-fold from liquid CO$_2$ to supercritical
CO$_2$ conditions. Little change in $D_{\text{eff}}$ was observed with CO$_2$ at 45 °C and 65 °C.
With 10/90 mole % methanol/CO$_2$, $D_{\text{eff}}$ increased 2.2-fold from 25 °C to 45 °C. A
2.5-fold increase in $D_{\text{eff}}$ was found between CO$_2$ and 10/90 mole % methanol/CO$_2$ at
45 °C. This again demonstrates the enhancement in extraction rate when
methanol/CO$_2$ was used. Furthermore, Table 3.8 shows that mass transport was not
greatly compromised when liquid conditions were used.

C. Experimental Advantages of Enhanced-Fluidity Liquids. Minor restrictor
plugging was encountered during the CO$_2$ replicates at all three temperatures. No
plugging was observed when methanol was present. Restrictor plugging is often a
problem when using supercritical CO$_2$ for wet samples. Methanol increases the
solubility of water in CO$_2$ and decreases ice formation. EFLE is also expected to work
well for the extraction or elution of phenols from an ODS extraction disk or cartridge
after SPE of a water sample.
Figure 3.6. Plot of $\ln \frac{m}{m_0}$ vs. time for the calculation of effective diffusion coefficients for o-cresol using CO$_2$ at (●) 25 °C, (▲) 45 °C, and (●) 65 °C, and 10/90 mole % methanol/CO$_2$ at (▼) 25 °C and (◆) 45 °C.
Table 3.8. Calculated characteristic times, $t_c$, and effective diffusion coefficients, $D_{eff}$, (± one standard deviation) for o-cresol ($n=3$).

<table>
<thead>
<tr>
<th></th>
<th>$t_c$ (min)</th>
<th>$D_{eff} \times 10^5$ (cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO$_2$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>6.54 ± 0.26</td>
<td>1.01 ± 0.04</td>
</tr>
<tr>
<td>45 °C</td>
<td>3.62 ± 0.29</td>
<td>1.82 ± 0.14</td>
</tr>
<tr>
<td>65 °C</td>
<td>3.58 ± 0.15</td>
<td>1.84 ± 0.06</td>
</tr>
<tr>
<td>10/90 mole % MeOH/CO$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>3.22 ± 3.08</td>
<td>2.05 ± 0.79</td>
</tr>
<tr>
<td>45 °C</td>
<td>1.44 ± 0.62</td>
<td>4.59 ± 0.82</td>
</tr>
</tbody>
</table>
Finally, a broader range of extraction temperatures can be utilized when exceeding the critical temperature of the mixture is not a criterion (i.e., experiments can be performed at either liquid or supercritical conditions). The basis for a viable extraction fluid in this study is one that exists as a single, homogeneous phase. Standard SFE equipment is applicable with enhanced-fluidity liquids as well. Analyte and/or matrix decomposition may be averted while using high solvent strength extraction fluids. High molecular weight and low solubility interferences may be left unextracted by keeping the extraction temperature low.

**SUMMARY**

The goal of this study was to compare the mass transport properties of supercritical fluids, enhanced-fluidity liquids, and conventional liquids for the extraction of phenolic and nitroaromatic pollutants. ODS was chosen as a simple, uniform matrix and spiked in a manner that allowed adsorption and equilibration to occur. This “model” surface allowed the separation of matrix effects from the properties of the extraction fluids and polar analytes. Efficient extractions were performed in shorter periods of time with 20/80 mole % methanol/CO₂ than with CO₂ and 10/90 mole % methanol/CO₂. For most of the analytes the extraction yield was unaffected by whether the fluid was in the supercritical or liquid phase region. Results with 10/90 and 20/80 mole % methanol/CO₂ were comparable to or better than those obtained using a 4 h Soxhlet extraction, and extractions were complete at least 20
times faster. This work represents the initial evaluation of enhanced-fluidity liquids for the extraction of polar pollutants. A complex environmental matrix and analytes that are more polar and less soluble in CO$_2$ are discussed in Chapters 4 and 5.
REFERENCES


CHAPTER 4
EXTRACTION OF PHENOLIC AND NITROAROMATIC POLLUTANTS
FROM HOUSE DUST USING CO$_2$, METHANOL/CO$_2$,
AND METHANOL/H$_2$O/CO$_2$ MIXTURES

INTRODUCTION

The extraction of environmental samples is often difficult due to the range of possible sites of interaction between the analytes and the matrix. Matrices such as soils, sediments, and dusts are composed of inorganic (sand, silt, and clay) and organic (humic and non-humic) substituents. Of the inorganic components, clays interact the most strongly with organic pollutants. The humic material is the adsorptive organic component and is comprised of humin, humic acids, and fulvic acids. Nonpolar analytes generally bind to the humin which is nonpolar and hydrophobic. Polar analytes adsorb to the acids and to clays. The non-humic material, composed of fats, waxes, resins, amino acids, and enzymes, is degraded with time. Polar pollutants frequently interact with matrix H$_2$O as well. H$_2$O may reside as a thin layer on matrix particles, in the matrix pores, or associated with clays and other minerals. The matrix porosity, pore size distribution, H$_2$O content, temperature, and pH as well as analyte
vapor pressure and solubility in H₂O all influence the rate and extent of adsorption/desorption of pollutants. Possible matrix-analyte interactions include van der Waals attraction, hydrophobic bonding, hydrogen bonding, bonding by charge transfer, ligand exchange and ion bonding, ion-dipole interactions, dipole-dipole interactions, and chemisorption. Matrices with a substantial content of H₂O and/or organic material are generally the most difficult to efficiently extract due to the complex and heterogeneous nature of the interactions involved.

When SFE is performed, CO₂, a nonpolar solvent, is frequently lacking the solvent strength necessary to break up matrix-analyte interactions. In addition, the minimal solubility of H₂O in CO₂ (~0.4-3 mole % at 25-100 °C) makes penetration of a H₂O layer on the matrix difficult, potentially trapping the analytes in the pore structure below. Polar analytes residing in the associated H₂O are unlikely to partition from the polar liquid to the nonpolar extraction fluid. The use of polar, organic cosolvents such as methanol increases the polarity of the extraction fluid and improves the partitioning of analytes through an adsorbed H₂O layer. The extraction of house dust, a matrix with a high H₂O and organic content, is investigated herein.

Previous Studies on Pollutants in Indoor Air and House Dust

The U.S. EPA established the National Ambient Air Quality Standards (NAAQS) to monitor outdoor air quality and OSHA developed permissible exposure limits (PELs) for hazardous chemicals during an 8 h day in the work place. However,
exposure to pollutants in homes and other buildings was not considered until the 1970s. Levels of pollutants measured indoors were often many times higher than those outdoors as a result of inadequate ventilation and air circulation. Since the average U.S. resident spends ~90% of the time indoors, the hazards of personal exposure to toxic chemicals in the home and other buildings are now of great interest and concern.6

Air is commonly tested using sorbents such as activated charcoal or Tenax which are then Soxhlet extracted or thermally desorbed, or via direct sampling in evacuated canisters followed by cryofocusing and GC analysis. Some of the pollutants identified in indoor air and possible sources include CO and NO₂ from gas stoves and automobiles, formaldehyde from insulation, carpet foam, and other building materials, polycyclic aromatic hydrocarbons (PAHs) from fossil fuels and tobacco smoke, and volatile organic compounds (VOCs) such as chloroform, m- and p-dichlorobenzene, and xylenes from spray cans, paints, repellents, and adhesives among others.7,8,9,10,11 Over 500 VOCs were found in four federal buildings during a recent study.10 Semi-volatile chlorinated pesticides such as chlordane were also detected in the air of residential dwellings at levels up to 7.3 times higher than outdoors.8 Homes with the highest levels were treated for termites up to 10 years prior to sampling, indicating that pollutants may persist for long periods of time.

The “track-in” of pollutants from outdoor sources followed by interaction with house dust was recently investigated.12 The transport of herbicides on shoes from
fields and yards up to a week after application was simulated by walking first through
treated turf then across a strip of carpet. The carpet was vacuumed and the collected
dust was extracted by Soxhlet. Dust was also collected from Columbus area homes
and analyzed for the phenoxyacid herbicides of interest. The herbicides, dicamba and
2,4-D (2,4-dichlorophenoxyacetic acid), were recovered at 16-29 ppm from the track-
in experiment and at native concentrations of 0.1-5 ppm in dust from area homes.12

Just as chlordane was present in indoor air up to 10 years after use,8 it is
anticipated that pollutants adsorbed to house dust will be trapped for extended periods
of time because natural degradation pathways due to sunlight, wind, rain, and soil
microbes are reduced or eliminated. The effects of personal exposure to pollutants in
the home and other buildings are now being realized. “Sick building syndrome” is one
possible outcome, where occupants of new and renovated buildings complain of eye,
nose, and throat irritation as well as dizziness, headaches, and nausea.10,13,14 Methods
to identify and monitor toxic pollutants present in a rapid and efficient manner are of
interest. The recovery of phenolic and nitroaromatic pollutants from house dust will
be discussed in this chapter.

Environmental Sources of Phenolics and Nitroaromatics

Common uses15,16,17 and median lethal dosages (LD₅₀)18 of the eleven phenolics
and nitroaromatics used in this study are listed in Table 4.1. Cresols are sold in
cleaning products such as Lysol® and nitrobenzene is found in some soaps and shoe
Table 4.1. Common uses and LD$_{50}$ values of the phenolics and nitroaromatics from references 15-18.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Common Uses</th>
<th>LD$_{50}$ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>disinfectant; antiseptic; pharmaceutical preservative; manufacturing of resins</td>
<td>317</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>----</td>
<td>670</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>disinfectant; solvent</td>
<td>121</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>disinfectant; fumigant; photographic developer; explosives</td>
<td>242</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>manufacturing of aniline; refining of lubricating oils; soaps; shoe polishes</td>
<td>780</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>----</td>
<td>3200</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>metabolite of 2,4-D herbicide</td>
<td>580</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>fungicide; bactericide; preservative</td>
<td>820</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>wood preservative; insecticide, acaricide (for mites and ticks), fungicide for dormant fruit trees; pH indicator; manufacturing of dyes</td>
<td>30</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>insecticide, fungicide for dormant fruit trees; defoliant</td>
<td>7</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>wood preservative; preharvest defoliant; insecticide for termites; molluscicide (for mollusks)</td>
<td>27</td>
</tr>
</tbody>
</table>
polishes. The chlorophenols and nitrophenols are commonly used in agriculture. Dinitrophenols prevent oxidative phosphorylation and the utilization of nutritional energy. Several deaths were attributed to dinitrophenols in the 1930s when the compounds were administered to patients to induce rapid weight loss. 2,4-Dichlorophenol is a metabolite of 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used herbicide for broad-leaf weed control (studied in Chapter 5). Pentachlorophenol, or PCP, has a variety of uses ranging from a wood preservative to a dessicant for cotton and seeds. PCP is one of a few chemicals that are considered “universally toxic,” or harmful to all living cells.16

Phenolics are also formed in the production of wood pulp and paper when lignin in partially degraded to monomeric phenols, guaiacols (methoxyphenols), and catechols (dihydroxybenzenes).19,20 Bleaching with elemental chlorine results in chlorination of the degradates. The chlorination of drinking H2O often produces trace levels of chlorophenols as well.21,22

The LD50 values of the eleven analytes range from 7 mg/kg 4,6-dinitro-o-cresol to 3200 mg/kg 2,4-dimethylphenol (see Table 4.1).18 The analytes are harmful upon inhalation and dermal contact and if swallowed. Target organs include the liver, kidneys, and central nervous system. The majority are cancer suspects. Seven of the eleven analytes tested are currently on the U.S. EPA Priority Pollutant list.23
Goals of this Study

In this study, house dust is fortified with phenol, 2-chlorophenol, o-cresol, m-cresol, nitrobenzene, 2,4-dimethylphenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol. As discussed in Chapter 3, the analytes are polar but their solubilities in CO₂ are high. For example, the solubilities of phenol and 2,4-dichlorophenol in liquid CO₂ at 25 °C are 3% and 14% by weight, respectively. Therefore, their extraction is expected to be controlled predominately by the ability of the extraction fluid to interrupt matrix-analyte interactions and/or penetrate an adsorbed H₂O layer associated with the matrix.

An adsorption and equilibration period of at least one month was used after fortifying to simulate a native sample. SFE and EFLE were tested. Variables studied include pressure, temperature, and effect of a static extraction step with CO₂ and 10/90 mole % methanol/CO₂. Other cosolvents including ethanol, 1-propanol, acetone, acetonitrile, and toluene were tested at the 10 mole % cosolvent level in CO₂ to evaluate the effects of cosolvent properties on the extraction. Increasing the methanol or methanol/H₂O composition to as high as 40 or 50 mole % in CO₂, respectively, was also tested at fixed pressure and temperature. The addition of methanol, H₂O, acid, or base directly to the sample, followed by a 10 min static step with CO₂, was studied. Classical Soxhlet extractions were performed for comparison.
EXPERIMENTAL

Materials

The eleven phenolics and nitroaromatics, also used in Chapter 3, were purchased from Aldrich (Milwaukee, WI) and Chem Service, Inc. (West Chester, PA). 2-Fluorophenol and 2,4,6-tribromophenol (surrogate standards), biphenyl (GC time reference compound), and p-t-butylphenol (internal standard) were also obtained from Aldrich. All chemicals were specified at 98% or higher purity and were used as received. Anhydrous, granular sodium sulfate (99.2% purity) and reagent grade sea sand were obtained from Jenniele Enterprises (Cincinnati, OH).

Methylene chloride (99.9%, Fisher Optima grade, Fairlawn, NJ), methanol (100.0%, J.T. Baker HPLC grade, Phillipsburg, NJ), ethanol (90% ethanol, 5% methanol, 5% isopropanol, Fisher HPLC grade), 1-propanol (98%, EM Science, Cherry Hill, NJ), acetone (99.6%, Fisher Optima grade), acetonitrile (100.0%, J.T. Baker HPLC grade), and toluene (99.9%, EM Science) were all used as received. H$_2$O was house distilled and deionized using a NANOpure II system (SYBRON/Barnstead, Boston, MA). SFE/SFC grade CO$_2$ without a helium pad was purchased from Air Products and Chemicals (99.99999% purity, Allentown, PA).

Methanol/H$_2$O mixtures were prepared by combining weighed portions of methanol and H$_2$O in a 500 ml bottle. The number of moles of each solvent was then calculated. The approximate methanol/H$_2$O volume ratios prepared and the subsequent mole % methanol/H$_2$O/CO$_2$ mixtures studied were 95/5 v/v (9.0/1.0/90.0
mole %), 92.5/7.5 v/v (16.9/3.1/80.0 and 25.4/4.6/70.0 mole %), and 90/10 v/v (32.1/7.9/60.0 and 40.1/9.9/50.0 mole %). The preparation of methanol/H₂O/CO₂ mixtures in the syringe pump is described in the SFE and EFLE Experiments section below.

House Dust Sample Collection

Dust was collected from door mats in McPherson and Celeste Laboratories at OSU using a High Volume Small Surface Sampler (HVS3, Cascade Stack Sampling System, Inc., Bend, OR), a modified Royal residential vacuum cleaner, that was borrowed from Battelle (Columbus, OH). The vacuum cleaner was equipped with a filtering system such that particles > 5 μm were collected in a removable polyethylene catch bottle, while particles < 5 μm were deposited in the standard vacuum bag. The cyclonic motion of the dust in the catch bottle allowed the small particles to fall out of the cyclone and caused larger material to clump on top when the vacuum was turned off. Very large particles such as rocks, leaves, paper, and carpet fibers were then easily removed from the catch bottle. The result was a sample of house dust that looked very similar in appearance to a finely ground sediment or soil.

Thermogravimetric Analysis (TGA)

A DuPont Instruments Model 951 Thermogravimetric Analyzer (TGA) was used to determine the H₂O and organic content of sea sand and house dust. The TGA
is sensitive to changes in mass caused by decomposition, oxidation, desorption, and evolution of volatile components upon heating. The sample (50 mg sand or 15 mg house dust) was loaded into a clean platinum pan, supported on a quartz rod connected to the balance, and inserted into the furnace. N₂ was used as the purge gas at 50 ml/min to flush out evolved gases. After purging air from the furnace for 10 min at 25 °C, the sample was heated at 10 °C/min to 1000 °C (the instrument's maximum temperature for routine use). % Weight loss of the sample during the heating process was plotted vs. temperature.

The evolution of H₂O bound tightly to minerals of the matrix was reported at temperatures ≥ 400 °C. A previous study of sediments showed that decomposition products such as CO₂, acetone, and VOCs were evolved at ~650 °C, while higher molecular weight aliphatic and aromatic compounds including humic material evolved unchanged at temperatures as high as 800-900 °C. Therefore, organic compounds evolve across virtually the entire temperature range and the weight loss for bound H₂O and organic matter must be considered together using this TGA instrument.

For sea sand, a single small step (0.57 ± 0.41 wt. %, n=6) was observed at ~400 °C, probably corresponding to bound H₂O. The sea sand showed no weight loss at 100 °C for H₂O and no organic content at higher temperatures. For house dust, a gradual weight loss was observed across the entire temperature range. Abrupt transitions that could be correlated to evolved H₂O were not detected at either 100 °C or ~400 °C. The total % weight loss from 25-1000 °C was 34.3 ± 1.1% (n=5) for the
house dust collected in the OSU buildings. For comparison, two other dust samples were also analyzed by TGA. A sample collected from Columbus area homes using the HVS3 vacuum cleaner for studies at Battelle had a combined H$_2$O and organic content of 44.1 ± 0.9 wt. % (n=4) by this method. A 57.3 ± 0.5% weight loss (n=5) was determined for dust taken from ordinary household vacuum cleaners. Therefore, the H$_2$O and organic content of the dust sample varies considerably depending on the collection site and method. All extractions in this study were performed on dust from the OSU buildings because a large quantity of dust was collected.

**Karl Fischer Titrations**

The H$_2$O content was also determined by Karl Fischer titration, where H$_2$O is titrated with a solution containing pyridine or proprietary amines, sulfur dioxide, and iodine in methanol until the first appearance of unused iodine is detected. A Mettler Model DL18 Karl Fischer titrator equipped with a 5 ml buret and a Model DO301 drying oven (Fisher Scientific, Pittsburgh, PA) were used. The drying oven was operated at the maximum temperature of 300 °C. N$_2$ was used as the oven purge gas at a flow rate of ~170 ml/min. A 250 mg sample of house dust was weighed into an oven-dried pan. A 10 min equilibration period was used after inserting the dust into the oven to allow the H$_2$O to be swept into the titration vessel containing ~60 ml pretitrated methanol. The sample was then titrated with pyridine-free Hydranal® Composite 2 (Fisher Scientific, Pittsburgh, PA), where 1 ml reagent titrates ~2 mg
H₂O. The average weight % H₂O evolved at 300 °C was 3.71 ± 0.35% (n=5). When considered together, the TGA and Karl Fischer titration results imply an organic content of ~30.6%.

**pH of the House Dust**

The pH of the house dust was determined by soaking 1.00 g dust in 10 ml distilled and deionized H₂O overnight. An Accumet® Model 10 pH meter (Fisher Scientific, Pittsburgh, PA) was calibrated using pH standards 4 and 7. The pH of the house dust in aqueous solution was ~6.5 (n=3).

**Sample Preparation**

A weighed portion of dust (usually 40 g) was saturated with methylene chloride in an amber glass bottle. The dust was then spiked at 15 μg/g with a 2 mg/ml stock solution of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and at 10 μg/g with a 2 mg/ml stock solution of the other eight analytes. The dust was stored at 4 °C in a sealed bottle and mixed daily, by swirling the bottle, for two weeks before evaporating the solvent with N₂ (~0.3 atm) until the dust was just slightly damp. The sample and adsorbed analytes were allowed to equilibrate for at least two more weeks. In this manner, a period of at least one month was allowed for adsorption of the analytes and aging of the spiked sample. This is a procedure similar to that reported to mimic native samples.²⁸
Soxhlet Extractions

Micro Soxhlet extractions were performed in triplicate for each batch of spiked dust using a 1.000 g sample and 15 ml methylene chloride (the solvent recommended in U.S. EPA Method 604 for the extraction of phenols\textsuperscript{29}). A 10-15 min solvent recycle time was used by adjusting the rate of heating. The extraction solvent was replaced after 2, 4, 8, and 24 h to follow the rate of extraction. The first extraction was allowed to proceed to 48 h and 72 h as well; however, no analytes were detected beyond the 24 h fraction and all other extractions were stopped after 24 h.

SFE and EFLE Experiments

An ISCO SFX™ 220 automated supercritical fluid extractor and a model 260D syringe pump (Lincoln, NE) were again used for all extractions. Extraction fluids tested and the appropriate phase diagram references for fluids other than those studied in Chapter 2 were methanol, methanol/H\textsubscript{2}O, ethanol,\textsuperscript{4,30} 1-propanol,\textsuperscript{4,30} acetone,\textsuperscript{4,31} acetonitrile, and toluene\textsuperscript{4} in CO\textsubscript{2}. On a mole fraction basis, an appropriate volume of liquid cosolvent (based on its density at room temperature) was added directly to the empty extraction pump. For methanol/H\textsubscript{2}O/CO\textsubscript{2} mixtures where the methanol/H\textsubscript{2}O mixture density was not known, a volume of methanol/H\textsubscript{2}O from the stock solution was carefully weighed and the number of moles of each component was calculated from the initial methanol/H\textsubscript{2}O mole ratio in the stock solution. A volume of CO\textsubscript{2} at constant density was then transferred from the second pump. After preparation,
methanol/CO₂ mixtures were pressurized to 238 atm while all other 10/90 mole %
cosolvent/CO₂ mixtures and the methanol/H₂O/CO₂ ternary mixtures were pressurized
to 408 atm to promote mixing. All mixtures were allowed to equilibrate for at least
12 h, and all extractions were performed in a well defined single phase liquid region of
the respective binary or ternary phase diagrams. The homogeneity of the mixture was
again monitored based on the volume of cosolvent collected from the extraction
chamber following the extraction. Consistent volumes of cosolvent were measured for
each extraction fluid mixture.

The 2.5 ml stainless steel extraction vessel was sealed with 0.5 μm stainless
steel frits on each end. A 1.000 g sample of dust (weighed to within ± 1 mg of the
target weight) was packed between 1.50 g sea sand below and 1.00 g sea sand above
the sample, filling the vessel completely. With flow of extraction fluid down through
the vertically oriented vessel, the sand below acted as a particle trap to prevent
clogging by compaction of the dust against the frit. The sand above filled the void
volume and kept the dust confined.

The collection vial contained 5 ml methylene chloride and 10 μg each 2-
fluorophenol and 2,4,6-tribromophenol (surrogate standards) and was chilled in an ice
bath for 5-10 min prior to use to facilitate trapping of the more volatile analytes. The
flow rate was maintained at 0.4-0.45 ml/min via 10-40 cm lengths of 30 μm i.d. fused
silica tubing (Polymicro Technologies, Inc., Phoenix, AZ). Minor restrictor plugging
during extractions with CO₂ and H₂O/CO₂ was alleviated by heating the tip of the restrictor (lifted out of the collection vial) with a heat gun for a few seconds.

After rapidly pressurizing the extraction vessel, a 1 min hold time was used to allow the pump to stabilize. A 10 min hold time (static step) was used to evaluate the effect of a static step with CO₂ and 10/90 mole % methanol/CO₂ and when modifier was added directly to the sample. A dynamic extraction of chosen volume followed. Fraction collection experiments were performed in triplicate by changing the collection vial after a specified volume had passed from the pump. During extractions with pure CO₂, collection vials were changed after 1.5, 3, 4.5, 6, 9, 12, 15, 18, 21, 24, 27, and 30 ml. When cosolvents were used and the extraction kinetics were greatly improved, fractions were collected at 1.5, 3, 6, 12, and 18 ml.

All extracts were concentrated to ~25-50 µl with a slow stream of dry, high purity N₂ (~0.2 atm) to minimize the volume of cosolvent present. The extracts were then transferred to autosampler vials and refilled to 1 ml with methylene chloride. p-t-Butylphenol (internal standard) and biphenyl (GC time reference compound) were added. When methanol/H₂O/CO₂ mixtures were used, extracts were dried over anhydrous, granular Na₂SO₄ during the concentration step and filtered through 1 cm Na₂SO₄ contained in a disposable pipet with a small plug of glass wool. Filtering through the pipet was tried initially but the short residence time was insufficient for complete drying of the solution; therefore, Na₂SO₄ was added during the evaporation step as well.
Extract Analysis

Analyses were performed using the GC/FID method described in Chapter 3 with minor adjustments to the oven program. The initial oven temperature of 40 °C (1 min hold) was followed by a 30 °C/min ramp to 100 °C (2 min hold). A 10 °C/min ramp to 280 °C (12 min hold) completed the 35 min program. The final hold time was necessary for elution of compounds coextracted from the dust.

Seven standards were prepared by serial dilution of the 2 mg/ml stocks. Concentration ranges of 1.5-18 µg/ml for 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol and 1-12 µg/ml for the other eight analytes were utilized. Standards were analyzed with each batch of extracts. Linear curve fits with correlation coefficients ($r^2$) > 0.99 were consistently found for the calibration curves of all analytes.

RESULTS AND DISCUSSION

Collection Efficiency, Surrogate Recovery, and Extraneous Matrix Material

The collection efficiency of the analytes in the methylene chloride solvent was tested and reported in Chapter 3. Recoveries of 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol were ~100%. Collection efficiencies for the other seven analytes were 80-90%.

Two surrogate standards, 2-fluorophenol and 2,4,6-tribromophenol, were used in this study. The surrogates were added to the solvent before extract collection and
were used to evaluate losses during extraction, concentration, and drying/filtering (for
the methanol/H₂O/CO₂ extracts). 2-Fluorophenol is more volatile than any of the
analytes and was recovered at 52-95% depending on the conditions and cosolvents
used. Recoveries were lowest for extractions with CO₂, particularly at elevated
pressures or temperatures, indicating purging losses from the collection vial due to
aerosol formation of the expanding CO₂. Losses during the evaporation step were also
possible in the absence of a less volatile cosolvent such as methanol. Recoveries of 2-
fluorophenol were generally 70-80% when a cosolvent was present. Yields of 2,4,6-
tribromophenol were 85-150% with recoveries > 100% resulting from a coeluting
extraneous compound.

The complex nature of the house dust matrix is apparent by the large number
of extraneous peaks in the chromatograms of the extracts. Fortunately, the majority
eluted after the analytes of interest, making quantitation by GC/FID feasible.
Chromatograms for spiked and unspiked dust that was extracted with 20/80 mole %
methanol/CO₂ at 238 atm and 50 °C are shown in Figure 4.1. The surrogates, internal
standard, and GC time reference compound are labeled on both chromatograms and
the analytes are marked in Figure 4.1A. The few extraneous peaks eluting at 6-12 min
did not significantly affect analyte or internal standard identification and quantitation.
Identification of 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4,6-dinitro-α-cresol, and
pentachlorophenol (eluting from ~14-19 min) was done by careful comparison to
standard retention times. The surrogate standard, 2,4,6-tribromophenol, was present as
Figure 4.1. Chromatograms for (A) spiked and (B) unspiked house dust extracted with 20/80 mole % methanol/CO₂ at 238 atm and 50 °C. The chromatographic peaks of interest are designated: (S) surrogate standard, (IS) internal standard, (R) GC time reference compound, and (x) analyte.
a shoulder on the front of a coeluting peak making quantitation of this compound considerably more difficult than the others. In addition, the chromatographic run time was extended by ~15 min after the analytes eluted to allow elution of extraneous matrix compounds.

**Effect of Extraction Variables with CO\(_2\) and 10/90 mole % Methanol/CO\(_2\)**

Extractions with CO\(_2\) were tested at supercritical conditions only. The critical temperature and pressure of 10/90 mole % methanol/CO\(_2\) are \(-50^\circ\text{C}\) and \(-95\ \text{atm},\) respectively (see Chapter 2). The 10/90 mole % methanol/CO\(_2\) mixture was tested as a supercritical fluid, an enhanced-fluidity liquid, and at conditions very near the critical temperature. These experiments evaluate the effectiveness of SFE and modified SFE, and the influence of the extraction fluid state (supercritical or liquid) on the extraction process.

*A. Effect of Extraction Pressure.* Extractions were performed in triplicate with CO\(_2\) (0-30 ml) at 50 °C and 170, 238, and 306 atm and with 10/90 mole % methanol/CO\(_2\) (0-18 ml) at 50 °C and 170, 238, 306, and 442 atm. The smaller extraction volume was used with methanol/CO\(_2\) due to the greatly improved extraction kinetics and detection of no additional analytes beyond this point during preliminary experiments. Table 4.2 lists the average overall % recoveries for the eleven analytes.

Figure 4.2 shows the % recovery, relative to the spiking level, vs. extraction fluid volume for phenol, a compound representative of the analyte set. Lines are
Table 4.2. Effect of pressure on the average overall % recoveries ± one standard deviation, relative to the spiking levels, for triplicate extractions using CO\textsubscript{2} (30 ml) and 10/90 mole % methanol/CO\textsubscript{2} (18 ml) at 50 °C. Surrogates and reference recoveries are the average of all fractions (n=36 for CO\textsubscript{2} and n=15 for methanol/CO\textsubscript{2}).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO\textsubscript{2}</th>
<th>10/90 mole % methanol/CO\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>170 atm</td>
<td>238 atm</td>
</tr>
<tr>
<td>Phenol</td>
<td>43 ± 9</td>
<td>49 ± 23</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>61 ± 14</td>
<td>63 ± 16</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>45 ± 10</td>
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<td>m-Cresol</td>
<td>46 ± 13</td>
<td>53 ± 20</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>85 ± 18</td>
<td>87 ± 16</td>
</tr>
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<td>2,4-Dimethylphenol</td>
<td>31 ± 10</td>
<td>24 ± 6</td>
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<td>2,4-Dichlorophenol</td>
<td>54 ± 15</td>
<td>61 ± 25</td>
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<td>2,4,6-Trichlorophenol</td>
<td>80 ± 24</td>
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<td>5.5 ± 3.6</td>
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<td>Pentachlorophenol</td>
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<td>0</td>
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<tr>
<td>Surrogates / Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Fluorophenol</td>
<td>58 ± 7</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>2,4,6-Tribromophenol</td>
<td>86 ± 9</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>102 ± 2</td>
<td>107 ± 5</td>
</tr>
</tbody>
</table>

149
Figure 4.2. % Recovery, relative to the spiking level, vs. extraction volume for phenol at 50 °C using CO₂ at (●) 170 atm, (▲) 238 atm, and (○) 306 atm, and 10/90 mole % methanol/CO₂ at (+) 170 atm, (△) 238 atm, (○) 306 atm, and (●) 442 atm.
drawn as a guide to the eye. The rate of extraction with CO₂ was gradual and did not reach a maximum during the 30 ml, ~70 min extractions. In contrast, the rate was greatly enhanced within the 18 ml, ~45 min extractions with 10/90 mole % methanol/CO₂. The average % RSDs in the overall recoveries at all pressures for the first eight analytes were 24% with CO₂ (at 30 ml) and 12% with 10/90 mole % methanol/CO₂ (at 18 ml). However, the precision in the 10/90 mole % methanol/CO₂ data sets varied from a low of 5% RSD at 238 atm to a high of 20% RSD at 306 atm. Recoveries of 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol were generally less precise.

One-way analysis of variance (ANOVA) calculations were performed at the 95% confidence level using Quattro Pro, version 6.01 (Novell Applications Group, Orem, UT). No statistical difference was found among the overall mean recoveries for phenol at the three CO₂ pressures or at the four 10/90 mole % methanol/CO₂ pressures, (see Figure 4.2). Two analytes, 4,6-dinitro-o-cresol and pentachlorophenol, showed a statistical difference in their means for the three pressures tested with CO₂. For both analytes, recoveries increased with increasing pressure. Pentachlorophenol was not detected at 170 atm or 238 atm, but was recovered with a 17% yield at 306 atm. Only one analyte, m-cresol, was found to have statistically different means with 10/90 mole % methanol/CO₂ at the four pressures. Based on high analyte recoveries and good precision using 10/90 mole % methanol/CO₂, a pressure of 238 atm was chosen for additional experiments.
For the majority of analytes, statistically higher recoveries were found with 10/90 mole % methanol/CO₂ than with CO₂ alone at 170 atm and 238 atm. Overall recoveries at 306 atm were comparable with 30 ml CO₂ and 18 ml of the 10/90 mole % methanol/CO₂ mixture. The greatest advantage of 10/90 mole % methanol/CO₂ was the enhanced extraction rate and therefore shorter extraction time.

B. Effect of Extraction Temperature. CO₂ extractions were performed at 238 atm and 50, 75, and 100 °C, while 10/90 mole % methanol/CO₂ extractions were done at 25 °C as well. Results for all eleven analytes are given in Table 4.3. A plot of % recovery, relative to the spiking level, vs. volume of extraction fluid for phenol is shown in Figure 4.3. Averages of 19% and 8% RSD were determined for the first eight analytes at all temperatures with CO₂ and 10/90 mole % methanol/CO₂, respectively. While the extraction rate improved at 100 °C with CO₂, the overall recovery was minimally affected. Only pentachlorophenol showed improvement in recovery (at 30 ml) with increasing temperature by ANOVA. With 10/90 mole % methanol/CO₂, a statistically significant difference was detected for all analytes except m-cresol. For most analytes, recoveries at 50 °C and/or 100 °C were ~10% higher than at 25 °C and/or 75 °C (although this is not readily explained). However, the extraction rates in Figure 4.3 were virtually identical regardless of temperature and fluid state (enhanced-fluidity liquid, near critical, and supercritical). Because the lower temperature was experimentally easier to use, a 50 °C extraction temperature was chosen for additional work.
Table 4.3. Effect of temperature on the average overall % recoveries ± one standard deviation, relative to the spiking levels, for triplicate extractions using CO$_2$ (30 ml) and 10/90 mole % methanol/CO$_2$ (18 ml) at 238 atm. Surrogates and reference recoveries are the average of all fractions (n=36 for CO$_2$ and n=15 for methanol/CO$_2$).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO$_2$</th>
<th>10/90 mole % methanol/CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 °C</td>
<td>75 °C</td>
</tr>
<tr>
<td>Phenol</td>
<td>49 ± 23</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>63 ± 16</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>49 ± 16</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>53 ± 20</td>
<td>53 ± 8</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>87 ± 16</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>24 ± 6</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>61 ± 25</td>
<td>64 ± 8</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>92 ± 33</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>35 ± 16</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>24 ± 10</td>
<td>18 ± 12</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Surrogates / Reference

<table>
<thead>
<tr>
<th></th>
<th>CO$_2$</th>
<th>10/90 mole % methanol/CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 °C</td>
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</tr>
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<td>55 ± 6</td>
</tr>
<tr>
<td>2,4,6-Tribromophenol</td>
<td>87 ± 9</td>
<td>102 ± 6</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>107 ± 5</td>
<td>104 ± 3</td>
</tr>
</tbody>
</table>
Figure 4.3. % Recovery, relative to the spiking level, vs. extraction volume for phenol at 238 atm using \( \text{CO}_2 \) at (♦) 50 °C, (▲) 75 °C, and (●) 100 °C, and 10/90 mole % methanol/\( \text{CO}_2 \) at (◇) 25 °C, (+) 50 °C, (∆) 75 °C, and (○) 100 °C.
C. Effect of a Static Extraction Step. The effect of a 10 min static step, prior to the 18 ml dynamic fraction collection, was also tested with CO₂ and 10/90 mole % methanol/CO₂ at 238 atm and 50 °C. Due to the longer interaction time between the extraction fluid and the matrix, a static step may be beneficial if analyte solubility and leaching from the matrix are limiting the extraction. Static/dynamic results were compared with those obtained for a dynamic extraction only. Results are given in Table 4.4 and the % recovery, relative to the spiking level, vs. volume of extraction fluid for phenol is shown in Figure 4.4. The static step with CO₂ considerably improved the extraction rate and precision of the triplicate experiments. The overall recovery after an 18 ml extraction was also greatly improved; however, when results for the 30 ml dynamic extractions with CO₂ were compared to the static/dynamic recoveries at 18 ml, no improvement in overall yield was observed for phenol. The static step with 10/90 mole % methanol/CO₂ had little effect on the rate or recovery of phenol but added 10 min to the overall extraction time.

Two compounds, 4,6-dinitro-o-cresol and pentachlorophenol, did show statistically higher results for the static step with CO₂ by t-test (95% confidence level). 4,6-Dinitro-o-cresol improved from 24% without a static step to 64% with a static step. Pentachlorophenol extraction drastically improved from 0% to 105%. For 10/90 mole % methanol/CO₂, five of the eleven analytes showed statistically different means. 2,4-Dinitrophenol recovery was higher with only a dynamic step (8.5% vs. 0% yields). Four analytes (o-cresol, 2,4-dichlorophenol, 4,6-dinitro-o-cresol, and
Table 4.4. Effect of a 10 min static extraction step on the average overall % recoveries ± one standard deviation, relative to the spiking levels, for triplicate extractions using CO$_2$ and 10/90 mole % methanol/CO$_2$ at 238 atm and 50 °C. Surrogates and reference recoveries are the average of all fractions (n=36 for CO$_2$, dynamic only and n=15 for CO$_2$, static/dynamic and methanol/CO$_2$).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>CO$_2$</th>
<th>10/90 mole % methanol/CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dynamic Only</td>
<td>Static / Dynamic</td>
</tr>
<tr>
<td>Phenol</td>
<td>49 ± 23</td>
<td>60 ± 4</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>63 ± 16</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>49 ± 16</td>
<td>53 ± 1</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>53 ± 20</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>87 ± 16</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>24 ± 6</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>61 ± 25</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>92 ± 33</td>
<td>69 ± 7</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>35 ± 16</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>24 ± 10</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>0</td>
<td>105 ± 1</td>
</tr>
</tbody>
</table>

Surrogates / Reference

<table>
<thead>
<tr>
<th></th>
<th>CO$_2$</th>
<th>10/90 mole % methanol/CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dynamic Only</td>
<td>Static / Dynamic</td>
</tr>
<tr>
<td>2-Fluorophenol</td>
<td>57 ± 8</td>
<td>71 ± 11</td>
</tr>
<tr>
<td>2,4,6-Tribromophenol</td>
<td>87 ± 9</td>
<td>130 ± 20</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>107 ± 5</td>
<td>124 ± 15</td>
</tr>
</tbody>
</table>
Figure 4.4. % Recovery, relative to the spiking level, vs. extraction volume for phenol at 238 atm and 50 °C using CO₂ (▲) without and (△) with a 10 min static step followed by dynamic extraction, and 10/90 mole % methanol/CO₂ (●) without and (○) with a 10 min static step followed by dynamic extraction.
pentachlorophenol) were positively affected by the static step. 4,6-Dinitro-o-cresol and pentachlorophenol yields were again greatly improved. Yields for the others were much less affected; for example, o-cresol recovery increased from 41% to 46%.

In summary, the addition of 10 mole % methanol in CO₂ or the use of a static step with CO₂ had a strong, positive impact on the rate of extraction but a lesser impact on the overall recoveries. The effects of pressure and temperature were also quite minimal. The average recovery for all eleven analytes with 10/90 mole % methanol/CO₂ at 238 atm and 50 °C was 50%, with a range of 8.5% for 2,4-dinitrophenol to 81% for 2,4,6-trichlorophenol. The limited ability of CO₂ to interrupt the strong matrix-analyte interactions present was indicated by the significantly slower extraction rates. The 10/90 mole % methanol/CO₂ mixture was tested above, below, and very near the mixture critical temperature of ~50 °C. Because analyte recoveries generally differed by ≤ 10% and extraction rates at the four temperatures were comparable (see Figure 4.3), the state of the extraction fluid (enhanced-fluidity liquid or supercritical fluid) appears to be unimportant.

Effect of other Cosolvents

Methanol is the cosolvent of choice for the majority of modified SFE experiments reported because methanol is polar and has a relatively low boiling point, making a concentration step feasible. Moreover, methanol/CO₂ phase diagram information is available. However, the role of the modifier in the extraction process
and the properties that make a modifier appropriate for a given matrix and set of analytes are poorly understood.

The effect of cosolvent on the solubility of a polar analyte, naproxen ((S)-6-methoxy-α-methyl-2-naphthaleneacetic acid), in CO₂ was recently investigated. The solubility enhancement found using 5.25 mole % cosolvent in CO₂ relative to CO₂ alone was appreciable for all six cosolvents tested. The cosolvent effect increased in the order ethyl acetate < acetone < methanol < ethanol < 2-propanol < 1-propanol, and correlated strongly with hydrogen bond accepting capabilities.

Eight static modifiers including methanol, methylene chloride, toluene, hexane, acetonitrile, aniline, diethylamine, and acetic acid were tested for the CO₂ extraction of polychlorinated biphenyls (PCBs) from river sediment and PAHs from urban air particulate. PCBs were most efficiently extracted with acidic or basic modifiers possessing a permanent dipole moment (methanol, acetic acid, and aniline). For low molecular weight PAHs, nearly all cosolvents increased recoveries although no trend based on possible interactions was observed. High molecular weight PAHs were most efficiently extracted with toluene, indicating a possible polarizability effect. The conclusions of the study were that the choice of modifier is dependent on the matrix and analytes being considered and that the role of the modifier is predominately to interact with the matrix-analyte complex and promote analyte desorption.

In this study, methanol, ethanol, 1-propanol, acetone, acetonitrile, and toluene were tested as 10/90 mole % cosolvent/CO₂ mixtures at 238 atm and 50 °C. Some
properties of these cosolvents, methylene chloride (used for Soxhlet extractions), and H₂O (used in the 9.0/1.0/90.0 mole % methanol/H₂O/CO₂ extraction fluid compared in this section and discussed in more detail in the next section) are listed in Table 4.5.

From an experimental perspective, the extracts were increasingly difficult to concentrate with N₂ as the boiling point of the cosolvent increased. Lower viscosities were expected to be beneficial in terms of ready percolation through the house dust particles, increased diffusivity, and extraction rate. However, the rates during 18 ml fraction collection experiments were identical (and comparable to those in Figures 4.2 and 4.3 with 10/90 mole % methanol/CO₂) regardless of cosolvent viscosity, implying that the favorable diffusional properties of CO₂ were maintained at the 10 mole % cosolvent level.

With the exception of toluene, the cosolvents are quite polar with relatively large dipole moments. The Kamlet-Taft solvatochromic parameters are also included in Table 4.5. Toluene has a large π* value (defining polarizability or dipolarity) only, and was tested because its chemical structure is similar to those of the analytes. The α and β parameters describe the hydrogen bond donating (acidity) and accepting (basicity) capabilities of the cosolvents. Methanol and H₂O have the highest α values and ethanol has the highest β value.

Overall recoveries using the 10/90 mole % cosolvent/CO₂ mixtures and 9.0/1.0/90.0 mole % methanol/H₂O/CO₂ mixture are given in Table 4.6. Soxhlet extraction results will be considered later. By one-way ANOVA calculations, only
<table>
<thead>
<tr>
<th></th>
<th>Boiling Point (°C)</th>
<th>Viscosity (cP)</th>
<th>Dipole Moment (D)</th>
<th>π*</th>
<th>Kamlet-Taft α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>64.7</td>
<td>0.55</td>
<td>1.70</td>
<td>0.60</td>
<td>0.93</td>
<td>0.62</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78</td>
<td>1.20</td>
<td>1.69</td>
<td>0.54</td>
<td>0.83</td>
<td>0.77</td>
</tr>
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<td>1-Propanol</td>
<td>97.2</td>
<td>2.3</td>
<td>1.68</td>
<td>0.52</td>
<td>0.78</td>
<td>0.45</td>
</tr>
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<td>Acetone</td>
<td>56.3</td>
<td>0.36</td>
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<td>0.71</td>
<td>0.08</td>
<td>0.48</td>
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<tr>
<td>Acetonitrile</td>
<td>81.6</td>
<td>0.38</td>
<td>3.85</td>
<td>0.75</td>
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<td>0.35</td>
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<tr>
<td>Toluene</td>
<td>110.6</td>
<td>0.59</td>
<td>0.36</td>
<td>0.54</td>
<td>0.00</td>
<td>0.11</td>
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<tr>
<td>Methylene chloride</td>
<td>40</td>
<td>0.44</td>
<td>1.14</td>
<td>0.82</td>
<td>0.30</td>
<td>0.05</td>
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<tr>
<td>H₂O</td>
<td>100</td>
<td>1.00</td>
<td>1.85</td>
<td>1.09</td>
<td>1.17</td>
<td>0.48</td>
</tr>
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</table>
Table 4.6. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of triplicate extractions at 238 atm and 50 °C using 10 mole % modifier (9.0/1.0 mole % methanol/H₂O) in CO₂. Surrogates and reference recoveries are the average of all fractions (n=15).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Methanol</th>
<th>MeOH/H₂O</th>
<th>Ethanol</th>
<th>1-Propanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>Toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>72 ± 2</td>
<td>73 ± 2</td>
<td>65 ± 4</td>
<td>69 ± 8</td>
<td>59 ± 1</td>
<td>60 ± 1</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>65 ± 2</td>
<td>69 ± 3</td>
<td>54 ± 3</td>
<td>55 ± 4</td>
<td>52 ± 1</td>
<td>52 ± 2</td>
<td>54 ± 1</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>41 ± 2</td>
<td>47 ± 5</td>
<td>48 ± 6</td>
<td>42 ± 3</td>
<td>46 ± 2</td>
<td>49 ± 2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>60 ± 3</td>
<td>60 ± 3</td>
<td>58 ± 1</td>
<td>57 ± 2</td>
<td>55 ± 2</td>
<td>61 ± 2</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>51 ± 3</td>
<td>72 ± 2</td>
<td>48 ± 5</td>
<td>45 ± 1</td>
<td>52 ± 1</td>
<td>52 ± 1</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>12 ± 1</td>
<td>11 ± 3</td>
<td>14 ± 4</td>
<td>8.2 ± 0.4</td>
<td>13 ± 3</td>
<td>5.8 ± 1.1</td>
<td>9.8 ± 1.5</td>
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<tr>
<td>2,4-Dichlorophenol</td>
<td>74 ± 3</td>
<td>71 ± 3</td>
<td>104 ± 4</td>
<td>88 ± 4</td>
<td>62 ± 1</td>
<td>71 ± 7</td>
<td>73 ± 2</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>81 ± 7</td>
<td>81 ± 3</td>
<td>87 ± 6</td>
<td>72 ± 4</td>
<td>72 ± 2</td>
<td>89 ± 8</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>8.5 ± 1.9</td>
<td>63 ± 18</td>
<td>6.8 ± 2.9</td>
<td>0</td>
<td>12 ± 2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>34 ± 10</td>
<td>59 ± 6</td>
<td>60 ± 19</td>
<td>28 ± 4</td>
<td>79 ± 5</td>
<td>46 ± 11</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>53 ± 14</td>
<td>74 ± 3</td>
<td>76 ± 8</td>
<td>77 ± 7</td>
<td>89 ± 2</td>
<td>69 ± 7</td>
<td>53 ± 6</td>
</tr>
</tbody>
</table>

**Surrogates / Reference**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Methanol</th>
<th>MeOH/H₂O</th>
<th>Ethanol</th>
<th>1-Propanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>Toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Fluorophenol</td>
<td>73 ± 9</td>
<td>71 ± 10</td>
<td>84 ± 7</td>
<td>81 ± 12</td>
<td>66 ± 7</td>
<td>71 ± 7</td>
<td>95 ± 11</td>
</tr>
<tr>
<td>2,4,6-Tribromophenol</td>
<td>155 ± 34</td>
<td>148 ± 34</td>
<td>125 ± 29</td>
<td>120 ± 20</td>
<td>131 ± 17</td>
<td>130 ± 33</td>
<td>151 ± 28</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>110 ± 12</td>
<td>108 ± 7</td>
<td>108 ± 12</td>
<td>120 ± 11</td>
<td>107 ± 5</td>
<td>149 ± 35</td>
<td>121 ± 10</td>
</tr>
</tbody>
</table>
*m*-cresol had identical overall recoveries with the seven cosolvents tested. The methanol/H₂O/CO₂ mixture yielded highest results for four analytes, acetonitrile for three analytes, and ethanol and acetone were each most efficient for two analytes. All of these cosolvents have large dipole moments (≥ 1.69 D) and substantial α and/or β values. Acetone and acetonitrile are hydrogen bond acceptors only; 4,6-dinitro-*o*-cresol and pentachlorophenol were most efficiently extracted with acetone, while *o*- and *m*-cresol and 2,4,6-trichlorophenol were recovered best with acetonitrile. The alcohols are capable of both donating and accepting hydrogen bonds; extraction recoveries were comparable with methanol, ethanol, and 1-propanol despite the considerable differences in viscosity among these solvents. With the exception of nitrobenzene, all of the analytes have a hydroxyl substituent that can also donate or accept hydrogen bonds. Therefore, polar cosolvents having large α and/or β values aided in the extraction of the polar phenolics by dipole effects and hydrogen bonding.

Phenol and 2,4-dimethylphenol were more efficiently recovered by 24 h Soxhlet extractions using methylene chloride than with any of the 10/90 mole % cosolvent/CO₂ mixtures. Methylene chloride has a high π⁺ value and an intermediate dipole moment and α value relative to the other solvents (see Table 4.5). Methylene chloride/CO₂ mixtures were tested but found unacceptable as extraction fluids because gradients were detected across the volume of the extraction pump, based on the volume of methylene chloride vented at the conclusion of the extractions.
Overall, differences in recoveries with the various cosolvents were quite small, although results with methanol/H₂O/CO₂ were consistently high for the entire analyte set. None of the cosolvents was particularly unsuitable for the extraction of the phenolic analytes. Lowest recoveries of various analytes were found with acetone, 1-propanol, acetonitrile, toluene, and methanol. Interestingly, 10/90 mole % methanol/CO₂, the extraction fluid most commonly used in modified SFE experiments, did not produce the highest absolute recovery for any of the analytes (although several were statistically identical).

Effect of Composition with Methanol/CO₂ and Methanol/H₂O/CO₂ Mixtures

Despite the improvements in extraction rate with 10/90 mole % methanol/CO₂ relative to CO₂, overall extraction yields were still quite low for a number of the analytes. Increasing proportions of methanol or methanol/H₂O in CO₂ were tested at enhanced-fluidity liquid conditions in an attempt to further increase the solvent strength of the extraction fluid.

Methanol/H₂O mixtures are commonly used in reversed-phase HPLC for the separation of polar solutes. The addition of H₂O increases the polarity of the mixture relative to methanol alone. Enhanced-fluidity liquid methanol/H₂O/CO₂ mixtures were successfully applied to chromatographic separations. Liquid methanol/H₂O/CO₂ mixtures were previously used for the extraction of morphinic alkaloids from poppy straw. Quantitative recoveries were obtained with 50/50 wt. %
methanol/CO₂ in 200 min and with 24/6/70 wt. % methanol/H₂O/CO₂ in 20 min at
197 atm and 40-45 °C. A 6/6/3/85 v/v methanol/H₂O/triethylamine/CO₂ mixture was
utilized for the recovery of morphinic alkaloids from urine.⁴² A 20 min extraction at
247 atm and 40 °C yielded ≥ 92% while recoveries with methanol/CO₂ mixtures were
not quantitative.

In this study, fraction collection experiments were performed in triplicate at
238 atm and 50 °C with 20/80, 30/70, and 40/60 mole % methanol/CO₂ and
9.0/1.0/90.0, 16.9/3.1/80.0, 25.4/4.6/70.0, 32.1/7.9/60.0, and 40.1/9.9/50.0 mole %
methanol/H₂O/CO₂. All extraction conditions were well within the single phase liquid
regions of the binary and ternary phase diagrams established in Chapter 2. A direct
comparison between methanol/CO₂ and methanol/H₂O/CO₂ mixtures with
compositions of 90, 80, 70, and 60 mole % CO₂ was then possible. Data for all eleven
analytes are given in Table 4.7.

The analytes tested can be divided into methylphenols, chlorophenols, and
nitroaromatics. % Recoveries, relative to the spiking levels, for o-cresol, 2,4,6-
trichlorophenol, and 4,6-dinitro-o-cresol with CO₂ (to 18 ml) and increasing
proportions of methanol and methanol/H₂O mixtures in CO₂ are shown in Figure 4.5.

The methylphenols and 2,4-dimethylphenol in particular were difficult to
extract (see Figure 4.5A). The cresols were recovered at 40-66%. All conditions
(including Soxhlet which will be discussed later) lead to recoveries of ≤ 36% for 2,4-
dimethylphenol. The highest yield was obtained with 32.1/7.9/60.0 mole %
Table 4.7. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of triplicate extractions at 238 atm and 50 °C for enhanced-fluidity liquid methanol/CO₂ and methanol/H₂O/CO₂ mixtures. All mixtures are given as mole % methanol/H₂O in CO₂. Surrogates and reference recoveries are the average of all fractions (n=15).

<table>
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<th>Analyte</th>
<th>10.0/0</th>
<th>9.0/1.0</th>
<th>20.0/0</th>
<th>16.9/3.1</th>
<th>30.0/0</th>
<th>25.4/4.6</th>
<th>40.0/0</th>
<th>32.1/7.9</th>
<th>40.1/9.9</th>
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</thead>
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<tr>
<td>Phenol</td>
<td>72 ± 2</td>
<td>73 ± 2</td>
<td>74 ± 9</td>
<td>81 ± 9</td>
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<td>87 ± 8</td>
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<td>2-Chlorophenol</td>
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<td>69 ± 3</td>
<td>76 ± 5</td>
<td>68 ± 10</td>
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<td>54 ± 5</td>
<td>53 ± 6</td>
<td>63 ± 6</td>
<td>61 ± 6</td>
</tr>
<tr>
<td>o-Cresol</td>
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<td>47 ± 5</td>
<td>50 ± 1</td>
<td>58 ± 9</td>
<td>47 ± 5</td>
<td>56 ± 7</td>
<td>40 ± 2</td>
<td>66 ± 8</td>
<td>59 ± 13</td>
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<tr>
<td>m-Cresol</td>
<td>60 ± 3</td>
<td>60 ± 3</td>
<td>63 ± 2</td>
<td>65 ± 11</td>
<td>57 ± 9</td>
<td>63 ± 4</td>
<td>49 ± 2</td>
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<td>Nitrobenzene</td>
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<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>34 ± 10</td>
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<td>35 ± 9</td>
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<tr>
<td>Pentachlorophenol</td>
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<th>20.0/0</th>
<th>16.9/3.1</th>
<th>30.0/0</th>
<th>25.4/4.6</th>
<th>40.0/0</th>
<th>32.1/7.9</th>
<th>40.1/9.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Fluorophenol</td>
<td>73 ± 9</td>
<td>71 ± 10</td>
<td>74 ± 11</td>
<td>83 ± 13</td>
<td>62 ± 12</td>
<td>65 ± 18</td>
<td>56 ± 11</td>
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<td>67 ± 12</td>
</tr>
<tr>
<td>2,4,6-Tribromophenol</td>
<td>155 ± 34</td>
<td>148 ± 34</td>
<td>159 ± 22</td>
<td>111 ± 18</td>
<td>124 ± 35</td>
<td>111 ± 17</td>
<td>102 ± 48</td>
<td>97 ± 24</td>
<td>114 ± 20</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>110 ± 12</td>
<td>108 ± 7</td>
<td>113 ± 17</td>
<td>116 ± 24</td>
<td>98 ± 4</td>
<td>101 ± 6</td>
<td>108 ± 16</td>
<td>113 ± 37</td>
<td>94 ± 3</td>
</tr>
</tbody>
</table>
Figure 4.5. % Recovery of (A) o-cresol, (B) 2,4,6-trichlorophenol, and (C) 4,6-dinitro-o-cresol, relative to the spiking level, at 238 atm and 50 °C using CO₂, methanol/CO₂ (solid bars), and methanol/H₂O/CO₂ (striped bars) mixtures. Methanol/H₂O compositions are 9.0/1.0, 16.9/3.1, 25.4/4.6, 32.1/7.9, and 40.1/9.9 mole % from left to right. Error bars signify one standard deviation.
Figure 4.5 (continued).
Figure 4.5 (continued).
methanol/H₂O/CO₂. To further test this apparent optimum composition, an elevated extraction temperature of 100 °C was used with 9.0/1.0/90.0 and 32.1/7.9/60.0 mole % methanol/H₂O/CO₂. No improvements in the overall recovery of 2,4-dimethylphenol were found with yields of 10-11% and 35-36% for the 9.0/1.0/90.0 and 32.1/7.9/60.0 mole % methanol/H₂O/CO₂ mixtures, respectively, at both 50 °C and 100 °C. The other analytes were unaffected by elevated temperature as well. Moreover, the recovery of 2,4-dimethylphenol dropped off again when the H₂O composition was increased with 40.1/9.9/50.0 mole % methanol/H₂O/CO₂.

The three methylphenols as well as phenol have pKₐ values ≥ 10 (see Chapter 3). Since the pH of the house dust in aqueous solution was 6.5, these analytes should be adsorbed to the matrix in the protonated, neutral form and may be strongly adsorbed to the humic and fulvic acids of the matrix.

Recoveries of the chlorophenols were consistently high with both methanol/CO₂ and methanol/H₂O/CO₂ mixtures (see Figure 4.5B). The pKₐ values for the chlorophenols range from a high of 8.6 for 2-chlorophenol to a low of 4.5 for pentachlorophenol. Recoveries tended to increase with decreasing pKₐ, implying that analytes present on the matrix as anions were more readily extractable. The two analytes, 2,4-dichlorophenol and 2,4,6-trichlorophenol, with pKₐ values nearest the pH of the dust (pH 6.5) were consistently extracted with high yields (averages of 76% and 89%, respectively, for all methanol/CO₂ and methanol/H₂O/CO₂ mixtures).
Nitrobenzene was most efficiently extracted with CO$_2$. Recoveries of 2,4-dinitrophenol and 4,6-dinitro-2-methylphenol (see Figure 4.5C), with pK$_a$ values of 4.1 and 4.7, were variable, but tended to be higher with methanol/H$_2$O/CO$_2$ mixtures than with CO$_2$ and methanol/CO$_2$. Again, these analytes were present as anions on the matrix. Because colloidal material of environmental matrices is negatively charged, adsorption of anions is generally deterred.$^{44}$

One-way ANOVA calculations on the methanol/CO$_2$ data (10, 20, 30, and 40 mole % methanol in CO$_2$) showed statistical differences in the recoveries of all but two analytes (phenol and 2,4,6-trichlorophenol), with 20/80 mole % methanol/CO$_2$ producing the highest results for the majority of the analytes. The Kamlet-Taft solvatochromic parameters $\alpha$ and $\beta$ increase by $\sim$20% from 10/90 to 20/80 mole % methanol/CO$_2$, and the solvent strength of 20/80 mole % methanol/CO$_2$ is $\sim$80% that of pure methanol.$^{45}$ Beyond 20/80 mole % methanol/CO$_2$, recoveries tended to drop. Based on solvent strength, this was not expected because the solvent strength continues to increase to that of pure methanol. However, the viscosity of the mixture increases significantly with added methanol such that penetration of inner pore sites may be slowed.$^{45}$

Results with methanol/H$_2$O/CO$_2$ showed improvements with increasing mole % H$_2$O over a greater composition range than methanol/CO$_2$. By one-way ANOVA, 2,4-dimethylphenol, 2,4-dichlorophenol, 4,6-dinitro-$o$-cresol, and pentachlorophenol
showed improved recoveries with increasing H₂O composition, while nitrobenzene
and 2,4-dinitrophenol were adversely affected by increasing the H₂O composition.

Pairwise comparisons (t-test, 95% confidence level) of methanol/CO₂ and
methanol/H₂O/CO₂ mixtures containing 90, 80, and 70 mole % CO₂ revealed that
recoveries were rather modestly improved with methanol/H₂O compared to methanol
alone for most analytes. At 60 mole % CO₂, seven of the eleven analytes had
statistically higher yields with 32.1/7.9/60.0 mole % methanol/H₂O/CO₂ than with
40/60 mole % methanol/CO₂. However, 2,4-dinitrophenol and 4,6-dinitro-o-cresol
were more efficiently extracted with methanol/H₂O/CO₂ than with methanol/CO₂ at all
compositions. The highest recovery of 2,4-dinitrophenol (63%) was found with
9.0/1.0/90.0 mole % methanol/H₂O/CO₂, while 25.4/4.6/70.0 mole %
methanol/H₂O/CO₂ was best for 4,6-dinitro-o-cresol (99%). The somewhat sporadic
yields and lower precisions for these two analytes relative to the others were
previously noted by Lopez-Avila et al.⁴⁶

For the entire analyte set, mixtures consisting of 16.9/3.1/80.0, 25.4/4.6/70.0,
and 32.1/7.9/60.0 mole % methanol/H₂O/CO₂ yielded the highest recoveries in the
shortest periods of time. The average recoveries of all eleven analytes with these
compositions were 70%, 66%, and 69%, respectively. The possible role of H₂O in the
extraction process will be discussed in the next section. Extractions were not
performed with < 50 mole % CO₂ for two reasons. First, the advantages of the low
viscosity fluid such as rapid diffusion into and out of the matrix are expected to be less

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prevalent. Second, with increasing mole % H₂O, drying the sample sufficiently for GC analysis became more challenging.

**Effect of Direct Addition of Modifier over an Extended Temperature Range**

Methanol/H₂O/CO₂ mixtures generally increased recoveries relative to the methanol/CO₂ mixtures tested. The possibility existed, however, that the H₂O alone was enhancing the extraction rather than the methanol/H₂O mixture. Due to the low solubility of H₂O in CO₂ at 238 atm and 25 °C (0.4 mole %), the preparation of H₂O/CO₂ binary mixtures in the syringe pump is not very feasible. A simple and frequently used alternative to binary or ternary mixture preparation is the addition of modifiers directly to the sample in the extraction vessel. A static step is normally employed so that interaction of the modifier, present initially at a high concentration, with the sample is maximized before it is swept out of the vessel. The direct addition of methanol for the extraction of phenolics from spiked florisil was previously reported with reasonable results.

Although the house dust already had a significant moisture content (3.7 wt. % or 2.1 mmoles H₂O), the effect of adding 100 µl H₂O (10% volume/sample weight) prior to extraction was tested. The addition of 100 µl (10% v/w) methanol was also studied. Extraction conditions were CO₂ at 238 atm and 50, 100, and 150 °C. A 10 min static extraction was followed by an 18 ml fraction collection. For comparison to the ternary methanol/H₂O/CO₂ mixtures, the addition of 100 µl (10% v/w) of 50/50
v/v methanol/H₂O solution was also tested at 50 °C. Data for all eleven analytes are given in Table 4.8.

Figure 4.6 shows the % recovery, relative to the spiking level, vs. volume of extraction fluid for 2,4-dichlorophenol with H₂O and methanol added at 50, 100, and 150 °C. In Figure 4.6A, improvement in the extraction rate with H₂O modifier at elevated temperatures may be attributable to the enhanced solubility of H₂O in CO₂ at 238 atm from 0.7 mole % at 50 °C to 2.9 mole % at 100 °C. However, the further increase to ~6 mole % H₂O at 150 °C provided no additional benefit. Overall recoveries were invariant at 66-69%. Experimentally, the amount of restrictor plugging due to ice formation decreased substantially at the higher temperatures. In Figure 4.6B, the addition of methanol lead to improved extraction rates compared to experiments involving the direct addition of H₂O and overall recoveries of 72-76% were found at all three temperatures.

The extraction rate and overall recovery for 2,4-dichlorophenol with 50/50 v/v methanol/H₂O at 50 °C were identical to those with H₂O at 50 °C. The viscosity of methanol/H₂O reaches a maximum at ~40-50 volume % methanol. The addition of methanol/H₂O to the sample resulted in lower recoveries than either methanol or H₂O alone for five of the eleven analytes (see Table 4.8). However, recoveries of 4,6-dinitro-o-cresol and pentachlorophenol were comparable or better than those with methanol or H₂O. When yields with 32.1/7.9/60.0 mole % methanol/H₂O/CO₂ (Table 4.7) are compared, the 50/50 v/v methanol/H₂O static modifier results are
Table 4.8. Average overall % recoveries ± one standard deviation, relative to the spiking levels, of triplicate extractions with CO₂ at 238 atm after the addition of methanol, H₂O, or methanol/H₂O directly to the sample. Surrogates and reference recoveries are the average of all fractions (n=15).

<table>
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<tr>
<th>Analyte</th>
<th>H₂O</th>
<th>Methanol</th>
<th>MeOH/H₂O</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>50 °C</td>
<td>100 °C</td>
<td>150 °C</td>
</tr>
<tr>
<td>Phenol</td>
<td>60 ± 7</td>
<td>68 ± 12</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>56 ± 5</td>
<td>54 ± 11</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>55 ± 3</td>
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<td>41 ± 1</td>
</tr>
<tr>
<td>m-Cresol</td>
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<td>57 ± 9</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>47 ± 5</td>
<td>55 ± 10</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>7.1 ± 0.9</td>
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<td>7.9 ± 2.6</td>
</tr>
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<td>2,4-Dichlorophenol</td>
<td>69 ± 6</td>
<td>66 ± 10</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>72 ± 13</td>
<td>81 ± 30</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>19 ± 7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>47 ± 13</td>
<td>35 ± 12</td>
<td>6.3 ± 0.9</td>
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<tr>
<td>Pentachlorophenol</td>
<td>104 ± 17</td>
<td>116 ± 28</td>
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**Surrogates / Reference**

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<th>150 °C</th>
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<td>2-Fluorophenol</td>
<td>68 ± 6</td>
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<td>66 ± 7</td>
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<tr>
<td>2,4,6-Tribromophenol</td>
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<td>152 ± 33</td>
<td>131 ± 30</td>
<td>122 ± 14</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>124 ± 15</td>
<td>113 ± 8</td>
<td>110 ± 12</td>
<td>125 ± 16</td>
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</table>
Figure 4.6. % Recovery, relative to the spiking level, vs. extraction volume for 2,4-dichlorophenol with (A) 100 μl H₂O and (B) 100 μl methanol added directly to the sample. A 10 min static step was followed by an 18 ml dynamic extraction with CO₂ at 238 atm and (★) 50 °C, (▲) 100 °C, and (●) 150 °C.
Figure 4.6 (continued).
an average of 13% lower. This seems to imply that there is a limit in the amount of 
H₂O that can be added based on the viscosity of the methanol/H₂O mixtures.

Elevated extraction temperatures were previously reported to increase overall 
recoveries by increasing the analyte volatility and providing the energy necessary for 
analyte desorption from the matrix. Temperatures > 100 °C were not tested with 
the binary and ternary extraction fluid mixtures because phase diagram information 
was not determined. However, the maximum extractor temperature of 150 °C was 
tested when the modifier was added directly to the sample. Increasing temperature 
had a negative impact on the recoveries of o-cresol, m-cresol, nitrobenzene, 2,4-
dinitrophenol, and 4,6-dinitro-o-cresol by two-way ANOVA calculations. 
Composition was the more important factor for the other six analytes with all showing 
higher recoveries with methanol addition.

Efficient extraction of chlorophenols (90-99%) from soil was previously 
reported using the direct addition of 10% H₂O followed by a 15 min dynamic 
extraction with CO₂ at 367 atm and 80 °C. Higher recovery of phenol from soil was 
found with 10 wt. % H₂O added than with 6.4 wt. % methanol added. Static extraction 
conditions were CO₂ at 148 atm and 37 °C. In the study described herein, the 
addition of methanol was more advantageous than H₂O for all analytes. This is 
possibly due to the relatively high H₂O content already associated with the matrix. 
Also, the results for the direct addition of methanol or H₂O were in general 10-20%
lower than those obtained with the methanol/\( \text{CO}_2 \) and methanol/\( \text{H}_2\text{O}/\text{CO}_2 \) extraction fluid mixtures (see Tables 4.7 and 4.8).

Therefore, the methanol/\( \text{H}_2\text{O} \) combination in the ternary extraction fluids offers advantages not observed with either modifier alone. \( \text{H}_2\text{O} \) is known to cause significant swelling of clay and plant materials at elevated pressures compared to organic solvents.\(^5\) With swelling, the matrix pore sites become more accessible to the extraction fluid. \( \text{H}_2\text{O} \) may be swelling the house dust matrix and increasing the penetration of methanol or the methanol/\( \text{H}_2\text{O} \) combination to the matrix-analyte interaction sites. \( \text{H}_2\text{O} \) also greatly increases the extraction fluid polarity relative to methanol alone.

**Effect of Varying the Matrix pH**

The pH of the house dust in aqueous solution was 6.5. The effect of varying matrix pH was tested by the addition of 100 \( \mu \text{l} \) of 1 M HCl or 1 M NaOH to the sample prior to extraction. This pH modification procedure was previously reported for the extraction of pesticides from top soil.\(^5\) Following a 10 min static step with \( \text{CO}_2 \) at 238 atm and 50 °C, an 18 ml fraction collection was performed. The acid/base recoveries were compared to those obtained with 100 \( \mu \text{l} \) \( \text{H}_2\text{O} \) added as a neutral pH indicator. The rate of extraction with acid or base was identical to that with \( \text{H}_2\text{O} \).

Figure 4.7 shows overall recoveries for some representative compounds. One-way ANOVA calculations revealed that four analytes (phenol, nitrobenzene,
Figure 4.7. % Recovery, relative to the spiking level, vs. matrix pH for phenol, o-cresol, 2,4-dichlorophenol, and 4,6-dinitro-o-cresol, shown from left to right for each pH condition.
2,4,6-trichlorophenol, and 4,6-dinitro-o-cresol) had statistically identical means with the three conditions. All other analytes showed higher recoveries at neutral pH than at acidic or basic pH.

As discussed previously, the pKₐ values of the analytes range from 4.1 for 2,4-dinitrophenol to 10.6 for 2,4-dimethylphenol. At pH 6.5, 2,4,6-trichlorophenol, 2,4-dinitrophenol, 4,6-dinitro-o-cresol, and pentachlorophenol are expected to be in the anionic form. At acidic pH, all of the analytes are neutral and potentially adsorb strongly to the matrix organic and colloidal matter. However, the solubility of the protonated acids (neutral) in CO₂ is expected to exceed the ionic forms. At high pH, all of the phenols are expected to be anions. Analyte solubility will be lower but hydrolysis of the matrix material may be possible. The possible advantages and disadvantages apparently offset one another and the overall recoveries were better at neutral pH than at either extreme. A similar trend in recoveries with acid and base added was observed in the previous study. Recoveries of eleven pesticides averaged 36% and 46% under acidic and basic conditions and 75% at neutral pH. The lower yields at acidic and basic conditions were attributed to instability of the pesticides due to protonation or hydrolysis.

Another potentially more important factor, however, is the pH of the extraction fluid. H₂O in the presence of CO₂ leads to the formation and dissociation of carbonic acid. A pH range of 2.80-2.95 was recently reported for H₂O/CO₂ at 25-70 °C and 70-
200 atm. Therefore, variations in the matrix pH may be quickly offset by the acidic pH of the extraction fluid.

Finally, the extraction of conjugated or covalently bound 2,4-dichlorophenol from plant material was reported. An acid or base pretreatment period of 4-24 h was required to partially hydrolyze the plant material, leading to recoveries of 18-22% from seeds and ≥ 100% from straw. A similar procedure was tested with the house dust. However, adjusting the matrix pH with HCl or NaOH 15 h prior to extraction provided no improvements in extraction yields.

**Soxhlet Extraction Results**

The % recoveries obtained by 24 h Soxhlet extraction in methylene chloride are listed in Table 4.9. Recoveries ranged from a low of 15% for 2,4-dimethylphenol to a high of 81% for pentachlorophenol. The % RSDs were somewhat poorer than those found by SFE and EFLE but were generally < 30%. For ten of the analytes, an average of 88% of the total quantity recovered was detected in the 0-2 h fraction. Additional recoveries of 11% and 1% were found in the 2-4 h and 4-8 h fractions, respectively. None of the analytes were detected in the 8-24 h fraction. For 2,4-dinitrophenol, recoveries were 27% and 73% for the 0-2 h and 2-4 h fractions, respectively. Overall, a 4 h Soxhlet extraction time would be sufficient for this particular matrix and set of analytes.
Table 4.9. Average % recoveries ± one standard deviation, relative to the spiking levels, of 24 h Soxhlet extractions with methylene chloride (n=12). % Recoveries, relative to the Soxhlet extraction results, for SFE and EFLE are also listed.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Recovery Relative to Soxhlet Yields</th>
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<tbody>
<tr>
<td></td>
<td>Soxhlet</td>
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<tr>
<td>Phenol</td>
<td>74 ± 17</td>
</tr>
<tr>
<td>2-Chlorophenol</td>
<td>54 ± 13</td>
</tr>
<tr>
<td>o-Cresol</td>
<td>42 ± 12</td>
</tr>
<tr>
<td>m-Cresol</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>59 ± 4</td>
</tr>
<tr>
<td>2,4-Dimethylphenol</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td>63 ± 14</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td>79 ± 24</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>52 ± 34</td>
</tr>
<tr>
<td>4,6-Dinitro-o-cresol</td>
<td>41 ± 14</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>81 ± 28</td>
</tr>
</tbody>
</table>

**Surrogates/Reference**
- 2-Fluorophenol: 82 ± 17
- 2,4,6-Tribromophenol: 144 ± 18
- Biphenyl: 121 ± 23
A comparison of extraction yields relative to the Soxhlet results is also made in Table 4.9. All SFE and EFLE experiments listed were performed at 238 atm and 50 °C. Recoveries for the 30 ml, ~70 min supercritical CO₂ extraction were good and represented a considerable time and solvent reduction compared to Soxhlet. When the 18 ml, ~45 min extractions with 10/90 mole % methanol/CO₂ are compared, recoveries declined slightly. The enhanced-fluidity liquid 20/80 mole % methanol/CO₂ and 32.1/7.9/60.0 mole % methanol/H₂O/CO₂ mixtures showed substantially improved results for all but one analyte (2,4-dinitrophenol). These conditions were chosen for comparison to demonstrate the reduction in extraction time with similar yields for 10/90 mole % methanol/CO₂ at near critical conditions, the increased recoveries with 20/80 mole % methanol/CO₂ at enhanced-fluidity liquid conditions, and the benefits of methanol/H₂O/CO₂ compared to methanol/CO₂ and CO₂ alone. But, as shown in Table 4.7, other methanol/H₂O/CO₂ compositions also resulted in higher yields than the methanol/CO₂ conditions listed. The reductions in time and solvent consumption and improvements in overall extraction yields and precision support the use of SFE and EFLE over the traditional Soxhlet method.

**SUMMARY**

The extraction of eleven phenolic pollutants from a wet and adsorptive house dust matrix was investigated. The initial evaluation of extraction temperature, pressure, and a static step with CO₂ and 10/90 mole % methanol/CO₂ showed that the
extraction rate could be greatly improved by the use of a static step with CO₂ or by the addition of methanol to CO₂. However, the average overall recovery of all eleven analytes with 10/90 mole % methanol/CO₂ was only 50%. Next, a range of 10/90 mole % cosolvent/CO₂ mixtures were tested. Highest recoveries were found using polar cosolvents such as methanol/H₂O, ethanol, acetonitrile, and acetone with hydrogen bond donating and/or accepting capabilities. Comparable recoveries were obtained for methanol, ethanol, and 1-propanol mixtures despite the substantially higher viscosity of 1-propanol. This implies that the mass transport properties of CO₂ are dominant and preserved at the 10 mole % cosolvent level. Enhanced-fluidity liquid methanol/CO₂ and methanol/H₂O/CO₂ mixtures provided improved recoveries relative to supercritical CO₂ and methanol/CO₂ extractions and conventional Soxhlet extraction. Recoveries averaged 61% with 20/80 mole % methanol/CO₂ and 69% with 32.1/7.9/60.0 mole % methanol/H₂O/CO₂, for example. The presence of H₂O alone in the extraction fluid did not greatly increase recoveries. H₂O may be swelling the matrix material, allowing more efficient penetration and interruption of matrix-analyte interactions with the very polar methanol/H₂O/CO₂ combination.

Phenol and the methylphenols, with pKₐ values ≥ 10, were difficult to extract. For example, the average recovery of m-cresol at all conditions tested was 57%. Highest yields of these analytes were observed with methanol/H₂O/CO₂ mixtures. 2,4-Dichlorophenol and 2,4,6-trichlorophenol, with pKₐ values nearest the pH of the house dust, were efficiently extracted at nearly all conditions tested. Nitrobenzene, an
analyte that is miscible with CO₂, was recovered rapidly and efficiently with CO₂ alone. 2,4-Dinitrophenol and 4,6-dinitro-o-cresol, with pKₐ values of 4.1 and 4.7, were best extracted with methanol/H₂O/CO₂ mixtures. For the entire analyte set, optimum extraction conditions were enhanced-fluidity liquid methanol/H₂O/CO₂ mixtures containing 60-80 mole % CO₂. Overall, the EFLE technique shows promise for the extraction of polar analytes and/or complex and adsorptive matrices compared to SFE and the conventional Soxhlet extraction technique.
REFERENCES


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CHAPTER 5
EXTRACTION OF PHENOXYACID HERBICIDES FROM HOUSE DUST USING CO₂ AND METHANOL/CO₂ MIXTURES

INTRODUCTION

The extraction of four herbicides, 3,6-dichloro-2-methoxybenzoic acid (dicamba), 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), from house dust is investigated using CO₂ and methanol/CO₂ mixtures at enhanced-fluidity liquid and supercritical conditions. Chemical structures of dicamba, 2,4-D, 2,4,5-TP, and 2,4,5-T are shown in Figure 5.1. Physical properties (formula weights, melting points, pKₐ values, and median lethal dosages (LD₅₀)), tradenames, and metabolites of the selected herbicides are included in Table 5.1.

Applications of Phenoxyacid Herbicides

The introduction of phenoxyacids as herbicides in 1942 represents one of the largest advances in weed control and agriculture. These herbicides are selective for broad-leaf weeds and function similar to auxins, or natural growth hormones.
Figure 5.1. Structures of (A) dicamba, (B) 2,4-D, (C) 2,4,5-TP, and (D) 2,4,5-T.
Table 5.1. Physical properties, tradenames, and metabolites of the phenoxyacid herbicides from references 1-6.

<table>
<thead>
<tr>
<th></th>
<th>FW (g/mole)</th>
<th>mp (°C)</th>
<th>pK_a</th>
<th>LD_{50} (mg/kg)</th>
<th>Tradenames</th>
<th>Metabolites</th>
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<tbody>
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<td>Dicamba</td>
<td>221.04</td>
<td>114-116</td>
<td>1.94</td>
<td>1040</td>
<td>Banex®&lt;sup&gt;®&lt;/sup&gt;</td>
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<td></td>
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<td>175-177</td>
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<td>2,4,5-TCPPA</td>
<td>methyl-2-(2,4,5-trichlorophenoxy)-propanoate</td>
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<td>Weedone</td>
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applied as defoliants, phenoxyacids promote cell elongation, the rapid uptake of H₂O, and eventual breakdown of cells in broad-leaf and woody plants. However, when applied at very precise times, phenoxyacids are beneficial to citrus and some apple crops by preventing preharvest fruit drop, delaying fruit maturity, and increasing fruit size.⁶,⁷

Two of the most widely used phenoxyacid herbicides are 2,4-D and 2,4,5-T. Formulated as a 1:1 mixture of their n-butyl esters, 2,4-D and 2,4,5-T comprised Agent Orange, a military defoliant applied at high concentrations in South Vietnam from 1965-1970.⁶,⁷ Although the pure phenoxyacids cause few adverse effects to humans and wildlife, the synthesis of 2,4,5-T from 2,4,5-trichlorophenol results in the formation of a highly toxic impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), when reaction temperatures exceed 160 °C.⁷ TCDD was present at up to 15 ppm in the Agent Orange formulations applied. The median lethal dosage (LD₅₀) of TCDD is 0.022-0.044 mg/kg, compared to LD₅₀ values of 300-1040 mg/kg for the phenoxyacids (see Table 5.1). TCDD causes skin rashes and liver necrosis upon direct exposure and was believed to contribute to a rise in birth defects in South Vietnam.

2,4,5-TP is used extensively for control of woody plants and aquatic weeds.⁶ Dicamba, although not strictly a phenoxyacid herbicide, is of similar chemical structure and functions much the same way for the control of annual and perennial weeds in grain crops and on pasture land.⁶
The herbicides are generally applied as esters or amine salts and are sprayed onto foliage in H₂O or oil based solutions. Once inside the plants, metabolism occurs via degradation of the acetic acid side chain, hydroxylation of the aromatic ring, or conjugation with plant material forming glucosides of hydroxyphenoxyacetic acids.

The portion of herbicide solution not interacting with plant material is left to degrade in the surroundings. The applied esters or amine salts are rapidly hydrolyzed to the acid forms, particularly in a warm, moist environment. Mechanisms of phenoxyacid breakdown in soils include photodegradation, thermal loss and volatilization, adsorption, and degradation by soil microorganisms. Photodegradative processes, shown in Figure 5.2 for 2,4-D, include conversion to a polymeric humic acid via 2,4-dichlorophenol intermediate, or the formation of 2,4-dihydroxyphenoxyacetic acid which then conjugates with organic material. The herbicide esters with ester carbon chains of ≤ 5 carbons are considered volatile, resulting in loss as the spray formulation dries. At a soil temperature of 60 °C, > 50% of 2,4-D acid was lost in one day and 55% of 2,4,5-T acid was lost within 7 days. The pKₐ values of the phenoxyacids are < 3 so that anions are present at the neutral pHs encountered with many environmental matrices. Soil colloidal material is also negatively charged, deterring adsorption. Organic matter of the soil and pores in inert material provide the principle sites of adsorption and residence. Finally, organisms including bacteria and actinomycetes degrade phenoxyacids via phenol and catechol intermediates to final products of CO₂, H₂O, and chloride ions. When these
Figure 5.2. Photodegradation of 2,4-D on soil resulting in either polymeric humic acid via 2,4-dichlorophenol intermediate, or 2,4-dihydroxyphenoxyacetic acid.
mechanisms of degradation are available, the phenoxyacid herbicides generally persist for periods of $\leq 6$ months.$^{6,7,8}$

The widespread use of the phenoxyacids warranted the establishment of tolerance levels by the U.S. Environmental Protection Agency.$^9$ For example, tolerance levels range from 1000 ppm 2,4-D in grass to 0.05 ppm 2,4-D and dicamba in milk.

**Extraction and Analysis of Phenoxyacids**

The efficient extraction and analysis of phenoxyacids is difficult due to low volatility, high polarity, and the tendency to form dimers.$^{10}$ Liquid-solid extractions including Soxhlet and shake flask techniques are slow and complex for these analytes.$^{11}$ The Soxhlet extraction described in U.S. EPA Method 3540 utilizes either methanol/toluene or acetone/hexane solvent mixtures and requires 8-24 h. As an alternative, U.S. EPA Method 8150A employs a shaking technique with acetone and diethyl ether followed by acidification and liquid-liquid extraction.

A derivatization step, typically to form an ester functionality, is necessary to improve the analyte volatility prior to gas chromatographic analysis.$^9,12,13$ Common reactions include esterification with acid and an alcohol, diazomethane, pentafluorobenzyl bromide (PFBBBr), or boron trifluoride in methanol.$^{12}$ However, disadvantages include long reaction times (up to 3 h using PFBBBr), side
products that make quantitation difficult, and carcinogenic starting materials (particularly diazomethane).\textsuperscript{14,15}

Accelerated solvent extraction (ASE) was recently tested as an alternative to liquid-solid techniques.\textsuperscript{16} ASE utilizes conventional liquid solvents at elevated temperatures and pressures to take advantage of increased analyte solubilities and kinetics of analyte desorption from the matrix at temperatures above the boiling points of the solvents. The extractions are generally performed for \(-12\) min and use \(-15\) ml solvent. The conditions tested were \(1:2\) (v/v) dichloromethane/acetone with \(4\%\) (v/v) \(\text{H}_3\text{PO}_4/\text{H}_2\text{O}\) added at \(136\) atm and \(100\) °C. Extracts were esterified with diazomethane and analyzed by GC with electron capture detection (ECD). Recoveries of eight herbicides from clay, loam, and sand averaged only \(36-72\%\) with \(11-55\%\) RSDs.

The use of an ion pairing/derivatizing reagent for the liquid-solid extraction of phenoxyacids was first reported by Chiang \textit{et al.}\textsuperscript{10,14} The reagent was added to reduce analyte polarity via ion pairing and derivatization, and to displace the analytes from the matrix by covering active sites and preventing readsoption. High yields (95-97\%) were obtained for the extraction of four phenoxyacids from soils using benzyltrimethylammonium chloride (BTMAC) in methanol as the solvent. The analytes were esterified at room temperature during the extraction and no additional derivatization step was required before analysis.

The supercritical fluid extraction of 2,4-D from glass wool was initially reported.\textsuperscript{17} Using \(\text{CO}_2\) at \(197\) atm and \(40\) °C, only \(34\%\) of the 2,4-D was recovered.
The yield improved to 74% when CO₂ saturated with ~1 mole % H₂O was used as the extraction fluid.

SFE of the phenoxyacid herbicides using the in situ ion pairing/derivatizing approach was then investigated. Most of the reagents, discussed below, are quaternary ammonium hydroxides or salts that initially ion pair with phenols, carboxylic acids, amines, and other polar functional groups. The resulting salts decompose and an esterification reaction occurs upon heating in the GC injection port. Thus, the reagent serves a dual purpose, aiding in the extraction of the polar analytes and derivatizing the analytes to a form appropriate for GC analysis.

Hawthorne et al. extracted 2,4-D and dicamba from river sediment and an agricultural soil. Trimethylphenylammonium hydroxide (TMPA) in methanol was added to the sample followed by extraction with CO₂ at 400 atm and 80 °C for 15 min static and 15 min dynamic. Quantitative recoveries (> 90%) were obtained only after three sequential extractions were performed with more TMPA in methanol added between extractions. The 4% and 5% organic content of the matrices, respectively, apparently also interacted with the TMPA, reducing its effectiveness. BF₃/methanol, added to the sample, was shown to methylate 2,4-D but not dicamba. However, when TMPA was added to the extract prior to analysis, dicamba was detected, possibly indicating that the methanol added to the sample promoted the extraction as well.

Rochette et al. extracted 2,4-D from fortified soils by SFE after the addition of static modifiers. Extraction conditions were CO₂ at 300 atm and 80 °C for 10 min
static and 20 min dynamic. Reagents tested include hexamethyldisilazane/trimethylchlorosilane (Tri-sil) for in situ silylation, BF$_3$/methanol for in situ methyl esterification, m-trifluoromethylphenyl trimethylammonium hydroxide (TFTMPA) for ion pairing, and phosphate, HCl, and CaCl$_2\cdot$2H$_2$O in methanol for ionic displacement. Most promising was the use of the strong cation displacing reagent, CaCl$_2\cdot$2H$_2$O in methanol, with ~87% recovery.

Finally, Lopez-Avila et al.$^{20}$ recovered seven phenoxyacids from spiked sand, topsoil, and clay soil with the addition of TMPA, BTMAC, benzyltriethylammonium chloride (BTEAC), and tetrabutylammonium hydroxide (TBA)/methyl iodide for ion pairing, and PFBBr/triethylamine for in situ derivatization, followed by extraction with CO$_2$. Recoveries ranged from 63-96% when TMPA in methanol was added for six of the seven analytes but 2,4,5-T was not detected. Using BTMAC and BTEAC, yields were ≤ 48%. The addition of TBA/methyl iodide followed by extraction with 5% methanol in CO$_2$ at 400 atm and 80 °C for 15 min static and 15 min dynamic was deemed the most useful with yields of 57-141% (recoveries > 100% were apparently due to the extraction of native herbicides from the matrices). However, the extracts could not be analyzed by GC/ECD due to the presence of excess methyl iodide.

Analysis by GC with mass spectral detection resulted in limits of detection of ~10 ng/µl, at least an order of magnitude higher than by GC/ECD.
Goals of this Study

In the study described herein, the extraction of four herbicides (dicamba, 2,4-D, 2,4,5-TP, and 2,4,5-T) from house dust is investigated. As mentioned above, these herbicides generally persist in environmental matrices for $\leq 6$ months when natural degradative pathways are possible. Inside homes and other buildings, the pollutants are potentially trapped for extended periods of time when photodegradation, thermal loss, and microorganisms are reduced or eliminated.

In most of the SFE experiments reported to date, improvements in analyte extraction were attributed to the ion pairing/derivatizing reagent; however, the relative importance of the solvent (most commonly methanol) as an extraction modifier was not thoroughly investigated. The effect of methanol was tested herein by forming binary mixtures of methanol and CO$_2$ in an effort to increase the polarity of the extraction fluid to a level similar to the polarity of the herbicides. CO$_2$ and mixtures consisting of 10, 20, 30, and 40 mole % methanol in CO$_2$ were tested at 442 atm and 25, 50, 100, and 150 °C. TMPA in methanol was added to the extract solutions after extraction to promote methyl esterification in the injection port during GC/ECD analysis. A pre-extraction step with hexane added directly to the sample followed by extraction with CO$_2$ was tested as a means of removing extraneous matrix material. The addition of methanol or TMPA in methanol directly to the sample followed by extraction with CO$_2$ was then used to recover the analytes. Results of these
experiments were compared to those of the previous studies with static modifiers. Soxhlet extractions with methanol were performed as well.

All three factors of the extraction triangle are important in this study. Favorable diffusional properties of the extraction fluids are needed to decrease the time of extraction relative to conventional techniques such as Soxhlet. The solubilities of the polar and relatively high molecular weight analytes in CO₂ are expected to be quite limited based on previous work (discussed in the Results section of this chapter). Analyte solubilities in methanol/CO₂ mixtures should be markedly improved. Finally, interactions between the polar and acidic analytes and the adsorptive house dust matrix are anticipated to be strong primarily due to the high organic and H₂O content of the dust.

EXPERIMENTAL

Materials

2,4-D (98%), 2,4,5-TP (97%), and 2,4,5-T (97%) were purchased from Aldrich (Milwaukee, WI). Dicamba (98.0%), 2,4-D methyl ester (99.5%), 2,4,5-TP methyl ester (99%), 2,4,5-T methyl ester (98.0%), and dicamba methyl ester (99%) were obtained from Chem Service, Inc. (West Chester, PA). 2,4,6-Tribromophenol (99%), used as a surrogate standard, and 1,4-dichlorobenzene (99+%), used as an internal standard, were also obtained from Aldrich. Trimethylphenylammonium hydroxide
Methanol (100.0%, J.T. Baker HPLC grade, Phillipsburg, NJ), acetone (99.6%, Fisher Optima grade, Fairlawn, NJ), and hexane (99.9%, Fisher Optima grade, Fairlawn, NJ) were used as solvents. SFE/SFC grade CO2 without a helium pad was purchased from Air Products and Chemicals (99.9999%, Allentown, PA).

Reagent grade sea sand (Jenniele Enterprises, Cincinnati, OH) was rinsed with acetone and dried at 150 °C overnight prior to use. As discussed in Chapter 4, house dust was collected from door mats inside McPherson and Celeste Laboratories at OSU using a High Volume Small Surface Sampler (HVS3, Cascade Stack Sampling Systems Inc., Bend, OR), a modified Royal residential vacuum cleaner. Particles < 5 µm were excluded by a filter. Large particles such as rocks, paper, and carpet fibers were removed from the collection bottle. The dust contained 30.6 wt. % organic, as determined by thermogravimetric analysis, and 3.7 wt. % H2O, as determined by Karl Fischer titration. The pH of the dust in aqueous solution was 6.5.

**Standard and Sample Preparation**

All glassware was acid rinsed prior to use to avoid adsorption of the phenoxyacids. A 2 mg/ml phenoxyacid stock solution was prepared in methanol. Eight phenoxyacid standards were made across the concentration range of 0.1-20 µg/ml by serial dilution of the stock. To a 1 ml volume of each standard in an
autosampler vial, 100 µl of 0.1 M TMPA in methanol and 5 µg 2,4,6-tribromophenol (surrogate) were added and the final volume was readjusted to 1 ml by gently concentrating with N₂ (~0.2 atm). A 10 µg quantity of 1,4-dichlorobenzene (internal standard) was added prior to analysis. A 2 mg/ml stock solution and eight standards were also prepared using the purchased herbicide methyl esters to determine the efficiency of the phenoxyacid derivatization reaction. All stock and standard solutions were stored at 4 °C when not in use.

A weighed portion of house dust (generally 40 g) was saturated with acetone in an amber glass bottle and fortified at 15 µg/g using the phenoxyacid herbicide stock solution. The sample was thoroughly mixed, then sealed and stored at 4 °C for two weeks before removing most of the solvent so that the dust was just damp. The sample and adsorbed analytes were then mixed daily by shaking the bottle and allowed to equilibrate for a period of ~6 months. This procedure is similar to that previously reported to mimic native samples.²⁸

SFE and EFLE Experiments

A. Comparison of Phenoxyacid and Methyl Ester Solubilities in CO₂. The phenoxyacids or the methyl esters were spiked at 20 µg/g using the 2 mg/ml stock solutions into the center of 1.00 g clean sand contained in a crimped filter paper funnel. The methanol solvent was gently evaporated with N₂ (~0.2 atm) before pouring the sand into the extraction vessel. All extractions were performed using the
SFX™ 220 automated supercritical fluid extractor and a model 260D syringe pump (ISCO, Lincoln, NE) previously described. A 12.5 ml dynamic extraction with CO₂ at 238 atm or 442 atm and 50 °C followed, with fractions collected at 2.5, 5, 7.5, 10, and 12.5 ml so that the 2.5 ml vessel was purged completely during each fraction. The extracts were concentrated to 25-50 μl with N₂ (~0.2 atm), transferred to autosampler vials, refilled to 1 ml with methanol, 100 μl of 0.1 M TMPA in methanol, and 10 μg internal standard added, and analyzed.

B. Extractions with CO₂ and Methanol/CO₂ Mixtures. Methanol/CO₂ mixtures were prepared on a mole fraction basis. The mixtures were pressurized to 442 atm and allowed to mix and equilibrate for at least 12 h. Extractions were performed with mixtures consisting of 10, 20, 30, and 40 mole % methanol in CO₂. All extractions were performed well within a single liquid or supercritical region of the phase diagram. The homogeneity of the mixture was again monitored based on the volume of methanol collected from the extraction chamber following the extraction. Consistent volumes of methanol were collected for each methanol/CO₂ composition.

A 1.000 g sample of dust (weighed to within ± 1 mg of the target weight) was packed between layers of clean sea sand in a 2.5 ml stainless steel extraction vessel. The sand was used as a particle trap to prevent plugging of the 0.5 μm frits used to contain the sample and to fill the void volume of the vessel. Extractions were performed in triplicate with each extraction fluid at a constant pressure of 442 atm and temperatures of 25, 50, 100, and 150 °C. After pressurizing the vessel, a 1 min hold
time was used to allow pump stabilization. A 30 ml dynamic extraction then followed, purging the vessel volume 12 times. In preliminary experiments, additional analyte recoveries with > 30 ml extraction fluid were negligible; therefore, the extraction was considered exhaustive using 30 ml fluid. The flow rate of extraction fluid out of the vessel was maintained at -0.5 ml/min by varying the length of 30 μm i.d. fused silica restrictor (Polymicro Technologies, Inc., Phoenix, AZ) so that a 30 ml dynamic extraction required ~60 min to perform. The extracted analytes were collected in 2 ml methanol, containing 5 μg 2,4,6-tribromophenol (surrogate), in a small vial. To monitor the rate of extraction as well as the overall recoveries, the collection solvent was changed after 2.5, 5, 10, and 20 ml with the extraction allowed to proceed to 30 ml.

To each extract was added 500 μl of 0.1 M TMPA in methanol. The extracts were concentrated to ~25-50 μl with a gentle stream of N₂ (~0.2 atm) and filtered through a small plug of glass wool contained in a disposable pipet. Before analysis, an additional 100 μl volume of 0.1 M TMPA in methanol and 10 μg 1,4-dichlorobenzene (internal standard) were added.

C. Evaluation of Hexane, Methanol, and TMPA in Methanol as Static Modifiers. In an attempt to remove extraneous material from the sample prior to extraction of the herbicides, a 200 μl volume of hexane was added directly to the dust layer in the extraction vessel and an extraction with CO₂ at 238 atm and 50 °C was performed for 5 min static and 5 ml dynamic. The sample was then extracted a second
time with methanol or 0.1 M TMPA in methanol added to the dust to recover the analytes. After the addition of 200 µl methanol or TMPA in methanol, a CO$_2$ extraction at 442 atm and 100 °C was done for 10 min static and 10 ml dynamic. When 500 µl methanol or TMPA in methanol was added, the extraction conditions tested were CO$_2$ at 442 atm and 150 °C for 15 min static and 15 ml dynamic. The analytes were collected in a single vial containing 2 ml methanol and treated in the manner described above for the CO$_2$ and methanol/CO$_2$ extracts. A total of 600 µl TMPA in methanol (static modifier plus volume added after extraction) was used.

**Soxhlet Extractions**

Soxhlet extractions were performed using a micro Soxhlet extractor equipped with a 30 ml flask and a H$_2$O-cooled condenser (Ace Glass Inc., Vineland, NJ). All glassware was acid rinsed prior to use. A 1.000 g dust sample was extracted using 15 ml methanol containing 5 µg 2,4,6-tribromophenol. A 10-15 min solvent recycle time was used. The solvent was replaced after 2, 4, and 8 h, then allowed to proceed to 24 h. The extracts were treated with 0.1 M TMPA in methanol, concentrated, and filtered using the procedure outlined above.

**Extract Analysis**

TMPA was used to ensure that the phenoxyacids were converted to the respective methyl esters via reaction in the hot injection port of the GC.$^{21,22,23,24}$
Analyses were performed using an HP 5890 Series II Plus GC equipped with an electron capture detector (ECD). The split-splitless injection port and ECD were maintained at 250 °C and 300 °C, respectively. A double tapered, deactivated injection liner was installed. After a splitless injection of 1 µl, the inlet purge valve was turned on at 0.75 min. A 30 m x 0.25 mm i.d. (0.25 µm film thickness) DB™-1701 fused silica capillary column (J&W Scientific, Inc., Folsom, CA) was used. The oven program was 50 °C (1 min hold), 20 °C/min to 180 °C (2 min hold), 5 °C/min to 230 °C (0.5 min hold), and 15 °C/min to 280 °C (4.67 min hold) for a total run time of 28.00 min. The analytes of interest eluted from ~180-210 °C, and compounds coextracted from the matrix eluted primarily at higher temperatures. Helium carrier gas was maintained at 20 psi throughout the entire run. Nitrogen was used as the ECD makeup gas and the total flow rate was adjusted to 60 ml/min. Standards prepared from the phenoxyacid stock solution and treated with TMPA were analyzed with each batch of extracts. Linear curve fits with correlation coefficients \( r^2 > 0.99 \) were consistently found for the calibration curves of all four analytes.

Several extracts were also analyzed without the addition of TMPA in methanol to check for \textit{in situ} methyl esterification due to the presence of methanol and carbonic acid (from CO\(_2\) and H\(_2\)O) during the extraction. An HP 5890 Series II Plus GC equipped with an HP 5972 mass selective detector was used. The split-splitless injection port and MS transfer line were maintained at 250 °C and 280 °C, respectively. A 30 m x 0.25 mm i.d. (0.25 µm film thickness) HP-5ms fused silica
capillary column (Hewlett-Packard Co., Wilmington, DE) was used. The oven program was 45 °C (2 min hold), 5 °C/min to 200 °C, then 15 °C/min to 280 °C (3 min hold) for a total run time of 41.33 min. Helium was used as the carrier gas. The methyl esters were carefully identified by retention times and fragmentation patterns. The most abundant fragments for each of the methyl esters were found at m/z ratios of 203 (dicamba), 199 (2,4-D), 196 (2,4,5-TP), and 233 (2,4,5-T).

RESULTS AND DISCUSSION

Derivatization Efficiency

The TMPA reagent was used to convert the acid herbicides to their respective methyl esters and the surrogate standard, 2,4,6-tribromophenol, to its methyl ether. The internal standard, 1,4-dichlorobenzene, was unaffected by TMPA. To determine the derivatization efficiency in the GC injection port, standards were prepared from the purchased phenoxyacids and methyl esters and analyzed by GC/ECD. Retention times, linear curve fits, and correlation coefficients listed in Table 5.2 matched very well. The limits of detection for the four analytes using the GC/ECD analysis method were ~0.3 ng/μl.

When the ratios of peak areas of standard to internal standard (A_s/A_is) were compared for the phenoxyacids and methyl esters, the derivatization efficiencies for the phenoxyacids averaged 125%. The variation from 100% was probably attributable to small differences in phenoxyacid and methyl ester masses in the two stock
Table 5.2. Calibration curves, correlation coefficients ($r^2$), and derivatization efficiencies (± one standard deviation) calculated for the eight standards prepared from the methyl ester and phenoxyacid herbicide stock solutions.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Methyl Ester Equation</th>
<th>$r^2$</th>
<th>Phenoxyacid Equation</th>
<th>$r^2$</th>
<th>Derivatization Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicamba</td>
<td>$y = 0.0346x - 0.0211$</td>
<td>0.9998</td>
<td>$y = 0.0381x - 0.0109$</td>
<td>0.9990</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>2,4-D</td>
<td>$y = 0.1396x - 0.0070$</td>
<td>0.9998</td>
<td>$y = 0.1227x - 0.0285$</td>
<td>0.9977</td>
<td>131 ± 25</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>$y = 0.0370x - 0.0374$</td>
<td>0.9997</td>
<td>$y = 0.0288x - 0.0356$</td>
<td>0.9976</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>$y = 0.0466x + 0.0151$</td>
<td>0.9990</td>
<td>$y = 0.0327x - 0.0053$</td>
<td>0.9995</td>
<td>160 ± 27</td>
</tr>
</tbody>
</table>
solutions. The addition of more TMPA in methanol to the phenoxyacid standards did not increase peak areas, and as expected, the addition of TMPA in methanol to the methyl ester standards did not affect their GC response. Therefore, the derivatization was efficient and complete using a ratio of TMPA to analytes of ~18:1 (w/w). The linear curve fits for the phenoxyacid standards were then used for quantitation.

Although a smaller quantity of TMPA in methanol was probably sufficient for derivatization of the standards, it was suspected that at least 100 µl TMPA in methanol would be required for the extracts due to the presence of extraneous matrix material (30.6 wt. % organic) that could also interact with the TMPA. The volume of 0.1 M TMPA in methanol added to the extracts was chosen by increasing the volume until the ratio of peak areas for the surrogate standard to internal standard for the extracts was comparable to the ratio found for the standards. A total of 600 µl TMPA in methanol (~141:1 (w/w) TMPA to analytes) was required due to the high matrix organic content. Throughout this study, quantitative recoveries of the surrogate standard indicated that analyte losses during extract collection, evaporation with N₂, and filtration were minimal and that the derivatization step during analysis was complete.

**Collection Efficiency and Filtration through Glass Wool**

To determine analyte losses in the collection solvent during the extraction step and the solvent concentration step, a 2 ml volume of methanol containing 10 µg each
herbicide and 5 μg surrogate was purged with 5 ml CO₂ at 238 atm and 50 °C for ~10 min. After the addition of 100 μl TMPA in methanol, the solution was evaporated to ~25-50 μl with N₂, transferred to an autosampler vial with the addition of internal standard, and analyzed. Analyte recoveries, relative to control standards that were not purged or concentrated, are listed in Table 5.3 and ranged from 97-99%.

A filtration step was necessary for the dust extracts because a white, waxy precipitate was present in the methanol solutions. Due to the very active nature of the analytes, losses from filtering through a small plug of glass wool were evaluated by comparing filtered and unfiltered standards. The glass wool plug in a disposable pipet was first rinsed with 2 ml methanol before adding the standard solution. Analyte recoveries, relative to the unfiltered standards, ranged from 87-96% (see Table 5.3). Additional extract clean-up methods such as solid phase extraction (SPE) were not utilized due to the very polar and adsorptive nature of the analytes in the phenoxyacid form.

**Comparison of Phenoxyacid and Methyl Ester Solubilities in CO₂**

An early study on the extraction of 2,4-D from glass wool showed that only 34% of the analyte could be recovered with pure CO₂ at 197 atm and 40 °C.¹⁷ The subsequent SFE experiments focused on reducing the analyte polarity via ion pairing or derivatization to promote extraction.¹⁸,¹⁹,²⁰ However, in two separate studies the solubility of 2,4-D in CO₂ at 40 °C and ~200 atm was reported as ~440 μg/ml and
Table 5.3. Average % recoveries ± one standard deviation, relative to control standards, for collection efficiency and filtration experiments (n=4), and for the extraction of the methyl esters and phenoxyacids from spiked sand using 12.5 ml CO$_2$ at 238 atm and/or 442 atm and 50 °C (n=4).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Collection Efficiency</th>
<th>Filtration through Glass Wool</th>
<th>Methyl Esters 238 atm</th>
<th>Phenoxyacids 238 atm</th>
<th>Phenoxyacids 442 atm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicamba</td>
<td>97 ± 2</td>
<td>96 ± 5</td>
<td>76 ± 4</td>
<td>44 ± 5</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>2,4-D</td>
<td>97 ± 4</td>
<td>87 ± 6</td>
<td>80 ± 6</td>
<td>21 ± 5</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>2,4,5-TP</td>
<td>99 ± 4</td>
<td>96 ± 5</td>
<td>94 ± 5</td>
<td>49 ± 6</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>97 ± 5</td>
<td>92 ± 4</td>
<td>81 ± 9</td>
<td>14 ± 3</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Surrogate</td>
<td>99 ± 5</td>
<td>104 ± 3</td>
<td>99 ± 4</td>
<td>103 ± 7</td>
<td>102 ± 5</td>
</tr>
</tbody>
</table>
-233 µg/ml, respectively.\textsuperscript{25,26} For comparison, the solubility of phenol in CO\textsubscript{2} at 238 atm and 25 °C is \(\sim 28000\) µg/ml.\textsuperscript{30} Still, for the trace levels of pollutants (15 µg/g) studied here, 2,4-D should be sufficiently soluble in CO\textsubscript{2}.

Large differences in the solubility of a related phenoxyacid in the acid and ester forms were previously noted. The solubility of 2-[4-(2,4-dichlorophenoxy)phenoxy]-propanoic acid (diclofop) is \(\sim 400\) µg/ml while the solubility of diclofopmethyl is \(\sim 8200\) µg/ml at 197 atm and 40 °C.\textsuperscript{25} Differences in the solubility of the acid and methyl ester forms of dicamba, 2,4-D, 2,4,5-TP, and 2,4,5-T in CO\textsubscript{2} were tested here at a moderate pressure and temperature of 238 atm and 50 °C using spiked sand. Because the sand was a clean, inert matrix, the analytes were expected to simply form a residue on the surface during the short time (\(\sim 5\) min) allowed between spiking and extraction. Analyte solubility in the bulk extraction fluid was the dominant factor in this experiment.

Plots of % recovery vs. extraction volume for 2,4-D and 2,4,5-TP are shown in Figure 5.3 and the overall % recoveries are included in Table 5.3. The yields of the methyl esters were \(\sim 2-6\) times higher than the respective acid forms using 12.5 ml CO\textsubscript{2} at 238 atm. A longer extraction would be necessary to completely solubilize the acids. Thus, for an extraction with CO\textsubscript{2} alone, ion pairing/derivatizing of the polar, acidic analytes is essential to high yields in a short extraction at mild conditions. However, when the extraction pressure was increased to 442 atm, overall recoveries of the phenoxyacids were \(\sim 2-3\) times higher than at 238 atm (see Table 5.3). Therefore, all

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Figure 5.3. Extraction of (○) 2,4,5-TP methyl ester, (◇) 2,4,5-TP acid, (▲) 2,4-D methyl ester, and (▲) 2,4-D acid from spiked sand using CO₂ at 238 atm and 50 °C.
subsequent herbicide extractions were performed at 442 atm to take advantage of this solubility enhancement at elevated pressure.

SFE and EFLE with CO₂ and Methanol/CO₂ Mixtures

Extraction conditions tested were CO₂ and 10/90, 20/80, 30/70, and 40/60 mole % methanol/CO₂ at 442 atm and 25, 50, 100, and 150 °C. CO₂ is a liquid at 25 °C and a supercritical fluid at temperatures > 31 °C. The critical temperature of 10/90 mole % methanol/CO₂ is ~50 °C; therefore, enhanced-fluidity liquid, near critical, and supercritical conditions were tested. Critical temperatures for the 20/80, 30/70, and 40/60 mole % methanol/CO₂ mixtures are approximately 70 °C, 90 °C, and 140 °C, respectively (see also Chapter 2). Thus, 20/80 and 30/70 mole % methanol/CO₂ at 25 °C and 50 °C and 40/60 mole % methanol/CO₂ at 25, 50, and 100 °C are enhanced-fluidity liquids, and the mixtures are supercritical at 100 °C and/or 150 °C when the respective critical temperatures are exceeded.

The average overall % recoveries ± one standard deviation, relative to the 15 μg/g spiking level, for triplicate 30 ml dynamic extractions are listed in Table 5.4. Recoveries of 2,4,6-tribromophenol, the surrogate standard, ranged from 93-114%, implying that the analyte losses between extraction and analysis were small and derivatization was efficient.

A. Results for Extractions with CO₂. Extraction yields with pure CO₂ were low, ranging from 1-39%. The effect of increasing temperature was quite minimal.
Table 5.4. Average overall % recoveries ± one standard deviation for triplicate supercritical fluid and enhanced-fluidity liquid extractions and six replicate Soxhlet extractions. Methanol/CO₂ mixtures are given on a mole % basis.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Dicamba</th>
<th>2,4-D</th>
<th>2,4,5-TP</th>
<th>2,4,5-T</th>
<th>Surrogate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>13 ± 5</td>
<td>3 ± 1</td>
<td>18 ± 6</td>
<td>1 ± 0.7</td>
<td>107 ± 12</td>
</tr>
<tr>
<td>50 °C</td>
<td>26 ± 10</td>
<td>19 ± 11</td>
<td>18 ± 8</td>
<td>7 ± 4</td>
<td>105 ± 16</td>
</tr>
<tr>
<td>100 °C</td>
<td>24 ± 1</td>
<td>10 ± 1</td>
<td>18 ± 2</td>
<td>6 ± 1</td>
<td>98 ± 6</td>
</tr>
<tr>
<td>150 °C</td>
<td>38 ± 6</td>
<td>20 ± 4</td>
<td>39 ± 6</td>
<td>14 ± 2</td>
<td>99 ± 6</td>
</tr>
<tr>
<td>10/90 MeOH/CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>79 ± 7</td>
<td>40 ± 13</td>
<td>95 ± 13</td>
<td>41 ± 11</td>
<td>112 ± 12</td>
</tr>
<tr>
<td>50 °C</td>
<td>84 ± 14</td>
<td>60 ± 10</td>
<td>102 ± 7</td>
<td>56 ± 4</td>
<td>111 ± 8</td>
</tr>
<tr>
<td>100 °C</td>
<td>86 ± 2</td>
<td>85 ± 9</td>
<td>81 ± 4</td>
<td>76 ± 1</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>150 °C</td>
<td>88 ± 0.3</td>
<td>78 ± 8</td>
<td>99 ± 5</td>
<td>88 ± 10</td>
<td>95 ± 13</td>
</tr>
<tr>
<td>20/80 MeOH/CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>84 ± 8</td>
<td>67 ± 7</td>
<td>103 ± 16</td>
<td>68 ± 7</td>
<td>113 ± 12</td>
</tr>
<tr>
<td>50 °C</td>
<td>98 ± 5</td>
<td>48 ± 9</td>
<td>101 ± 14</td>
<td>38 ± 3</td>
<td>108 ± 12</td>
</tr>
<tr>
<td>100 °C</td>
<td>88 ± 3</td>
<td>87 ± 12</td>
<td>91 ± 4</td>
<td>88 ± 14</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>150 °C</td>
<td>83 ± 2</td>
<td>87 ± 13</td>
<td>89 ± 3</td>
<td>95 ± 8</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>30/70 MeOH/CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>25 °C</td>
<td>105 ± 10</td>
<td>65 ± 18</td>
<td>81 ± 16</td>
<td>60 ± 6</td>
<td>114 ± 13</td>
</tr>
<tr>
<td>50 °C</td>
<td>82 ± 13</td>
<td>60 ± 6</td>
<td>80 ± 9</td>
<td>59 ± 7</td>
<td>108 ± 11</td>
</tr>
<tr>
<td>100 °C</td>
<td>93 ± 4</td>
<td>60 ± 13</td>
<td>81 ± 9</td>
<td>57 ± 13</td>
<td>107 ± 8</td>
</tr>
<tr>
<td>150 °C</td>
<td>84 ± 6</td>
<td>70 ± 7</td>
<td>81 ± 5</td>
<td>75 ± 11</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>40/60 MeOH/CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 °C</td>
<td>85 ± 3</td>
<td>51 ± 3</td>
<td>84 ± 3</td>
<td>51 ± 5</td>
<td>107 ± 8</td>
</tr>
<tr>
<td>50 °C</td>
<td>100 ± 12</td>
<td>66 ± 5</td>
<td>69 ± 12</td>
<td>44 ± 7</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>100 °C</td>
<td>47 ± 5</td>
<td>62 ± 7</td>
<td>37 ± 4</td>
<td>33 ± 5</td>
<td>96 ± 21</td>
</tr>
<tr>
<td>150 °C</td>
<td>50 ± 2</td>
<td>67 ± 8</td>
<td>40 ± 2</td>
<td>35 ± 3</td>
<td>114 ± 10</td>
</tr>
<tr>
<td>Soxhlet Extractions</td>
<td>24 h, MeOH</td>
<td>35 ± 16</td>
<td>48 ± 10</td>
<td>43 ± 13</td>
<td>37 ± 8</td>
</tr>
</tbody>
</table>
although statistically higher recoveries were found at 150 °C compared to 25 °C by t-test at the 95% confidence level (Quattro Pro, version 6.01, Novell Applications Group, Orem, UT) for all four analytes. The CO₂ density decreases from 1.0 g/ml at 25 °C to 0.65 g/ml at 150 °C, leading to a decrease in solvent strength. However, analyte volatility improves with increasing temperature; the melting points of the analytes vary from 114-116 °C for dicamba to 175-177 °C for 2,4,5-TP (see Table 5.1), suggesting that temperatures > 100 °C may positively affect the extraction. Increasing temperature also aids in overcoming the energy of desorption. These factors account for the improved recoveries at 150 °C. However, analyte solubility in CO₂, demonstrated above, is somewhat limited on a short time scale and CO₂ is lacking the solvent strength necessary to disrupt interactions between the polar analytes and the house dust matrix resulting in low extraction yields.

B. Results for Extractions with Methanol/CO₂ Mixtures. As shown in Table 5.4, analyte recoveries improved considerably when methanol/CO₂ mixtures were used. General trends observed with extraction fluid composition, temperature, and analyte structure will be considered. Overall yields with 10/90 mole % methanol/CO₂ ranged from 40-102%. Two of the analytes, dicamba and 2,4,5-TP, were found at consistently high levels regardless of extraction temperature and fluid state (liquid, near critical, or supercritical). In contrast, 2,4-D and 2,4,5-T were more efficiently extracted at 100 °C and 150 °C (supercritical). Highest recoveries for the entire analyte set were found using 20/80 mole % methanol/CO₂ at either 100 °C or
150 °C, where the yields ranged from 83-95%. With 30/70 mole % methanol/CO₂, recoveries of dicamba and 2,4,5-TP were still relatively high, but yields of 2,4-D and 2,4,5-T were lower than with 20/80 mole % methanol/CO₂ at 100 °C and 150 °C. With the exception of dicamba and 2,4,5-TP at 25 °C and dicamba at 50 °C, yields for all four analytes were significantly lower using 40/60 mole % methanol/CO₂ at both enhanced-fluidity liquid and supercritical conditions.

The differences in recoveries based on analyte structures are also shown graphically in Figure 5.4. Recoveries of dicamba and 2,4,5-TP (shown in Figure 5.4A) were roughly constant using 10/90, 20/80, and 30/70 mole % methanol/CO₂ regardless of temperature. Extraction yields of 2,4-D and 2,4,5-T (shown in Figure 5.4B) improved with increasing temperature and were generally maximized using 10/90 or 20/80 mole % methanol/CO₂ at supercritical conditions. The acid side chains of dicamba and 2,4,5-TP are sterically hindered by other substitutents, possibly limiting the extent of interactions with the matrix adsorptive sites. In contrast, the acid side chains of 2,4-D and 2,4,5-T are more accessible for matrix-analyte interactions, making their extraction more difficult.

C. Statistical Comparison of Results. Two-way analysis of variance (ANOVA) calculations at the 95% confidence level were performed using Systat for Windows, version 5.0 (Systat, Inc., Evanston, IL) with extraction fluid composition and temperature as the factors. When CO₂ data were included in the calculations, composition was the more important factor for all analytes. Without CO₂, temperature
Figure 5.4. Average overall % recoveries, relative to the spiking level, vs. mole % methanol in the extraction fluid for (A) 2,4,5-TP and (B) 2,4,5-T at 442 atm and 25, 50, 100, and 150 °C, shown from left to right for each composition. Error bars signify one standard deviation.
Figure 5.4 (continued).
was the more important factor for 2,4-D while composition was still the more important factor for the other three analytes. Composition, temperature, and the cross term between composition and temperature were all significant based on the F-ratio for all four analytes. The cross term was similar in magnitude to temperature for dicamba and 2,4,5-TP, but considerably smaller than temperature for 2,4-D and 2,4,5-T.

The t-test at the 95% confidence level was used to detect statistical differences between the apparent optimum extraction conditions (i.e., 10/90 and 20/80 mole % methanol/CO₂ at elevated temperatures). For the temperature dependent analytes (2,4-D and 2,4,5-T), extraction yields were lower at 50 °C than at 100 °C and 150 °C with both 10/90 and 20/80 mole % methanol/CO₂. Recoveries at 100 °C and 150 °C were different for 2,4,5-TP using 10/90 mole % methanol/CO₂ but identical for the other three analytes with 10/90 mole % methanol/CO₂ and for all four analytes with 20/80 mole % methanol/CO₂. When 10/90 and 20/80 mole % methanol/CO₂ were compared at the higher temperatures, the 20/80 mole % methanol/CO₂ mixture produced a higher recovery for 2,4,5-TP at 100 °C, 10/90 mole % methanol/CO₂ was more efficient for dicamba and 2,4,5-TP at 150 °C, and 10/90 and 20/80 mole % methanol/CO₂ gave equivalent yields for the other analytes at 100 °C and/or 150 °C. Therefore, overall recoveries with 10/90 and 20/80 mole % methanol/CO₂ at temperatures of 100 °C and 150 °C were generally comparable and higher than with the other conditions tested.
Experimentally, 20/80 mole % methanol at 100 °C and 150 °C was much easier to use than 10/90 mole % methanol/CO₂ at the same temperatures. When 10/90 mole % methanol/CO₂ was used at supercritical conditions, the fused silica restrictor became brittle and often broke during the 30 ml extraction. Although the cause is unknown, this problem with 10/90 mole % methanol/CO₂ is routinely encountered by other researchers in our group as well. Restrictor breakage was not observed with any of the other extraction fluids or temperatures tested (including 10/90 mole % methanol/CO₂ at liquid conditions). Freezing at the restrictor tip was also greatly reduced as the proportion of methanol (≥ 20%) in the extraction fluid was increased, resulting in a more steady and reproducible flow rate.

The addition of increasing proportions of methanol to CO₂ is expected to increase the polarity and solvent strength of the resulting methanol/CO₂ mixtures eventually to that of pure methanol. Yet, recoveries were highest with 20/80 mole % methanol/CO₂ indicating that solvent strength is not the only factor influencing the extraction. According to measured Kamlet-Taft solvatochromic parameters, α and β (defining hydrogen bond donating and accepting capabilities of the solvent), the solvent strength of 20/80 mole % methanol/CO₂ is ~80% that of pure methanol.³³ With mixtures containing higher proportions of methanol, the viscosity of the mixtures increases rather substantially such that penetration of inner pore sites may be less efficient. For example, the viscosity increases from 0.09 cP with pure CO₂ to ~0.13 cP with 20/80 mole % methanol/CO₂ and ~0.20 cP with 40/60 mole %
methanol/CO₂ at 170 atm and 25 °C. The viscosity of pure methanol at these
conditions is 0.57 cP.¹³

D. Comparison of Extraction Rates. The extraction rates were also evaluated.
In Figure 5.5, the effect of temperature on the extraction rate of dicamba using 10/90
and 30/70 mole % methanol/CO₂ is shown. Using 10/90 mole % methanol/CO₂
(Figure 5.5A), the rate of extraction improved significantly upon increasing the
temperature from 25 °C to 50 °C; the rate of extraction improved modestly with
further increases to 100 °C and 150 °C. The extraction rates were nearly identical at
the four temperatures using 20-40 mole % methanol in CO₂ and were comparable to
the rates at 100 °C and 150 °C in Figure 5.5A. This is shown in Figure 5.5B using
30/70 mole % methanol/CO₂. Although extractions with near critical and supercritical
fluids were faster than with the enhanced-fluidity liquid for 10/90 mole %
methanol/CO₂, the rates were virtually independent of state (liquid or supercritical) for
30/70 mole % methanol/CO₂ where the extraction fluid solvent strength was high.

The variation in rate with extraction fluid composition is depicted in Figure 5.6
for 2,4-D at 25 °C and 150 °C. At 25 °C (Figure 5.6A), the rates increased with
increasing proportions of methanol, implying that the solvent strength was also
increased. At 150 °C (Figure 5.6B), extraction rates were considerably improved
compared to 25 °C and were nearly identical for the four methanol/CO₂ mixtures.

These results clearly show that differences in extraction rates were not the
cause of the diminished yields for 30/70 and 40/60 mole % methanol/CO₂.
Figure 5.5. % Recovery, relative to the spiking level, vs. extraction volume for dicamba using (A) 10/90 mole % methanol/CO$_2$ and (B) 30/70 mole % methanol/CO$_2$ at 442 atm and (●) 25 °C, (▲) 50 °C, (●) 100 °C, and (▼) 150 °C.
Figure 5.5 (continued).
Figure 5.6. % Recovery, relative to the spiking level, vs. extraction volume for 2,4-D at 442 atm and (A) 25 °C and (B) 150 °C using (+) CO₂, (▲) 10/90 mole % methanol/CO₂, (●) 20/80 mole % methanol/CO₂, (▼) 30/70 mole % methanol/CO₂, and (♦) 40/60 mole % methanol/CO₂.
Figure 5.6 (continued).
Extractions were rapid with 30/70 mole % methanol/CO₂ at both enhanced-fluidity liquid and supercritical conditions (Figure 5.5B), and extraction rates were comparable or better with increasing methanol compositions at a constant temperature (Figure 5.6). Again, inaccessibility to inner pore sites due to increased mixture viscosity may be causing the lower overall yields.

**GC/MS Analysis of the House Dust Extracts**

A common methyl esterification method for phenoxyacids involves refluxing in methanol and acid.¹² Matrix H₂O in the presence of CO₂ likely leads to the formation of carbonic acid during the extraction. A pH range of 2.80-2.95 was recently reported for H₂O/CO₂ at 70-200 atm and 25-70 °C.¹⁴ Therefore, the extractions with methanol/CO₂, particularly at the elevated temperatures, potentially provided the necessary conditions for *in situ* methyl esterification. To test this possibility, extractions were performed with 20/80 mole % methanol/CO₂ at 442 atm and 100 °C using a 10 ml dynamic extraction and the extracts were collected in a single vial. The extracts were then analyzed by GC/MS before and after the addition of TMPA in methanol. Before TMPA in methanol was added, recoveries of the methyl esters were 0%, 3%, 10%, and 16% for 2,4,5-TP, dicamba, 2,4-D, and 2,4,5-T, respectively, for three replicates. After the addition of TMPA for methyl esterification in the hot injection port of the GC, analyte recoveries ranged from 71-97%. A large chromatographic peak for N,N-dimethylaniline (most abundant fragment, m/z = 121)
was also observed, indicating that TMPA gave up a methyl group in the esterification reaction.\textsuperscript{10}

However, \textit{in situ} methyl esterification cannot be ruled out as a mechanism aiding the extraction because the presence of H\textsubscript{2}O in the extract solution after dissociation of carbonic acid may lead to rapid hydrolysis of the esters back to the acid form.\textsuperscript{9} Thus, the TMPA reagent was necessary for the efficient derivatization of the phenoxyacids during GC analysis. It was also reported that the pK\textsubscript{a} values of the herbicides increase from < 3 in H\textsubscript{2}O to \textasciitilde 7 in methanol.\textsuperscript{4} The result is an increased abundance of the nonionic acid forms of the analytes.\textsuperscript{35} By lowering the extraction fluid pH with the formation of carbonic acid, the phenoxyacids should exist primarily in the nonionic form, potentially making the analytes more soluble and readily extractable with polar fluids such as the methanol/CO\textsubscript{2} mixtures.

\textbf{Soxhlet Extractions}

Because methanol was the only cosolvent tested in the SFE and EFLE experiments, pure methanol was used as the Soxhlet extraction solvent (rather than the methanol/toluene mixture described in U.S. EPA Method 3540)\textsuperscript{11} for comparison. Results for six replicate extractions are listed in Table 5.4. Relative to the 15 \textmu g/g spiking level, recoveries ranged from 35-48\% with poor precisions. The surrogate recovery was low at 82\% and did not improve with the addition of more TMPA, implying that the remainder was lost during the extraction or concentration step. The
rate of extraction was also tested; 71% of the recovered analytes were detected in the 0-2 h fraction, 21% in the 2-4 h fraction, 6% in the 4-8 h fraction, and only 2% in the 8-24 h fraction. Therefore, an extraction time of 8 h would be sufficient for this particular application. In contrast, methanol/CO₂ extractions at 25 °C (see Table 5.4) resulted in recoveries that were as much as twice those obtained by Soxhlet and were complete in ~1 h.

Evaluation of Hexane, Methanol, and TMPA in Methanol as Static Modifiers

A. Pre-extraction with Hexane and CO₂. The house dust matrix consisted of 30.6 wt. % organic material. Coextracted matrix material resulted in the use of a large excess of TMPA derivatizing reagent, a filtering step to remove a waxy precipitate from the methanol collection solvent, and the presence of numerous chromatographic peaks that may shorten the column lifetime and require cleaning of the injection liner and ECD. A pre-extraction step, selective for the extraneous material, prior to recovery of the phenoxyacids would be beneficial. Extractions with pure CO₂ yielded little extraneous material compared to extractions with methanol/CO₂. Therefore, hexane was tested as a static modifier since it is nonpolar and the phenoxyacids are not readily soluble in it. The house dust was first treated with 200 µl hexane and extracted at mild conditions using CO₂ at 238 atm and 50 °C. A 5 min static step was followed by a 5 ml dynamic extraction. To recover the analytes, the sample was then treated with methanol or TMPA in methanol and extracted with CO₂ at 442 atm. Extractions
were performed using 200 µl modifier at 100 °C for 10 min static and 10 ml dynamic, and with 500 µl modifier at 150 °C for 15 min static and 15 ml dynamic.

Figure 5.7 shows GC/ECD chromatograms for the extraction of house dust with 500 µl methanol at 150 °C without a pre-extraction step (Figure 5.7A), the pre-extraction step with 200 µl hexane added (Figure 5.7B), and the extraction with 500 µl methanol at 150 °C after pre-extraction (Figure 5.7C). Qualitatively, the removal of analytes during the pre-extraction step (Figure 5.7B) is minimal, and the number of extraneous peaks in Figure 5.7C is greatly reduced relative to Figure 5.7A although the analyte peak heights and areas are comparable.

B. Quantitative Results for Extractions with Static Modifiers. Analyte recoveries in the pre-extraction and extraction steps, relative to the 15 µg/g spiking level, are listed in Table 5.5. The pre-extraction step with hexane yielded ≤ 4.9% of the analytes. Extractions with 200 µl methanol or TMPA in methanol added recovered only 27-68% of the analytes. Furthermore, recoveries with methanol and TMPA in methanol were statistically identical, indicating that the addition of TMPA as an ion pairing reagent did not enhance analyte extraction. Extractions with 500 µl methanol or TMPA in methanol added yielded 29-59% of the analytes. Dicamba was more efficiently extracted with methanol while extractions with methanol and TMPA in methanol were statistically identical for the other three analytes. Additionally, recoveries with 200 µl and 500 µl methanol were identical as were those with 200 µl and 500 µl TMPA in methanol, with the exception of dicamba due to the low recovery
Figure 5.7. GC/ECD chromatograms of the extracts from (A) extraction with 500 μl methanol added without a pre-extraction step, (B) pre-extraction with 200 μl hexane added, and (C) extraction with 500 μl methanol added after pre-extraction. Chromatographic peaks are identified as (1) 1,4-dichlorobenzene (internal standard), (2) dicamba methyl ester, (3) 2,4,6-tribromophenol methyl ether (surrogate standard), (4) 2,4-D methyl ester, (5) 2,4,5-TP methyl ester, and (6) 2,4,5-T methyl ester.
Table 5.5. Average % recoveries ± one standard deviation for 12 CO₂ pre-extractions with hexane added and triplicate CO₂ extractions with methanol and 0.1 M TMPA in methanol added directly to the sample.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Dicamba</th>
<th>2,4-D</th>
<th>2,4,5-TP</th>
<th>2,4,5-T</th>
<th>Surrogate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane Pre-extraction</td>
<td>1.3 ± 1.1</td>
<td>4.9 ± 1.6</td>
<td>1.5 ± 0.4</td>
<td>1.3 ± 0.8</td>
<td>93 ± 6</td>
</tr>
<tr>
<td>200 μl Modifier, 100 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>68 ± 11</td>
<td>28 ± 3</td>
<td>48 ± 5</td>
<td>36 ± 5</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>TMPA in Methanol</td>
<td>63 ± 7</td>
<td>27 ± 5</td>
<td>56 ± 12</td>
<td>34 ± 5</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>500 μl Modifier, 150 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>59 ± 6</td>
<td>29 ± 3</td>
<td>55 ± 12</td>
<td>36 ± 4</td>
<td>106 ± 12</td>
</tr>
<tr>
<td>TMPA in Methanol</td>
<td>30 ± 7</td>
<td>33 ± 14</td>
<td>40 ± 12</td>
<td>41 ± 18</td>
<td>99 ± 6</td>
</tr>
</tbody>
</table>
with 500 µL TMPA in methanol. These results imply that the presence of TMPA, the extraction temperature, and the length of the static and dynamic steps had no significant impact on the overall yields. It is expected that once the modifiers were purged from the vessel early in the dynamic extraction, little additional recovery of analytes occurred with the CO₂ extraction fluid.

The conditions tested with the static addition of methanol and TMPA in methanol were similar to those previously reported, allowing direct comparison with those studies and with the methanol/CO₂ results discussed earlier. The recoveries with static modifiers found in this study were comparable to those reported by Hawthorne et al. (23%) for a single extraction of 2,4-D from spiked river sediment. In their work, two additional extractions were required for quantitative yields. Rochette et al. obtained lower recoveries (14-19%) for 2,4-D with TFTMPA added. Lopez-Avila et al. recovered six of seven herbicides using TMPA in methanol with comparable or somewhat higher yields (63-96%) although 2,4,5-T was not detected. Results reported herein show that methanol was responsible for the analyte extraction and the presence of TMPA ion pairing reagent was inconsequential to the overall recoveries, at least for this particular matrix. The use of methanol/CO₂ binary mixtures proved to be a better alternative to static modifiers in this study. Recoveries were up to three times higher with methanol/CO₂ extraction fluids than with methanol or 0.1 M TMPA in methanol added directly to the sample (see Tables 5.4 and 5.5).
Therefore, the ideal extraction conditions for the recovery of phenoxyacids from house dust found in this study involve a CO₂ pre-extraction step with hexane added directly to the sample to remove extraneous material, followed by recovery of the analytes with 20/80 mole % methanol/CO₂. These steps were not combined herein, however, because either two extraction pumps (one for CO₂ and the other for the methanol/CO₂ mixture) or an efficient and reproducible means of mixing large proportions of methanol with CO₂ supplied from two pumps (not currently available) would be required. Still, the pre-extraction technique is an attractive alternative to reduce subsequent sample clean-up steps (reducing solvent waste) and improve chromatographic performance (both in terms of column lifetime and cleanliness of the injection liner and detector).

**SUMMARY**

A simple and efficient method for the extraction of phenoxyacid herbicides from house dust was developed using methanol/CO₂ mixtures. Optimum conditions were found to be 20/80 mole % methanol/CO₂ at 442 atm and 100 °C or 150 °C with recoveries ranging from 83-95%. The use of methanol/CO₂ mixtures eliminated the need for sequential extractions by supplying a constant composition of polar cosolvent throughout the entire extraction. TMPA was added to the extracts and the phenoxyacids were derivatized to the methyl esters predominately in the GC injection port, avoiding the use of hazardous reagents such as diazomethane. Analysis by
GC/ECD resulted in limits of detection of ~0.3 ng/μl, at least an order of magnitude better than by GC/MS. A simple pre-extraction step with CO₂, using hexane as a static modifier, was also studied as a means of reducing the extraneous matrix material present in the extract. The use of methanol and TMPA in methanol as static modifiers showed that the presence of the TMPA ion pairing reagent did not enhance the extraction of analytes from this particular matrix. Moreover, the methanol/CO₂ mixtures yielded recoveries up to three times greater than with methanol or TMPA in methanol added directly to the sample and twice those obtained by Soxhlet extraction.
REFERENCES


CHAPTER 6

ALTERNATIVE APPROACHES TO THE EXTRACTION
OF SOLID AND LIQUID ENVIRONMENTAL MATRICES

INTRODUCTION

Supercritical fluid extraction (SFE) was developed as a direct alternative to conventional liquid-solid extraction techniques such as Soxhlet and sonication. As discussed in Chapter 1, frequently cited advantages of SFE include shorter extraction times, reduced solvent use, and the potential for analyte selective extractions by varying the pressure, temperature, or modifier used. Over the past five years, the shortcomings of SFE and modified SFE experiments with CO₂ and organic cosolvents were illuminated. Low extraction yields of many polar and/or high molecular weight analytes were encountered, particularly from wet and adsorptive matrices. Technical problems such as restrictor plugging and inefficient trapping of the analytes after extraction were also observed. Finally, the large number of experimental variables, initially seen as an advantage, slowed the practical use of SFE because standard extraction conditions for a range of analytes and matrices were not established, thereby requiring a method development step for each sample.
Alternative approaches, such as enhanced-fluidity liquid extraction (described in Chapters 3-5), are being investigated. This literature review discusses other alternatives that are currently in use as an attempt to “bridge the gap” between liquid extraction techniques and SFE for both solid and liquid matrices. Experimental approaches are categorized herein based on whether changes were made to the extraction fluid, the analytes, the matrix, or the experiment/instrumentation. Positive and negative attributes of each technique are considered.

“BRIDGING THE GAP”

Altering the Extraction Fluid

As previously described, CO₂ is the most widely used extraction fluid because it is inert, nontoxic, available in high purity, and has a low critical temperature (31.1 °C) and pressure (72.9 atm). However, CO₂ lacks a permanent dipole moment and has a polarity comparable to liquid pentane or hexane. Possible alternative fluids are limited because of chemical reactivity (N₂O), environmental hazards (Freons), or critical temperatures and pressures too high for convenient use (H₂O and organic solvents such as methanol). Despite these apparent disadvantages, the search for a polar extraction fluid warranted their investigation and several are discussed here. Critical properties and dipole moments of these fluids are listed in Table 6.1. Enhanced-fluidity liquid extraction would also be included in this category.
Table 6.1. Critical properties and dipole moments from reference 1 of the extraction fluids discussed.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>$T_c$ (°C)</th>
<th>$P_c$ (atm)</th>
<th>Dipole Moment (debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$</td>
<td>31.1</td>
<td>72.9</td>
<td>0</td>
</tr>
<tr>
<td>N$_2$O</td>
<td>36.5</td>
<td>71.7</td>
<td>0.17</td>
</tr>
<tr>
<td>Freon-22 (CHClF$_2$)</td>
<td>96</td>
<td>48.5</td>
<td>1.29</td>
</tr>
<tr>
<td>Freon-23 (CHF$_3$)</td>
<td>25.9</td>
<td>46.9</td>
<td>1.65</td>
</tr>
<tr>
<td>Methanol</td>
<td>240</td>
<td>78.5</td>
<td>1.70</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>374.1</td>
<td>218.3</td>
<td>1.85</td>
</tr>
</tbody>
</table>
A. Nitrous Oxide. Nitrous oxide, N₂O, was one of the first tested alternatives to CO₂. N₂O has similar critical properties but is slightly more polar with a small, permanent dipole moment of 0.17 D.¹ In an early application, 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) was extracted from sediment.⁵ Using identical extraction conditions, recoveries with N₂O and CO₂ averaged 91% and 48%, respectively. However, when 2% methanol was added to N₂O and CO₂, recoveries improved to 100% and 93%. N₂O was tested in numerous other studies as well.⁶⁻¹³ In most cases, extraction rates with N₂O were moderately faster than with CO₂ but overall recoveries were identical. N₂O should be avoided when matrices have a high organic content due to the danger of oxidation and explosion. The small advantages achieved with this fluid clearly do not warrant the risk and most researchers now refrain from using it.

B. Freon-22. Chlorodifluoromethane or Freon-22 is nontoxic, nonflammable, and has a large dipole moment of 1.29 D. But, it is quite expensive and has a relatively high critical temperature of 96 °C.¹ Freon-22 is believed to be less ozone depleting than Freon-11 and Freon-12, but still damaging because it contains chlorine. Seven steroids including cortisone (shown in Figure 6.1) were extracted using CO₂ and Freon-22 with recoveries of 16% and 100%, respectively.¹⁴ PAHs and nitrated PAHs were recovered from diesel exhaust particulate matter more efficiently with Freon-22 than with CO₂ or 10% methanol in CO₂, although results with 10% toluene in CO₂ were comparable.¹⁵ Other examples where Freon-22 yielded higher recoveries than
Figure 6.1. Structures of selected analytes discussed in the text: (A) cortisone, (B) sulfometuron methyl, (C) chlorsulfuron, and (D) morphine.
CO₂ and N₂O include the extraction of polychlorinated biphenyls (PCBs) from river sediment, polycyclic aromatic hydrocarbons (PAHs) from petroleum waste sludge, elemental sulfur from bituminous coal, and α-pinene from pine needles. The stronger interaction between the polar extraction fluid and matrix H₂O was credited with the increased recoveries.

C. Freon-23. Trifluoromethane, also known as fluoroform or Freon-23, has a large dipole moment of 1.65 D, a critical temperature and pressure lower than those of CO₂, and is believed to be less hazardous to the atmosphere than Freon-22 because it does not contain chlorine. Freon-23 was evaluated for the extraction of sulfonyl urea herbicides and PAHs. Despite the polar nature of the extraction fluid, sulfometuron methyl and chlorsulfuron (shown in Figure 6.1) were recovered from spiked Celite at only 43% and 52%, respectively. Recoveries with pure CO₂ were 30% lower, although > 90% yields were obtained with 2% methanol in CO₂. Freon-23 was 15-30% less efficient for the removal of PAHs from clay than either CO₂ or Freon-22. Recoveries of polar pesticides including triazines, OPPs, and carbamates from glass beads averaged 15% higher with Freon-23 than with CO₂.

D. Methanol. Methanol is a common liquid solvent with a large dipole moment of 1.70 D. The high critical temperature of 240 °C generally deters the use of methanol as a supercritical fluid. However, supercritical methanol was tested in one study for the recovery of bound (nonextractable) ¹⁴C pesticide residues from soils and plants. Recoveries were generally > 60% and were comparable to or better than a
high temperature distillation technique. But, reactions between methanol and some of
the pesticides or their metabolites occurred at the 250 °C extraction temperature used
(for example, atrazine was converted to its methoxy analog).

E. Subcritical and Supercritical H₂O. Hawthorne and coworkers recently
demonstrated the feasibility of using H₂O as an extraction fluid in the subcritical liquid
or gaseous state.¹⁹,²⁰ The critical temperature and pressure of H₂O are 374.1 °C and
218.3 atm, respectively.¹ Supercritical H₂O is corrosive in the presence of oxygen
leading to both analyte degradation and instrumental problems. For this reason,
Hawthorne's work centered mostly around milder, subcritical conditions.

The high polarity of H₂O is reflected by the large dielectric constant, ε, of 79 at
ambient conditions. At the critical point however, ε is ~5-15 which is comparable to
liquid methylene chloride, making supercritical H₂O an attractive solvent for nonpolar
organic pollutants.¹⁹,²⁰ For example, the solubility of benzo[e]pyrene in H₂O at 99 atm
increased ~25 million-fold by elevating the temperature from 25 °C to 350 °C.¹⁹
Extracted analytes were collected in a few milliliters of chloroform with the exiting
H₂O extraction fluid forming a layer above the chloroform in the collection vial. After
initially separating the liquid layers, the H₂O layer was extracted two more times with
chloroform, and the combined chloroform extract was analyzed.

The variation in dielectric constant with temperature made class selective
extractions based on polarity possible. Chlorinated phenols, PAHs, and alkanes were
fractionated from an urban air particulate and a certified soil by raising the temperature

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from 50 °C to 250 °C to 400 °C. The dielectric constant decreases from 71 to 29 to 8 at the three temperatures.\textsuperscript{19} PCBs were recovered from an industrial soil and a river sediment at trace levels at 50-100 °C but quantitatively at 250-300 °C in 15 min at liquid conditions and in 5 min with steam at 300 °C.\textsuperscript{20}

This technique appears promising. H\textsubscript{2}O is an inexpensive, environmentally sound extraction solvent. Because the extraction fluid polarity is controllable with temperature, analytes of a given polarity can be recovered selectively from other analytes and extraneous matrix material. However, most standard SFE instruments currently available do not operate at temperatures > 150 °C and the possibility of corrosion due to oxidation is a considerable risk. The extra liquid-liquid extraction step to recover the analytes in the collection vial is also both time and solvent consuming.

\textbf{Altering the Analytes}

When the analytes have low solubilities in CO\textsubscript{2} or the matrix-analyte interactions are strong, the addition of a reagent capable of chemical derivatization, complexation, or ion pair formation is a viable new approach. The reagent works either by modifying the analytes so that the resulting compounds are more soluble and/or less polar, or by interacting with adsorptive sites on the matrix thereby displacing the analytes and preventing readsorption. Thus, some of the examples cited in this section are also applicable to the next section where altering the matrix is
This technique was first applied to organic pollutants and was termed supercritical fluid derivatization and extraction (SFDE).\textsuperscript{21} Recently, the methodology was extended to organometallic compounds and metal ions as well, making many of these analytes CO\textsubscript{2} extractable for the first time.

\textit{A. Organic Analytes.} Tri-sil silylation reagent, a 2:1 (v/v) mixture of hexamethyldisilane and trimethylchlorosilane, was one of the first reagents tested. The extractions of caffeine from roasted coffee beans and Japanese tea,\textsuperscript{21} lipophilic material (fatty acids and terpines) from marine sediment,\textsuperscript{21} PAHs from harbor sediment and urban dust standard reference materials,\textsuperscript{22} and 2,4-dichlorophenoxyacetic acid (2,4-D), a broadleaf weed control herbicide, from sand and soil\textsuperscript{23} were studied. The PAH recoveries were six times higher with Tri-sil added than with CO\textsubscript{2} alone and two times higher than with 10\% methanol added. However, low yields of 2,4-D (31\%) were attributed to the interference of matrix moisture in the silylation reaction.

Neat N,O-bis-trimethylsilyltrifluoroacetamide (BSTFA, shown in Figure 6.2) was added to fly ash for the extraction of PAHs, halogenated phenols and aromatics, and dioxins.\textsuperscript{24} Although some of the analytes do not form trimethylsilyl esters, all recoveries were enhanced in the presence of BSTFA relative to CO\textsubscript{2} alone or with methanol added to the sample.

Acetic anhydride, TEA, and H\textsubscript{2}O were used to recover chlorophenols from fortified and contaminated soils,\textsuperscript{25} catechols, guaiacols, vanillins, and syringols from sediments,\textsuperscript{26} and pentachlorophenol (PCP) from leather.\textsuperscript{27,28} The derivatization was
Figure 6.2. Structures of selected reagents discussed in the text: (A) BSTFA, (B) NaDDC, (C) FOD, and (D) t-butyl substituted dibenzobistriazolo-crown ether.
most effective for the highly chlorinated phenols like PCP but was amenable to di- and trichlorophenols as well. Acetic anhydride and pyridine were used to extract o-, m-, and p-cresol from soil as ethers.\textsuperscript{29,30}

A variety of reagents were tested for the SFDE of phenoxyacid herbicides. Phenoxyacids are prime candidates for this technique because derivatization to a more volatile and less polar form is required prior to GC analysis. Boron trifluoride,\textsuperscript{23,31} pentafluorobenzylbromide (PFBB\(_\text{Br}\)),\textsuperscript{32} trimethylphenylammonium hydroxide (TMP\(_\text{A}\)),\textsuperscript{31} tetrabutylammonium hydroxide (TBA) and methyl iodide,\textsuperscript{33} tetrahexylammonium hydrogen sulfate and methyl iodide,\textsuperscript{34} and methyl iodide alone\textsuperscript{35} were all studied. Three sequential extractions with more TMP\(_\text{A}\) added were necessary for quantitative recoveries of 2,4-D and dicamba when the matrix organic content was high.\textsuperscript{31} Yields of seven analytes from sand, clay soil, and topsoil ranged from 57-141\% using TBA and methyl iodide although excess methyl iodide prevented analysis by GC/ECD.\textsuperscript{33} 2,4-D and 2,4,5-T were also extracted as methyl esters when methyl iodide and tetrahexylammonium hydrogen sulfate, a phase transfer reagent, were added to spiked diatomaceous earth, soil, urine, and rice.\textsuperscript{34} The phase transfer reagent was later found to be unnecessary. Methyl iodide alone recovered organic acids such as 2,4-D, 2,4,5-T, and PCP from an anion exchange resin at 78-101\% yields.\textsuperscript{35}

Transesterification with methanol in CO\(_2\) was demonstrated. Triglycerides in soybeans and soybean seeds, evening primrose seeds, and peanuts were converted to fatty acid methyl esters over methanol-treated alumina in the presence of CO\(_2\).\textsuperscript{36} Resin
and fatty acids were extracted from sediment with the addition of methanol and formic acid. Recoveries improved from \( \leq 3\% \) with pure \( \text{CO}_2 \) to 88-267\% relative to Soxhlet results.\(^{37}\)

Ion pairing reagents were also tested. TMPA was utilized for the extraction of sulphonamides from diatomaceous earth with 82-108\% yields.\(^ {38}\) Secondary alkanesulfonate (SAS) and linear alkylbenzenesulfonate (LAS) surfactants were recovered from sewage sludge as TBA ion pairs.\(^ {39}\) Three consecutive extractions with more TBA added lead to a 2.5-fold increase in yields over methanol modifier alone. Finally, the addition of a strong cation displacer, \( \text{CaCl}_2 \cdot 2\text{H}_2\text{O} \) in methanol, yielded 87\% of 2,4-D from soil.\(^ {23}\)

Strong advances were made in the extraction of some polar analytes by the use of SFDE. Additionally, even analytes that did not react with the reagents were more efficiently extracted because the reagents covered the adsorptive matrix sites. However, problems encountered include the need for multiple extractions with more reagent added when the matrix organic content is high, ineffective reagents in the presence of matrix \( \text{H}_2\text{O} \) or other modifiers, and excess reagent complicating analysis.

\textit{B. Organometallic Analytes.} SFE was applied almost exclusively to the recovery of organic analytes until the past few years. However, organometallic compounds are frequently used in environmental applications. For example, uses of organotin compounds include agricultural and wood preservative fungicides, antifouling paint biocides, fire retardants, thermal stabilizers for poly(vinyl chloride),
and catalysts in the production of silicones and polyurethane foams. Most of the analytical efforts are focused on monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) due to toxicity and lack of a suitable method for MBT analysis. The use of SFE for the recovery of organometallics is still in its early stages with the majority of references reported in the last three years. Examples with and without the addition of a complexing reagent are included in this section to calibrate the reader to the present state of the technique.

Di- and tri-substituted tins were recovered from marine paint, potatoes, and almonds using on-line SFE/SFC with 0.3% (v/v) formic acid in CO₂. TBT and triphenyltin (TPhT) were extracted from a fish tissue certified reference material using 10/90% methanol/CO₂; only 44% of TBT and 23% of TPhT were recovered. The use of 20/80% (v/v) methanol/CO₂ lead to the 82% recovery of TBT from spiked soil and the 70-75% recovery of TBT from a certified reference sediment. Aqueous ethylation with sodium tetraethylborate prior to solid phase extraction (SPE) of H₂O samples and SFE of the extraction disks with pure CO₂ resulted in 55-115% yields of butyl-, phenyl-, and cyclohexyltins. The Sn-C₂H₅ bond was thermally more stable than the Sn-Cl bond and lowered the analyte polarity, increasing the solubility in CO₂.

While the results described above without the addition of a reagent were promising, in situ complexation reactions were also investigated. Sodium diethylidithiocarbamate (NaDDC, shown in Figure 6.2) was one reagent tested for ionic organotins. Recoveries of trisubstituted species improved from 50-75% with CO₂.
alone to 70-90% with NaDDC added prior to extraction.\textsuperscript{45} Recoveries of dimethyltin, DBT, diphenyltin, and MBT roughly doubled from < 20% to ~40% with NaDDC added followed by extraction with CO\textsubscript{2} or 5/95% methanol/CO\textsubscript{2}. In another study, the recovery of MBT was improved to 62% using NaDDC and 10/90% methanol/CO\textsubscript{2} while TBT and DBT were quantitatively recovered.\textsuperscript{46} The authors attributed the low recovery of MBT to either exhaustion of NaDDC during the extraction or loss of the now volatile analyte during collection in a small vial of solvent. The addition of diethylammonium diethyldithiocarbamate (DEA-DDC) followed by extraction with 5/95% methanol/CO\textsubscript{2} resulted in recoveries of <48% of monosubstituted, 7-106% of disubstituted, 75-119% of trisubstituted, and 76-123% of tetrarousubstituted species from soils and sediments.\textsuperscript{40}

\textit{In situ} Grignard reactions were tested because organotin species are relatively involatile and derivatization is required prior to GC analysis. The sample must be very dry to prevent deactivation of the Grignard reagent. Treatment with C\textsubscript{2}H\textsubscript{5}MgCl and extraction with CO\textsubscript{2} lead to recoveries of 70% MBT, 92% DBT, and 102% TBT from an SPE disk.\textsuperscript{47} \textit{In situ} Grignard hexylation followed by CO\textsubscript{2} extraction yielded 76-114% recoveries for di- and trisubstituted species but only 15% and 40% for MBT and MPhT, respectively.\textsuperscript{48}

The extraction of ionic alkyllead from sediment and urban dust was accomplished via methanol addition and CO\textsubscript{2} extraction with yields of 96% trimethyllead, 106% triethyllead, and 80% diethyllead.\textsuperscript{49} Complexation with DDC
before analysis was used. Given the favorable results for in situ complexation of DDC with tin described above, the addition of DDC to the sample prior to extraction appears quite feasible for lead as well.

Although recoveries of tri- and tetrasubstituted organotins were consistently high, the extraction of monosubstituted and some disubstituted species was plagued by low and sometimes irreproducible results. Still, the recoveries were promising relative to previously used methods. The extraction of alkylleads was recently reported, and SFE with or without in situ complexation will undoubtedly be extended to other organometallic species in the near future.

C. Metal Analytes. The extraction of metals by SFE was initially ignored due to the extremely low solubility of charged species in supercritical CO₂. Interactions between the ionic analytes and nonpolar solvent are very weak and charge neutralization is required. Chelating or complexing reagents are commonly used to recover metals from H₂O. The potential for in situ chelation or complexation in CO₂, forming a neutral species, followed by SFE was successfully investigated over the past five years.

DDC forms stable complexes with over 40 metals and nonmetals. However, M-DDC solubility in CO₂ is quite low. The substitution of fluorine for hydrogen, producing a ligand such as bis(trifluoroethyl)dithiocarbamate (FDDC) was shown to improve metal-ligand (M-FDDC) solubilities by 2-3 orders of magnitude.
FDDC was used for the extraction of Cu$^{2+}$ from Celite and directly from H$_2$O, Hg$^{2+}$ from spiked filter paper, and Cu$^{2+}$, Co$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$ from spiked filter paper, sand, and silica gel. Extraction fluids were CO$_2$ or 5/95% methanol/CO$_2$. The addition of H$_2$O to the sample aided extraction by facilitating chelation and transport of the complex, and covering matrix sites reducing readsorption. Recoveries were generally near 100%, although % RSDs were sometimes high and ranged from 2-70%.

The ligand tetrabutylammonium dibutylthiocarbamate (DBDTC) was also found to be more soluble than DDC. DBDTC-saturated CO$_2$ was used for the recovery of Zn$^{2+}$, Cd$^{2+}$, and Pb$^{2+}$ from H$_2$O, divalent metals from freeze-dried and fresh bovine liver, and Cd$^{2+}$ from metallothioneins dissolved in H$_2$O (although Cd$^{2+}$ recovery from the solid protein was unsuccessful).

Fluorinated β-diketones, such as 2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedione (FOD, shown in Figure 6.2), are commercially available and form stable complexes with trivalent species. The efficient extraction of lanthanides and actinides is of increasing importance for nuclear waste analysis and management. Uranyl ion (UO$_2$$^{2+}$), La$^{3+}$, Eu$^{3+}$, and Lu$^{3+}$ were recovered from spiked filter paper with FOD and H$_2$O added followed by extraction with CO$_2$ or 5/95% methanol/CO$_2$. Recoveries were < 1% in the absence of the ligand with CO$_2$ extraction and 91-99% with FOD added and methanol modification. The metals were also extracted at very
acidic pH, outside of the pH range where complexation was expected with liquid solvent extraction.

In an effort to eliminate the methanol modifier, tributyl phosphate (TBP) was added with various β-diketones to test the synergistic effect. With TBP alone in CO₂, < 4% of the lanthanides were detected from spiked filter paper. Recoveries of 92-98% were found in the presence of various β-diketones and TBP. Because the coordination number of trivalent species is generally 8-9, TBP probably fills vacancies not occupied by the β-diketone, preventing readsorption to the matrix. La³⁺, Eu³⁺, and Lu³⁺ were recovered at 75-89% yields from H₂O with the β-diketone thienyltrifluoroacetylacetone (TTA) and TBP added. Likewise, Th⁴⁺ and UO₂²⁺ were quantitatively recovered from filter paper, mine H₂O, and soils using TBP and FOD or other β-diketones without methanol modification. The extraction of UO₂²⁺ from Kaolin (Al₂SiO₅(OH)₄), however, required the use of 5% ethanol in CO₂ with FOD and TBP added for a 93% yield. In contrast, the effect of the β-diketone was minimal for the recovery of lanthanides from a 6 M HNO₃, 3 M LiNO₃ solution using a 30% (v/v) TBP/CO₂ mixture. TBP reportedly acted as both a chelating agent and a solvent modifier. Recoveries of 85-92% for Sm³⁺, Eu³⁺, Gd³⁺, and Dy³⁺ and 44-72% for La³⁺, Ce³⁺, Lu³⁺, and Yb³⁺ were found.

Finally, a t-butyl substituted dibenzobistriazolo-crown ether (see Figure 6.2) was used to selectively extract Hg²⁺ in the presence of other divalent metals such as Cd²⁺, Mn²⁺, and Zn²⁺. The crown ether is selective based on ionic radius-cavity size.
compatibility. The extraction fluid was 5/95 mole % methanol/CO₂ and the presence of H₂O again aided extraction. Recoveries of 98%, 95%, and 94% from filter paper, sand, and H₂O, respectively, were reported while < 4% of the other divalents were extracted. However, Au³⁺ was also partially extracted in the presence of Hg²⁺.

The recovery of metals by SFE provides a mechanism for a cleaner, more selective extract which is desirable for quantitation by analysis methods such as ion chromatography and GC with atomic emission detection. Organic solvent use is also substantially reduced. Chelating reagents commonly used for liquid extraction were found to work quite well. Numerous developments are anticipated in this area over the next few years.

**Alterning the Matrix**

The extraction of liquid samples using supercritical CO₂ is difficult due to experimental problems of sample containment and restrictor plugging. Because H₂O and CO₂ have low solubilities in one another,⁶³ significant time is required for analyte partitioning between the nearly immiscible phases. The direct SFE of H₂O was achieved with good results, however, and is briefly reviewed herein. Freeze drying a liquid sample and subsequently extracting the residue is a rather novel approach with some applications. A very promising alternative, solid phase extraction followed by supercritical fluid elution, is also discussed. SPE/SFE utilizes common sorbents to preconcentrate the analytes before elution using standard SFE instrumentation.
Solvent use is greatly reduced compared to liquid elution and the time for extraction is abridged relative to SFE of the original H$_2$O sample. Also included in this section is the use of temperatures $\geq$ 200 °C for the extraction of solid matrices. Elevated temperatures are expected to provide the activation energy needed for analyte desorption from the matrix sites. As pointed out in the previous section, some of the derivatizing/ion pairing reagents used also modify the matrix by interacting with adsorptive sites and preventing readsorption of the analytes.

A. Direct SFE of Liquid Matrices. The direct SFE of a liquid sample first employed a phase separator for the recovery of phenol and 4-chlorophenol from urine.$^{64}$ Consistent with SFE of solid matrices, Hedrick and Taylor then modified 10 cm x 1 cm i.d., 7-8 ml stainless steel vessels (Keystone Scientific, Bellefonte, PA and Suprex, Pittsburgh, PA)$^{65,66,67,68}$ The vessel (shown in Figure 6.3A) was filled half full and positioned vertically with flow of CO$_2$ down from the top through an inlet tube inserted below the H$_2$O surface. CO$_2$ flowed out of the vessel bottom via a tube inserted above the H$_2$O level in the cell. A series of 6-port switching valves and a recirculation pump were used to move supercritical CO$_2$ in a loop through the matrix. The CO$_2$ extract was then sampled and analyzed on-line or trapped in a liquid solvent or on a solid phase support for later analysis.

Analytes that are polar, of high molecular weight, and even very H$_2$O soluble may be efficiently extracted provided that the analytes are also soluble in CO$_2$ and sufficient time is allowed.$^{66}$ Examples of analytes recovered from aqueous matrices
Figure 6.3. Extraction vessels designed by (A) Hedrick and Taylor and (B) Barnabas et al. for the direct SFE of liquid matrices.
include diisopropyl methylphosphonate (DIMP),\textsuperscript{65,66} triprolidine and pseudoephedrine,\textsuperscript{65,66,67} phenolics,\textsuperscript{68} and phenoxyacid herbicides after the addition of 10\% methanol or acetonitrile as modifiers.\textsuperscript{69} Additionally, phenol was extracted with CO\textsubscript{2} from 6 M H\textsubscript{2}SO\textsubscript{4} into H\textsubscript{2}O for subsequent analysis.\textsuperscript{66}

This vessel was also used extensively for the extraction of metals from H\textsubscript{2}O. Due to the low solubility of metal ions in supercritical CO\textsubscript{2}, a ligand was added either to the extraction fluid or directly to the sample to form a neutral complex that was then readily extractable. Dithiocarbamate-based ligands, particularly fluorinated species, were utilized for divalent metals.\textsuperscript{50,54,55} Fluorinated \(\beta\)-diketones with or without TBP were used for lanthanides and actinides.\textsuperscript{58,59,61} A crown ether was added for the selective extraction of Hg\textsuperscript{2+} in the presence of other divalent metals.\textsuperscript{62} In all cases, <10\% of the analyte was extracted without ligand and recoveries of 50-100\% were reported when \textit{in situ} chelation was possible. A more detailed discussion of the chelation procedure and cited references was given in the previous section.

Barnabas \textit{et al.} later used a 50 ml, stainless steel vessel design shown in Figure 6.3B.\textsuperscript{70} An HPLC solvent filter was placed on the end of the CO\textsubscript{2} inlet tube which was submerged in the solution. CO\textsubscript{2} exited the vessel via a tube positioned above the H\textsubscript{2}O, effectively sampling the headspace. Although a considerably larger sample volume (~45 ml) could be used than in the Hedrick and Taylor vessel, the extraction time was greatly increased as well. Recoveries of the organochlorine pesticides (OCPs) lindane, aldrin, and dieldrin ranged from 40-75\% after a 2 h
Alcohol phenol ethoxylate (APE, shown in Figure 6.4), a non-ionic surfactant, was removed from H₂O at 60% yield in 2 h.

The direct SFE technique suffers from several limitations however. First, although the solubility of H₂O in CO₂ is quite low (≈0.2-3 mole % at 25-100 °C), any H₂O that is transported from the vessel may contribute to restrictor plugging. This H₂O also causes problems in the analyte collection step. If the analytes are collected in a few milliliters of nonpolar organic solvent, a two-phase aqueous and organic mixture may result, and the matrix and analytes are simply transported from one vessel to another. H₂O can also activate solid phase traps, lowering the trapping efficiency.

Second, the Hedrick and Taylor extraction vessel holds ≈3-5 ml H₂O and the Barnabas extraction vessel holds ≈45 ml solution. When trace levels of pollutants are present, the extraction of a much larger sample volume (such as 1 L) may be necessary in order to concentrate the analytes sufficiently to exceed the limit of detection imposed by the analysis method. Third, the pH of H₂O in contact with CO₂ at pressures of 70-200 atm and temperatures of 25-70 °C was recently measured at 2.80-2.95. Therefore, bases are protonated and are much more soluble in H₂O than CO₂. If the analyte pKₐ is > 10, reactions with CO₂ are possible (for example, nitrogenous bases are converted to carbamates). Hedrick and Taylor found that a hydrocarbon, lipophilic moiety of considerable size (i.e., caffeine or larger) is necessary for a base to be CO₂ extractable.
Figure 6.4. Structures of selected analytes discussed in the text: (A) alcohol phenol ethoxylate (APE), (B) mebeverine alcohol, (C) rotenone, and (D) RGH 2981 peripheral blood flow enhancer.
B. Freeze Drying the Sample. An interesting alternative to the direct SFE of a H₂O sample is to freeze dry the liquid and extract the residue.⁷³,⁷⁴ This may be particularly attractive when analytes such as OPPs and carbamates decompose in H₂O upon prolonged storage. A H₂O sample spiked with atrazine and related herbicides was freeze dried with 0.6% glycine added as a stabilizer.⁷³ The residue was then extracted with CO₂. All of the analytes except fenitrothion were stable for at least one month, and recoveries exceeded those obtained by liquid-liquid extraction of the original sample. SFE was also applied to freeze-dried skim milk for the recovery of PCBs in Arochlor 1242. The residue was mixed with florisil and extracted with CO₂.⁷⁴

C. Solid Phase Extraction/Supercritical Fluid Elution (SPE/SFE). Solid phase extraction (SPE) is a favorable method for the concentration and/or purification of trace levels of pollutants from liquid matrices. SPE utilizes a small quantity of a support material coated with a chromatographic stationary phase and contained in a cartridge or disk. The analytes of interest are adsorbed while interferences are not retained (or in some cases the reverse is true). The analytes are then eluted with ~15-30 ml of an appropriate solvent. A solvent concentration step typically follows prior to analysis.

SPE substantially reduces the volume of organic solvent required compared to liquid-liquid extraction methods, but elution with supercritical fluids offers a further solvent reduction. Moreover, SPE/SFE reduces or eliminates some of the difficulties encountered in the direct SFE of H₂O including containing the sample in the extraction
vessel and restrictor plugging associated with the minimal solubility of H₂O in CO₂.

SPE/SFE also provides a preconcentration step for the analysis of trace levels of pollutants. Whereas the concentration or removal of an aqueous/organic eluent after SPE is time consuming, the supercritical fluid eluent is easily removed from the extract since CO₂ goes off as a gas into the air.

Octadecyl (C₁₈) sorbents are by far the most widely used SPE materials. Examples of SPE/SFE using C₁₈ are the isolation of a drug metabolite, mebeverine alcohol (shown in Figure 6.4), from dog plasma,⁷⁵ morphinic alkaloids from H₂O and urine,⁷⁶ PCB congeners from milk and blood serum,⁷⁷ and rotenone (see Figure 6.4), a natural product pesticide used for controlling fish populations, from H₂O.⁷⁸ In several cases, CO₂ modification with methanol or acetonitrile was necessary for rapid and efficient elution. Recoveries were generally quantitative.

Class selective extractions based on polarity were also possible. Three OCPs (lindane, aldrin, and dieldrin) and three OPPs (dichlorvos, diazinon, and malathion), shown in Figure 6.5, were selectively recovered from a C₁₈ disk.⁷⁹ The disk was first eluted with pure CO₂ for the removal of OCPs then with CO₂ after the addition of methanol directly to the disk for elution of the OPPs. Recoveries exceeded 70% for all six analytes. The selective extraction aided in GC analysis because the nitrogen phosphorus detector used for the OPP analysis was less responsive in the presence of chlorinated species such as the OCPs, which were then quantitated by electron capture detection. In a similar study, three OCPs (heptachlor, isodrin, and dieldrin) were first
Figure 6.5. Structures of the OCPs (A) lindane, (B) aldrin, and (C) dieldrin, and the OPPs (D) dichlorvos, (E) diazinon, and (F) malathion.
extracted using CO₂ before six herbicides including simazine and diuron were then removed with 10/90% methanol/CO₂. The OCPs were analyzed by GC and the herbicides were quantitated by HPLC with recoveries of 85-100% for all analytes.

C₁₈ was also used for the SPE/SFE of PCBs and hexachlorocyclohexanes, phenols, PAHs, OCPs, non-ionic surfactants, and 43 semivolatiles including PAHs, PCBs, phthalate esters, and OCPs. Recoveries of 3 OCPs ranged from 77-99% and were on average ~20% higher than by direct SFE, described in the previous section. Recoveries of the non-ionic surfactant, APE, improved from 60% directly from H₂O to 100% after SPE/SFE.

Organotins were recovered at ppb levels from synthetic seawater after retention on a C₁₈ disk and an in situ Grignard reaction. Limits of detection for the three analytes were 6-16 ng/L. Similarly, organotins were recovered from seawater, harbor H₂O, and river H₂O after derivatization with sodium tetraethylborate and enrichment on a C₁₈ disk.

Styrene-divinylbenzene (SDB) proved better than C₁₈ for the extraction of phenols, averaging 83% recovery. A C₈ disk followed by elution with 2% methanol in CO₂ was used for the recovery of sulfoeturon methyl and chlorsulfuron (shown in Figure 6.1) at > 90%. Organic solvent use was reduced by 75% over liquid elution. Nitrotoluene explosives were concentrated on a phenyl sorbent and eluted with CO₂ or toluene/CO₂. Finally, method development and evaluation of various SPE cartridges and disks for PAHs, and pesticides and phthalate esters were reported.
Advantages of SPE/SFE are numerous. While pre-treatment of the disk or cartridge for sorbent activation can not be avoided and generally consumes 10-20 ml organic/aqueous solvent, elution with CO$_2$ or modified CO$_2$ greatly reduces the subsequent solvent usage. By choosing a sorbent selective for the analytes of interest, a cleaner extract results. The need for a solvent concentration step is greatly reduced or eliminated as well. Finally, trace levels of pollutants can be concentrated from a large volume of H$_2$O to a small quantity of sorbent, reducing the volume of CO$_2$ needed and eliminating the problems associated with the direct SFE of H$_2$O. With the advent of more SPE phases, class selective extractions from liquid samples will become even more common.

D. High Temperature SFE. The use of elevated extraction temperatures (≥ 200 °C) was initially avoided because CO$_2$ density decreases with increasing temperature at constant pressure. However, analyte solubility in CO$_2$ is influenced by both CO$_2$ density and the analyte vapor pressure (which improves with increasing temperature). Furthermore, the energy of desorption needed to release the analytes from the matrix should be more easily overcome at higher temperatures.

Analyte fractionation via temperature variation was recently investigated with good results. A mixture of PCBs and PCDDs was spiked onto florisil. The PCBs were first extracted with CO$_2$ at 238 atm and 80 °C for 15 min. The PCDDs were then removed with CO$_2$ at 612 atm and 200 °C during an 80 min extraction. To extend the technique to native samples, fly ash was initially extracted with CO$_2$ at 640 atm and

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250 °C for 80 min to remove all of the analytes. The extract was then loaded onto florisil with liquid elution of interfering components followed by CO₂ fractionation of the PCBs and PCDDs at the above listed conditions. The two steps were then performed simultaneously by connecting a florisil column directly to the outlet of the extraction vessel containing the fly ash matrix.

Temperatures of 50 °C, 200 °C, and 350 °C were tested for the extraction of PAHs, N- and S-heterocyclics, chlorinated phenols, and pesticides from soil and soot. Increasing the temperature from 50 °C to 200 °C with CO₂ had a positive effect on all analytes. A 350 °C extraction temperature resulted in yields only comparable to the 50 °C extractions although no thermal degradation was detected. Extractions at 350 °C were more difficult to perform due to leaks around the vessel seals and low molecular weight PAHs were formed from matrix organics. To determine the role of CO₂, thermal desorption with a nitrogen purge was tested. Recoveries of 0-50% with nitrogen were compared to 51-100% recoveries with CO₂ at 200 °C from a railroad bed soil, indicating that the solvating power of CO₂ was important to the extraction.

In other studies, aliphatic and aromatic hydrocarbons were recovered from shale more efficiently at 350 °C than at 50 °C, 150 °C, and 250 °C, PCDDs from fly ash and PAHs from marine sediment were detected at higher yields at 200 °C than at 120 °C or 40 °C, PCBs from river sediment and PAHs from urban air particulate were effectively recovered only when the temperature was raised from 50 °C to
200 °C,\textsuperscript{96} and PAHs were more efficiently extracted from marine sediment, diesel soot, and air particulate at 200 °C than at 80 °C.\textsuperscript{91} In this last study, the effect of temperature was found to be matrix independent.\textsuperscript{91}

The most severe limitations currently slowing the use of elevated temperatures are technical in nature. Most of the commercial SFE instruments have a maximum operating temperature of 150 °C. While the use of a GC oven is quite feasible, appropriate reusable seals for the extraction vessel are not commonly available. However, the benefits of high temperature extraction, including higher recoveries, decreased extraction time, possibility of a matrix independent effect, and potential for selective extraction or fractionation, make the technique worth further investigation.

**Altering the Instrumentation/Experiment**

A high pressure Soxhlet extractor typically utilizes liquid CO\textsubscript{2} as the extraction fluid instead of conventional liquid solvents. Conversely, microwave extraction and accelerated solvent extraction use smaller quantities (generally < 30 ml) of traditional liquid-solid extraction solvents at elevated pressures and temperatures. In these techniques, reduced liquid solvent usage and shorter extraction times compared to conventional techniques are clearly the goals. Furthermore, standardized extraction conditions were developed, minimizing the need for a method development step for each sample which is currently required in SFE.
A. High Pressure Soxhlet Extraction. A high pressure Soxhlet extractor was first described and utilized by Jennings and later patented by J&W Scientific (Folsom, CA). The extractor replaces conventional liquid solvents with substances such as CO\textsubscript{2}, N\textsubscript{2}O, and pentane that can be liquefied at temperatures of 0-20 °C and pressures up to 102 atm. CO\textsubscript{2} is the most commonly used solvent. The glass Soxhlet extractor is enclosed in a stainless steel pressure vessel containing an appropriate quantity of solvent such as dry ice or CO\textsubscript{2} from a gas cylinder. Both the glass extractor and surrounding vessel are pressurized. Inserting the vessel in a 40-45 °C H\textsubscript{2}O bath causes vaporization of the dry ice until the CO\textsubscript{2} hits an ice H\textsubscript{2}O cold finger above the extraction thimble containing the sample, condenses, and drips into the thimble. Liquid CO\textsubscript{2} collects in the thimble and eventually spills over through the siphon tube to the boiling flask. As cycling of vaporization and condensation occurs, the pressure inside the vessel (~41-48 atm) is below the critical pressure of CO\textsubscript{2}. To stop the extraction, the unit is placed in dry ice to drop the pressure inside. The pressure is then vented and the sample residue in the boiling flask is removed by syringe or by rinsing with a small quantity of solvent.

Examples shown include the extraction of volatile components from bananas and PAHs from coal fly ash. Quantitative recoveries of morphine from blood, caffeine from kola nuts, and quinine from plants were obtained. A variety of spices including clove, ginger, and sandelwood were extracted from plant materials in 2-2.5 h vs. 4-24 h by steam distillation with comparable yields. More recently, OCPs and
PCBs were recovered from certified potato, carrot, olive oil, butter, and lyophilized fish tissue samples, but tributyltin was recovered from spiked sediment at only 21% yield.

Although this technique was available in 1979, relatively few references are found in the literature. The minimal operator supervision required once the extraction is under way is a positive point in comparison to SFE. Organic solvent use is virtually eliminated and a more selective extraction, based on analyte solubility in CO₂, is anticipated compared to liquid Soxhlet solvents. However, analyte solubility is necessary but often insufficient for extraction when matrix-analyte interactions are strong. Since the instrument can not operate at a variety of temperatures, one of the important variables in SFE is excluded. The initial cost of the extractor and the need for a dry ice source possibly also deterred its use relative to the conventional technique.

B. Microwave Extraction. Microwave energy was explored as a means of rapidly extracting analytes without degrading or digesting the entire sample. The physics of microwave energy as well as general applications to analytical and environmental chemistry were recently reviewed. In the microwave extraction of a solid matrix, the sample is saturated with a common organic or organic/aqueous solvent and irradiated for a short period of generally 0.5-10 min. After cooling, the supernatant is decanted and the matrix is rinsed several times. The combined extract is centrifuged, concentrated, or purified as necessary prior to analysis. Solvents lacking
a dipole moment (i.e., hexane) do not efficiently absorb microwave energy. Therefore, at least 10% polar solvent is generally required. The absorption of energy by the solvent results in the disruption of weak hydrogen bonds, improved solvent penetration, and enhanced solvation.103

Environment Canada holds a patent on the microwave extraction technique and the trademark rights on a Microwave Assisted Process (MAP™).104 This process uses a Kenmore microwave/convection oven and an energy magnetron generator. The patent describes the extraction of natural products such as garlic and mint using hexane, ethanol, or methylene chloride in several heating stages to avoid solvent boiling.

OCPs including endrin and dieldrin were extracted from spiked sediment using MAP™ with a 1:1 mixture of isooctane and acetonitrile.105 Results were comparable to 6 h Soxhlet extractions. Other examples include the removal of crude fat from food products and OPPs (bromophos and parathion) from spiked soils using 30 s irradiation intervals.106 Microwave extraction required 1/100 the time for Soxhlet extraction and analyte degradation, sometimes a problem with Soxhlet, was avoided. The extraction of the lupin alkaloid sparteine and 14C labelled RGH 2981 (see Figure 6.4), a peripheral blood flow enhancer, and its metabolite from seeds and rat faeces was also studied.103 Methanol/H2O mixtures containing acetic acid or ammonia in varying proportions were tested. Results were comparable to or better than those by a shake flask (liquid-solid) technique.
A CEM Model MES 1000 microwave system (CEM, Matthews, NC), consisting of a magnetron tube and sample chamber or oven with a turntable holding up to 12 vessels, was also developed. Extraction vessels are polyetherimide, lined with perfluoroalkoxy or Teflon. The extraction solvent (~30 ml) was a 1:1 mixture of hexane and acetone. Extractions were typically performed on 5-10 g samples at 115 °C for 10 min. Recoveries of PAHs, base/neutral compounds, phenols, and OCPs from marine sediments and soils, PCBs from clay soil, topsoil, and sand, phthalate esters, OCPs, and PAHs from marine sediment, and 187 compounds and 4 Arochlors from topsoil were studied. Imidazolinone herbicides were extracted from soil at 1-50 ppb levels using an ammonium acetate/ammonium hydroxide buffer at pH 10. The average recovery was 92%.

In other studies, PAHs were extracted from highly contaminated soils using methylene chloride or hexane/acetone. Yields were comparable to or better than 6 h Soxhlet extractions in methylene chloride and required 50% less solvent. Atrazines and principle degradates were recovered from soils at > 85% by first irradiating the soil sample in 25 ml H₂O followed by 0.35 N HCl in triplicate. The fungal metabolite ergosterol and fatty acids were removed from a variety of agricultural products including mushrooms, corn, and grain dust using a methanol/NaOH mixture. Fatty acids were simultaneously extracted and saponified by this process.
A Microdigest Model A301 microwave digester (Prolabo, France) was used for the leaching of organotin species from sediments using 0.5 M acetic acid in methanol.¹¹⁹ Recoveries of MBT, DBT, and TBT from two reference materials were ≥ the certified values. When the reference materials were leached at a comparable temperature in the absence of a microwave field, ~50% of DBT and TBT and < 10% of MBT were found, validating the need for the microwave energy. Butyl- and phenyltins were also leached from certified sediments using 0.5 M ethanoic acid in methanol with recoveries exceeding the certified values.¹²⁰

Recently, microwave energy was used for the direct extraction of H₂O samples. MAP™ was applied to the headspace analysis of volatiles such as benzene, toluene, ethylbenzene, chlorobenzene, xylenes, and dichlorobenzenes at ppb to ppm levels.¹²¹ The vapor above the water was sampled using a standard headspace sampler. PCBs were extracted from 500 ml H₂O containing 10 g NaCl and 50 ml isoctane at ~70 °C.¹²² Isooctane was chosen as a cosolvent due to its near immiscibility with H₂O and because it readily dissolves PCBs. The addition of NaCl caused "salting out" but also aided in uniform heat transfer within the vessel. The organic phase was then analyzed with recoveries of 68-85%. Finally, chlorinated benzenes at ppt levels were removed from 1 L H₂O with NaCl added.¹²³ After heating, helium was used to purge the analytes from the vessel into a small vial of hexane.

Advantages noted in the above references include shorter extraction times and minimal solvent usage relative to Soxhlet or sonication, the ability to extract multiple
samples at once, portability and potential for use in the field, and trace analysis of even somewhat polar analytes in H₂O. Disadvantages include degradation of some thermally labile components and lack of selectivity in the extraction such that a clean-up step is frequently necessary prior to analysis.

C. Accelerated Solvent Extraction. Accelerated solvent extraction (ASE™) was introduced by Dionex Corporation (Sunnyvale, CA) in 1994. ASE™ uses the solvents traditionally used for liquid-solid extraction techniques at elevated pressures (102-136 atm) and temperatures (50-200 °C) for solid and semisolid samples. The technique capitalizes on improved analyte solubilities and kinetics of analyte desorption from the matrix at temperatures above the boiling points of the solvents. The sample in a stainless steel vessel is saturated with solvent and heated. The most frequently utilized conditions are a 1:1 mixture of hexane and acetone at 136 atm and 100 °C. During a 5-10 min static extraction period, the heated, expanding solvent is vented to a collection vial. Fresh solvent is introduced at the end of the static step prior to a compressed nitrogen purge to flush the extraction cell. A 10 g sample requires ~12 min and ~15 ml solvent for extraction.

Extracted analytes include OPPs and phenoxyacids from clay, loam, and sand, PAHs from marine sediment and contaminated soils, 56 basic, neutral, and acidic compounds from soils, and PCBs from sewage sludge and oyster tissue. Recoveries in all cases were comparable to or better than the certified values.
or those obtained by conventional methods, and no matrix dependence in the overall extraction yields was observed.

The same methodology was applied to the extraction of 16 PAHs from lignite and bituminous coal fly ashes using standard SFE equipment. Extractions with methylene chloride at 136 atm and 150 °C yielded > 70% recoveries of mid-molecular weight PAHs. High molecular weight PAHs were recovered at > 62% and low molecular weight PAHs were recovered at yields ≤ Soxhlet extraction.

Advantages of ASE™ over conventional techniques include avoidance of localized heating and numerous washes with sonication, and greatly decreased time and solvent consumption over Soxhlet and wrist shake. Furthermore, the ASE 200 (Dionex Corporation, Sunnyvale, CA) is automated with autosampler and collection trays holding up to 24 samples. A potential disadvantage is that the extractions tend to be exhaustive, leading to nonselective extracts requiring additional clean-up.

**SUMMARY**

Supercritical fluid extraction and traditional liquid extraction methods represent opposite ends of the spectrum, with advantages and shortcomings recognized for both techniques. Neither is ideal for all analytes from all matrices. The alternative approaches that developed predominantly over the past five years to bridge the gap between SFE and liquid extraction were discussed in this chapter. Some of the most promising alternatives appear to be the use of subcritical H₂O at a variety of
temperatures for polarity-selective extractions, *in situ* complexation or chelation for the extraction of organometallics and metal ions that are not ordinarily soluble in CO₂, solid phase extraction followed by supercritical fluid elution for aqueous samples, and microwave extraction and accelerated solvent extraction as direct alternatives to long and cumbersome Soxhlet extractions. All of the methods described focus on reduced solvent usage and shorter extraction times. Some methods, such as *in situ* chelation and high temperature SFE, are potentially analyte selective while others including microwave extraction and ASE offer an exhaustive extract that requires further cleanup prior to analysis. Since a single technique that is universal for all samples is still not available, alternatives will continue to develop to meet the needs for sensitive, precise, and environmentally sound extraction methods.
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CHAPTER 7

CONCLUSIONS

The use of supercritical fluid extraction (SFE) is making great advances in overcoming the problems of competing liquid-solid techniques such as Soxhlet and sonication. Extraction times and organic solvent use are greatly reduced. However, SFE is not perfect. Supercritical CO₂ often lacks the solvent strength needed to solubilize polar and/or high molecular weight analytes and to interrupt the variety of possible matrix-analyte interactions. While the addition of small proportions (1-10%) of polar organic modifiers helps significantly, the technique is still limited and liquid-solid extraction methods are not obsolete.

Enhanced-fluidity liquid extraction (EFLE), investigated herein, is just one of many possible alternatives being developed (see Chapter 6). The goal of EFLE is to combine the positive attributes of supercritical fluids such as low viscosity and rapid diffusion, with the high polarity and solvent strength of conventional liquids needed for analyte solubility and the interruption of matrix-analyte interactions. Thus, EFLE considers and potentially optimizes each interrelated factor in the extraction triangle.
A weakness common to both modified supercritical fluids and enhanced-fluidity liquids is that phase diagram information is not always available. A relatively simple and precise method for the determination of liquid-liquid, liquid-vapor, and supercritical fluid-vapor phase transitions was described in Chapter 2. The single phase regions for methanol/CO₂, acetonitrile/CO₂, methanol/H₂O/CO₂, and acetonitrile/H₂O/CO₂ were mapped at temperatures of 25-100 °C and pressures ≤ 306 atm. This information was vital to the selection, preparation, and use of single phase, homogeneous extraction fluid mixtures.

A method to determine mixture densities simultaneously with the phase transitions would be useful. This should be possible by measuring the volume displacement of the pneumatic assembly needed to form single phase mixtures in the view cell. Initial measurements with CO₂ or other pure fluids such as nitrogen or argon would elucidate the accuracy of this technique.

Since many polar environmental pollutants are acids or bases, varying the pH of the extraction fluid via buffers commonly used in reversed-phase HPLC may prove beneficial. Just as varying proportions of H₂O could be added to methanol/CO₂ and acetonitrile/CO₂ mixtures, limits to the proportions of added buffer are expected as well. Determining the variation, if any, in those limits relative to H₂O alone would be quite interesting and applicable to both enhanced-fluidity liquid HPLC and extraction. For example, the variation in phase behavior resulting from the presence of inorganic
components that are not soluble in CO₂ may be insignificant, but currently is unknown.

In Chapter 3, EFLE was tested for the extraction of polar phenolics and nitroaromatics from an octadecyl polysiloxane sorbent material. Because the analytes are sufficiently soluble in even pure CO₂ and the matrix provided uniform, well-defined matrix-analyte interaction sites, a comparison of mass transport properties for supercritical fluids and enhanced-fluidity liquids was possible. The effect of temperature was minimal over the 25-65 °C range studied, and the fluid state, liquid or supercritical, for methanol/CO₂ mixtures was found to be virtually inconsequential to extraction rates and recoveries. Relative to SFE and EFLE, results for 4 h Soxhlet extractions were comparable in yields but required up to 40 times longer to complete. Therefore, the enhanced-fluidity liquids behaved considerably more like supercritical fluids than conventional liquids in terms of mass transport in this study.

In Chapter 4, the extraction of phenolics and nitroaromatics from house dust, an environmental matrix containing a high H₂O and organic content, was considered. The extraction rates with supercritical CO₂ were slow indicating that the fluid was unable to rapidly overcome the strong matrix-analyte interactions. Extraction rates were greatly improved with all cosolvent/CO₂ mixtures tested. Recoveries increased with increasing extraction fluid solvent strength. For example, average recoveries for the entire analyte set were 49% with supercritical CO₂, 50% with near critical 10/90 mole % methanol/CO₂, 61% with liquid 20/80 mole % methanol/CO₂, and 69% with
When methanol and H₂O were tested as static modifiers, methanol yielded higher results than H₂O alone, and recoveries were significantly lower (~10-20%) than with binary and ternary mixtures. The enhanced-fluidity liquid methanol/CO₂ and methanol/H₂O/CO₂ mixtures better promoted the interruption of matrix-analyte interactions and possibly prevented analyte readsorption by interacting with matrix active sites.

Finally in Chapter 5, the extraction of phenoxyacid herbicides from house dust was studied to test all three controlling factors (i.e., mass transport, matrix-analyte interactions, and analyte solubility). While previous studies focused on in situ ion pairing or methyl esterification to make the analytes less polar and more extractable, increasing the polarity of the extraction fluid to a level comparable to that of the analytes was tested here. Recoveries with pure CO₂ were < 40%. Highest yields for all four analytes (83-95%) were obtained with supercritical 20/80 mole % methanol/CO₂. Recoveries of 2,4-D and 2,4,5-T improved with increasing temperature but recoveries of dicamba and 2,4,5-TP were invariant with temperature at both supercritical and enhanced-fluidity liquid conditions. The addition of the derivatizing reagent in methanol directly to the sample had no positive effect relative to the addition of methanol alone, at least for this particular sample. Recoveries with methanol/CO₂ mixtures were roughly twice those of Soxhlet extraction and up to three times greater than those obtained with static modifiers. Therefore, methanol
modification enhanced analyte solubility and effectively interrupted matrix-analyte interactions.

The properties of enhanced-fluidity liquids discussed in Chapter 1 were tested and found to be advantageous to the extraction of polar analytes relative to SFE and liquid-solid extraction techniques. The extraction rates and recoveries with 20/80 mole % methanol/CO₂ were consistently better than those with 10/90 mole % methanol/CO₂ at both liquid and supercritical conditions. Extractions with supercritical 10/90 mole % methanol/CO₂ were also experimentally more difficult to perform. The fused silica restrictor became brittle and frequently broke during the extractions. This problem was not encountered with 10/90 mole % methanol/CO₂ at liquid conditions or with any of the other mixtures at either liquid or supercritical conditions.

Yields declined when 30/70 and 40/60 mole % methanol/CO₂ were used. This was not expected because the solvent strength of the methanol/CO₂ mixtures increases with increasing methanol composition beyond that of the 20/80 mole % mixture. The relatively small but possibly significant increases in mixture viscosity at 30/70 and 40/60 mole % methanol/CO₂ may be limiting accessibility to matrix pore sites. It is also possible that surface tension, affecting the matrix wettability, increases significantly with these mixtures. Supercritical fluids have zero surface tension while conventional liquids often have substantial surface tension. Further studies of the properties of enhanced-fluidity liquids at a variety of temperatures and pressures, and
better characterization of the matrix composition (organic and inorganic components), pore size distribution, and porosity should improve the understanding of these results.

A possible future application of EFLE is in the recovery of thermally labile analytes such as phenylurea herbicides and carbamate fungicides. These analytes are polar, thereby requiring a high solvent strength extraction fluid, but decompose at elevated temperatures, limiting the use of one of the most important variables in SFE. By working in a single phase liquid region of the phase diagram, high temperatures can be avoided. Also, because 20/80 mole % methanol/CO₂ was a suitable extraction fluid for both the phenolics and the phenoxyacids, developing a standard procedure or at least recommendations for initial extraction conditions may be possible. However, additional polar analytes and other environmental matrices such as soils, sediments, urban dusts, and fly ashes should first be considered.

Finally, SFE and EFLE could be integrated to pre-extract extraneous matrix material using supercritical CO₂ then recover the analytes of interest with high solvent strength extraction fluids such as enhanced-fluidity liquids. This was briefly tested in Chapter 5 with good results. Although two extractions are required, the elimination of a solvent-consuming clean-up step after extraction would be quite favorable.

Overall, EFLE was shown to be a viable alternative or addition to SFE for polar analytes. The extractions were rapid and consumed little additional solvent compared to modified SFE. Solvent use was also comparable to or less than alternative techniques, such as accelerated solvent extraction and microwave
extraction, discussed in Chapter 6. From the results of these studies, the future of EFLE looks promising and research on EFLE should continue.
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