THE EXTRACTION OF ANALYTES FROM AQUEOUS SOLUTION USING SUPERCRITICAL FLUIDS

by

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Abstract

Supercritical fluid extraction (SFE) is becoming an attractive alternative to conventional solvent extraction for many reasons. These reasons include advantages of speed, the ability to be automated more easily than conventional solvent extractions and the lack of solvent disposal after the extraction has been performed. At this date SFE is performed in a multitude of ways with no one method out performing the others. The different ways in which SFE is performed is reviewed.

Supercritical fluid extraction of analytes from aqueous solution has not received much attention. The design of a system which allows for the extraction of analytes from aqueous solution has been explored in this thesis. Several related areas (injection techniques for supercritical fluid chromatography and on-line SFE) were also developed. The injection port of a supercritical fluid chromatograph was modified to provide better (more reproducible) sample introduction. For a 100 ppm 3,5-nitrobenzamide solution in methylene chloride the area reproducibility was increased from 3.2 % RSD for the unmodified valve to 0.74 % RSD for the modified valve. The method also resulted in a more narrow solvent front as well as an increase of 10% in the number of theoretical plates of the system.

On-line SFE\SFC was explored as one possible configuration for the extraction of analytes from aqueous solution. Solvent elimination injection (SEI), was developed for SFC. The difference in vapor pressure between the analytes and solvent allowed for the solvent to evaporate and be transported form the system while the analytes were collected on various traps. After evaporation of the injection solvent the analytes were flushed onto a chromatographic column. SEI allowed for the reproducible injection of larger volumes of sample (solvent and analyte) into the system. SEI allowed for different hardware configurations to be tested without performing an actual supercritical fluid extraction.

An off-line solid phase trapping system for SFE was developed. The system trapped the analytes from the SFE effluent onto a solid phase extraction cartridge. The cartridge could then be rinsed in a normal fashion to elute the analytes of interest. Trapping in this way was found to allow for faster extraction rates than liquid trapping. The efficiency of the trapping mechanism was found to be dependent upon the temperature of the trap, the chemical functionality of the phase bonded to the silica and the nature of the analytes.

A system which allowed for the extraction of moderate volumes (3-5 mL) of aqueous solution was developed. A test solution of phenols was used to evaluate the system. The extractability of the phenols was found to be a function of pressure of the system and the chemical nature of the phenol. A decrease in extractability of the phenols was found to take place at pressures greater than 250 atm. The distribution coefficient of phenol was found to increase steadily through 400 atm. A decrease in surface area of the supercritical fluid passing through the aqueous solution was thought to be responsible for the apparent contradiction in behavior.

This thesis is dedicated to
Leah J. Mulcahey

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There are a number of people who have helped make this thesis come into being. The first and by no means the least is my father Jack Hedrick. It was under his guidance that I could be found tapping packing material into copper tubing for GC columns at the tender age of 12. Dr. Don Jones at Western Maryland College and Dr. Chuck Root at Bucknell University managed to teach me some chemistry between then and the time this thesis began. I'd like to thank Dr. Larry Taylor for giving me almost free reign in the lab to pursue what I felt was interesting. It made for a very enjoyable few years.

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CHAPTER 1

INTRODUCTION

Analytical techniques for both qualitative and quantitative analysis have become both fast and automated. The result of these efforts has been an increased throughput of samples that can be analyzed by a given technique. Sample preparation, however, has not received equal attention by either instrument companies or research facilities. Sample preparation is often thought of as a necessary evil that must be performed before the "real analysis" can take place. As a result, sample preparation is now the most time consuming and costly portion of many analyses.

The goal of any sample preparation is to yield the analytes of interest in a form that can be readily analyzed. In the simplest of cases where chromatography is to be employed, the analyte needs only to be vaporized or dissolved in a solvent compatible with that of the analysis. Complex samples such as sediments, food stuffs, waste water and biological materials require somewhat more involved sample preparation.

Supercritical fluids (SFs), as a medium for selectively isolating components from complex mixtures and matrices by extraction and/or chromatography, are currently being studied worldwide. Interest in supercritical fluid extraction (SFE) has surpassed supercritical fluid chromatography (SFC) as it provides, for the first time, a viable alternative to conventional and widely used soxhlet extraction. SFs afford advantages over conventional liquids in the areas of efficiency of separation per unit time[1], the time required for methods development[2] and the environmental consequences of the use and disposal of liquid organic solvents[3].

The near gas-like transport properties (i.e.: high diffusivity and low viscosity) of SFs insure "fast" mass transfer within difficult-to-access matrices as compared to conventional organic solvents. The mass transfer properties of SFs are enhanced further

by their near zero surface tension which facilitates their facile penetration into (and out of) porous material. The physical properties of supercritical fluids are well documented and will not be discussed here[4,5,6,7].

Conventional soxhlet extractions in which the analyte is extracted with boiling solvent may degrade the analytes of interest. The "gentle" extraction conditions used in SFE, as compared to soxhlet extraction, provide a greater assurance that chemical degradation or reaction does not take place during the extraction. In other words, the analytes that are isolated by SFE may be more representative of what was initially in the sample, compared to compounds isolated by soxhlet extraction.

Current SFE efforts cover a multitude of matrices and chemical types. Some of the studies include: extraction of cholesterol from eggs[8], alkaloids from natural products[5], various lipid bearing materials[9,10,11,12], extraction of drugs and drug metabolites from animal feed, urine, and serum[13], extraction of pollutants from soils[14,15], sediments[16,17], and animal tissue[18] as well as the stabilization of propellants[19]. While SFE has been found to be useful on both the process and the analytical scales, much research needs to be done in order to understand, optimize and apply the technology. Such studies will, no doubt, further serve to expand the scope of this field.

The primary goal of the work done in this thesis over the past few years has been to determine whether the direct extraction of aqueous samples using a supercritical fluid can be performed. At this moment there is little data in the literature regarding the direct extraction of material from liquid matrices with supercritical fluids. A more commonly used technique for the extraction of aqueous samples is to pass the aqueous solution through a solid phase adsorbent. The analytes are adsorbed to the surface of the solid phase and can then be extracted using conventional SFE procedures. Our approach will be to determine the feasibility of direct liquid-fluid extraction.

This thesis contains a review of current methods (to 1990) for SFE (Chapter 2). Later chapters deal with work done in our laboratory leading to the successful extraction of material from aqueous matrices. Chapter 3 deals with injection considerations for SFC . Commonly practiced injection techniques will be shown to be imprecise and to lead to band broadening in the resulting chromatogram. Modification of the currently practiced method of injection onto a 1 mm packed column was seen as necessary in order to provide precise and reliable quantitation for any subsequent SFC that would be done after the extraction. The development of a solid phase trapping system for SFE is discussed in Chapter 4. A knowledge of the trapping behavior for the analytes of interest was necessary so that when SFE was performed any problems with the extraction could be distinguished from the subsequent trapping and rinsing of the analytes. At the time that extractions were being performed in our laboratory a commercial unit that employed solid phase trapping was not available. A commercial unit has, however, been introduced since then. The commercial unit now available can, unfortunately, only extract solids and semisolids. Chapter 5 deals with the extraction of phenols from water, while Chapter 6 is a collection of applications involving the extraction of material from aqueous matrices. The compounds extracted in Chapters 5 and 6 were chosen for their applicability to current analytical problems by those supporting this work.

Chapter 2

TYPES OF SUPERCRITICAL FLUID EXTRACTION

The different methods by which SFE can be performed on an analytical scale have not been well documented. Currently supercritical fluid extraction does not refer to a single technique. Instead, it refers to many different techniques used by researchers and technicians. In most instances, the only common factor between laboratories that perform SFE is the use of supercritical fluids to solvate analyte(s) at some point in the procedure. The main differences in SFE techniques are: (1) how the supercritical fluid comes into contact with the analyte; (2) how the analyte is subsequently isolated from the sample matrix; and (3) the fate of the analyte once it is isolated from the sample matrix.

Hardware

Much of the hardware and vocabulary used for SFE is similar regardless of the mode of extraction. Figure 1 shows the minimum hardware required for performing supercritical fluid extraction. Some sort of solvent delivery system is necessary for all types of SFE. Both syringe and reciprocating pumps can be used for this purpose. A temperature controlled zone is required since the critical temperature for most SFs is above room temperature. Stainless steel or fused silica tubing is used to connect the various parts of the extraction apparatus. All systems employ an extraction vessel which is used to contain the sample matrix to be extracted. The extraction vessel is usually a stainless steel cylinder of varying length and inner diameter. Ideally the sample should occupy the extraction vessel with little or no dead volume. Any void in the vessel or tubing between the vessel and restrictor represents a volume which will dilute the extracted sample. The dilution of extracted sample does not in itself hinder the extraction

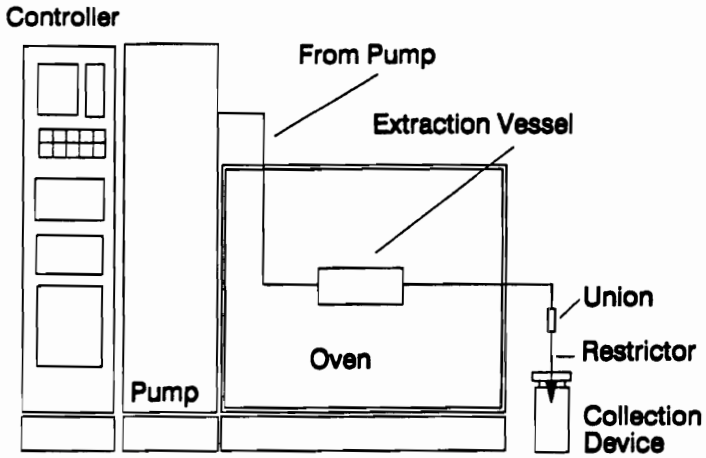


Figure 1

General Hardware Needed for a Supercritical Fluid Extractor

process but the void volume of the system must be cleared in order to remove the analyte from the system. The additional fluid volume required to sweep the vessel translates into added cost to each analysis, not only in SF consumed but also in the time required to perform the analysis.

In order to provide an effective high pressure seal, extraction vessels are usually less than 3 cm in diameter. Larger vessels require multi-seal systems in order to operate at high pressures[20]. Stainless steel frits are placed at either end to contain the sample matrix within the vessel. The seals themselves are usually Kel-F or Teflon. Silicone rubber seals are not used as they swell and may dissolve in some SFs.

Most forms of SFE require a vessel outlet restrictor. The restrictor acts to provide a back pressure to the system so that high pressures may be maintained throughout the system. Fused silica capillaries are the simplest restrictors and provide a fixed mass restriction. Mechanical restrictors (for the most part needle valve type arrangements) with variable orifices have also been used to provide back pressure on the system.

The device used to collect the analytes after they exit the restrictor can assume many forms. In the simplest case it can simply be an empty or solvent filled container. Other more elaborate forms of collection such as solid phase trapping and "micro-trapping" for subsequent analysis by on-line methods can also be employed.

Static and Dynamic Methods

Figure 2 shows the different ways in which SFE can be performed. The basic division is between static (or equilibrium) and dynamic modes. More specifically, extraction with SFs can be performed in the dynamic mode, static mode or some combination of the two. The dynamic mode allows for fresh SF to continuously pass over the sample matrix. Analyte is extracted (moved) from the sample matrix to some other

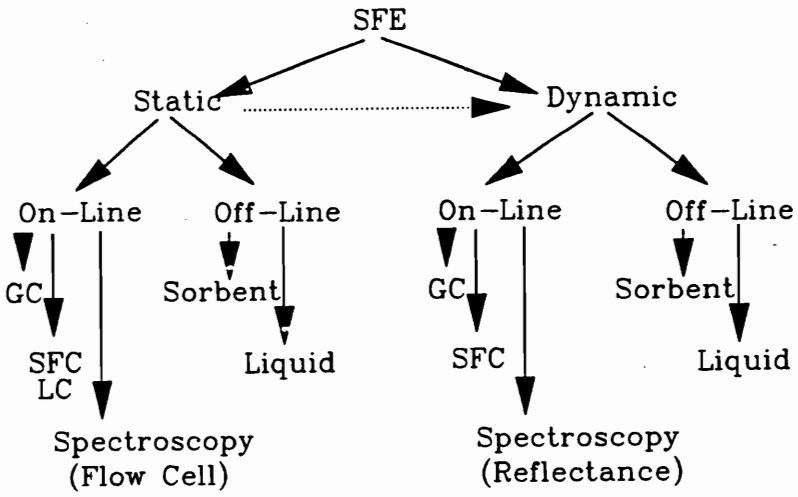


Figure 2

Various SFE Methods

point. The static mode employs a fixed amount of SF, either to recycle through the analyte/matrix or to access the analyte/matrix by a diffusion limited process. Given sufficient time, the analyte will achieve equilibrium between the supercritical fluid and the sample matrix. Beyond the division between static and dynamic the different forms of SFE are differentiated only by the fate of the extractable components.

Static and dynamic forms of extraction can be further broken down into categories dependent upon the fate of the extracted material. On-line and off-line methods exist for both static and dynamic extraction. On-line methods are those in which the extraction and following chromatographic or spectrometric analysis are done in a single analysis. The analyte is never isolated in a conventional manner. Off-line methods, however, require a trap/accumulator. Off-line methods of analysis also require at least two separate pieces of instrumentation - one for the extraction and another for the analysis.

Dynamic On-Line Extraction

On-line SFE/chromatographic methods have generated a good deal of interest because of the ability to perform trace analysis of organics quickly and easily. The heart of the system is the mechanism by which the analytes are trapped. Dynamic on-line methods all focus the extracted material onto a relatively small area. Once the extraction is complete the extract is then backflushed and/or thermally desorbed onto the appropriate chromatographic column for analysis. Both GC and SFC have been used for the instrumental method.

For SFC the extract is often collected on the inner surface of cooled tubing. A schematic of the general type of hardware used for the procedure is shown in Figure 3. The SF decompresses into a tee junction. One end of the tee junction leads to a

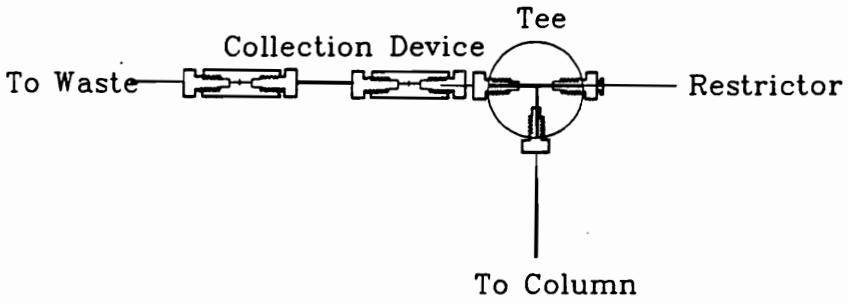


Figure 3

Typical Decompression tee for On-Line SFE\SFC

chromatographic column and the other leads to waste. Ideally, decompressed SF exits through the waste line, while the analytes are trapped in a narrow band on the surface of the collection device. The system is then backflushed with SF, thereby loading the analytes onto a column where chromatography is performed. During the backflush procedure SF may pass through both the restrictor and what had been the waste line. The analyte is subsequently solvated and directed into the chromatographic column.

Many different designs and details for an on-line SFE/chromatography system can be envisioned. Some of the variables for on-line SFE/chromatography include temperature of the trapping surface, the inner diameter of the trapping capillary, the nature of the capillary (stainless steel or fused silica), placement of the restrictor in relation to the trap, the actual hardware used in making the trap, etc. The tee design must be modified to minimize dead volume if dynamic SFE is to be followed by capillary SFC. Lee and coworkers[21] were successful in this regard by extracting ouabain (3-[(6-Deoxy-a-L-mannopyranosyl)oxy]-1,5,11a,14,19-pentahydroxycard-20(22)-enolide) in high yields with little or no decrease in chromatographic performance.

On-line SFE/GC is performed in much the same manner as described for SFE/SFC. The tee junction arrangement is conceptually the same but instead of being a simple tee junction it is the heated split/splitless injection port of a gas chromatograph. The purge and split outlets act to vent the decompressed SF while the analyte is trapped directly at the head of a cryogenically cooled column. After the extraction is complete any residual CO_2 is purged from the system and the normal GC temperature program is then performed. Alternately, the SF used for the extraction may be decompressed directly onto a large bore column (320-530 μm i.d.) or retention gap by way of a direct injection port[22]. The gaseous material would then exit through leaks in the direct injection port, since the injection port does not seal completely with a fused silica restrictor in the septum.

SFE/GC has developed more quickly than SFE/SFC. In part this can be attributed to the fact that at least half of the tandem technique is mature and well understood. SFE/GC also profits from the fact that volatile and semivolatile materials are readily extracted and that there are a number of GC methods that are routine, leading to easily adapted SFE/GC analyses.

Hawthorne and coworkers[23,24,25] have performed considerable work with the SFE/GC of polyurethane foam traps for air particulates and urban dust as well as marine sediments and incinerator fly ash. Excellent recoveries of polyaromatic hydrocarbons (PAH's) have been reported for the air particulate analysis. Somewhat lower recoveries have been reported for the extraction of PAH's from soils and sediments. Hawthorne and coworkers have probed the effectiveness of N_2O as well as CO_2 as extraction solvents.

The major difficulty in the design of on-line methods is the mechanical difficulty in creating a trapping system which accumulates all of the analyte in a tight band. Aside from the difficulty of design, a major problem of the SFE/GC or SFE/SFC is that it is quite easy to overload the system with a large quantity of analyte. Analytes that are present in concentrations greater than 10 ppm in the sample can block the trap tubing or interfere with the subsequent analysis if sample sizes are too large. Typical samples are generally less than 50 mg. Even at this small size, analyte present at 10 ppm will lead to 0.5 μg of analyte being loaded onto the chromatographic system regardless of whether a packed or capillary column is being used. Should sample inhomogeneity be a problem, many different SFE/GC or SFE/SFC analyses would need to be performed in order to provide an accurate representation of the sample. For samples which contain trace levels of organics in a homogeneous form, however, on-line techniques offer a means by which detection of organics at low concentrations can be achieved because the bulk of the extract is transferred directly to a chromatograph.

Dynamic Off-Line Extraction

Off-line dynamic extraction is arguably the most widely used SFE technique[26]. Analyte is simply moved from point A (analyte in matrix) to point B (analyte separated from matrix). The greatest difference in the way off-line SFE is performed is how the analyte is collected.

The simplest form of off-line extraction is collection of the analyte directly into a liquid organic solvent. The restrictor exits into a solvent vial where the SF decompresses. The analyte is trapped in the solvent while the SF escapes to the atmosphere. When CO_2 and N_2O are used as SFs there can be a great deal of cooling associated with the decompression of the SF. It is possible for the collection fluid to freeze and for small pieces of the organic ice to foul the restrictor tip. Either of these occurrences will have a detrimental effect on the extraction. The fluid flow through the system, which can affect the quality of the analysis, is usually less than 1/2 mL/min of SF. While this flow rate may seem rather slow, upon decompression of the fluid 300-600 mL/min of gas is produced. This large volume of gas produced at the restrictor outlet can cause violent bubbling of the solvent which can lead to aerosol formation and subsequent analyte loss to the atmosphere. The liquid solvent used for collection must match the polarity of the analytes being extracted so that the analyte will be soluble in the collection solvent. Methanol is frequently used as a solvent for polar analytes. Dichloromethane, on the other hand, is most frequently used with nonpolar analytes. In general, the use of dichloromethane is to be avoided because it freezes easily due to the cooling produced by the decompressing gas.

Recoveries and precision of extraction reported using liquid trapping depends upon the analyte and matrix being analyzed. Hawthorne and coworkers[27] have shown good precision and high recoveries for PAH's from urban dust, river sediment and fly ash

when collecting into a liquid trap. Reported recoveries varied according to the compound being extracted and by the SF used to perform the extraction. N_2O with 10% MeOH as modifier provided the best results in terms of overall recovery. Lopez-Avilia et al.[28] showed that CO_2 with 10% MeOH modifier performed the extraction of similar analytes (to those reported by Hawthorne) from similar matrices equally well. The problem that exists with modified fluid use is that the liquid solvent used for collection must be compatible with the modifier used. CH_2Cl_2 was used in both cases (Hawthorne and Lopez) as a collection solvent. Obviously when MeOH is used as a modifier, hexane or CCl_4 can not be used as a collection solvent as a two phase system would result. Flow rates and the amount of CO_2 used was not reported for both cases cited. In both papers cited, internal standard was added to the collection solvent before the extraction was performed in order to correct for the gross loss of analyte due to bubbling. Any loss due to bubbling would presumably remove the internal standard as well as the analyte so that no net effect on the analysis would result.

Collection of extracted analyte on a solid support in the off-line mode is also an option. Here the restrictor exits the extraction vessel onto a bed of solid sorbent material which may or may not be cryogenically cooled. Once the extraction is complete the sorbent is rinsed with organic solvent to remove the desired analyte. With this method of accumulation much faster flows of SF may be employed as there is no liquid to violently bubble out of the trap. However, breakthrough of analyte from the trap may occur if extracted analyte amount is high or if the analyte is sufficiently volatile.

The solvent used to rinse the analyte from the trap must be chosen carefully. A small rinse volume is desirable in the case of trace analysis so that the analyte is as concentrated as possible for the analysis to follow. Rinse solvent must first be able to solvate the analytes then move them efficiently through the trap. Standard solid phase

extraction (SPE) procedures can be used, but it must be remembered that many of the compounds that are quite soluble in SFs have limited solubility in organic solvents. Higher molecular weight alkanes are a good example. Beyond C_{30}, alkanes show limited solubility in hexane and chlorinated solvents, although they are quite soluble in SFs such as CO_2 and pentane. CS_2 would be a much more appropriate rinse solvent for this type of analyte.

Schneiderman et al.[29] used SiO_2 as a trap for extracted anthraquinone from wood pulp. The silica was rinsed with MeOH/acetone to remove the trapped analyte once the extraction was deemed complete. Recoveries greater than 95% were reported.

Static On-Line Extraction

Static on-line extraction methods are limited in that a high pressure gas must be monitored, in situ, or introduced into an analytical system. One obvious way to monitor the system is to construct a high pressure flow cell in the system so that the solvated components can be monitored spectrometrically[30]. Figure 4 shows such a system. The system consists of a controller, a pump, a simple valving scheme and an associated spectrometric detector. The valve allows the system to be pressurized and then isolated from the pump, creating a closed system. As drawn, the extracted analytes must migrate to the flow cell in a diffusion limited process. The distance that the extract must travel can be decreased dramatically by building the flow cell directly into the housing of the extraction chamber. The first experiments in SFE were done in this manner[31]. The recent development of high pressure recirculation pumps allows for the spectrometric device to be placed some distance away from the extraction chamber. Additionally, the mechanical mixing provided by the recirculation pump ensures homogeneity of the SF phase so that precise sampling can be achieved.

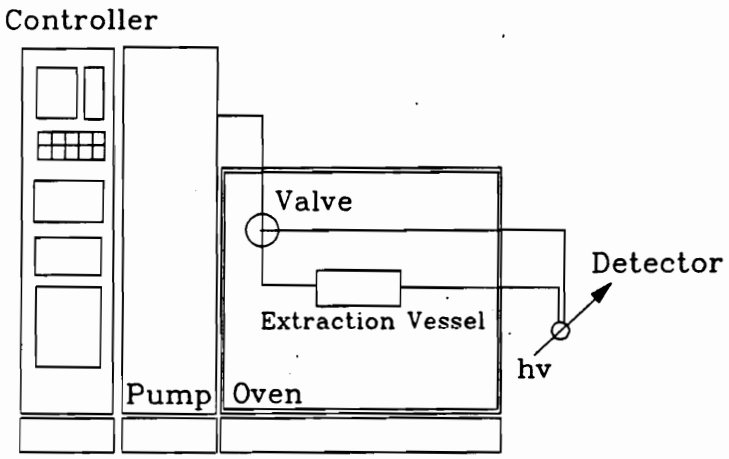


Figure 4

Static Extraction with Spectrometric Monitoring

Loading a small aliquot of material directly onto an SFC system also provides a means by which the high pressure gas can be easily managed[32,33,34]. Figure 5 shows a system similar to the previous spectrometric monitoring mode, but the difference is that the spectroscopic detector is replaced by a sampling valve that allows for a portion of the SF phase to be loaded onto a chromatographic system.

Chromatographic methods of analysis other than SFC require special handling of the system. In the case of liquid chromatography, static on-line methods can be used if the HPLC system is maintained at a pressure consistent with that of the extraction. A backpressure regulator placed post-detector easily accomplishes this pressure requirement. Static SFE followed by on-line gas chromatography requires that the injected SF be allowed to decompress, usually into the hot injection port of the GC.

Static Off-Line

Ideal static off-line extraction is difficult to perform. The problem that arises with a strictly static off-line extraction occurs when the system is depressurized and the analyte is collected. Since the analyte is never trapped, it is impossible to cleanly remove the analyte from the extraction vessel. As the system depressurizes, analyte falls out of solution, coating both the matrix as well as the sides of the extraction vessel. For this reason static extraction is most often used in series with dynamic extraction for off-line analysis. The combination of static and dynamic extraction modes is performed by the use of various valves. The extraction starts in strictly a static mode. There is no net flow through the system. When the extraction has proceeded for a given amount of time the system is put into a dynamic mode by the switching of valves. Fresh SF enters the vessel, replacing the SF which has exited through a restrictor. A net flow now passes through the

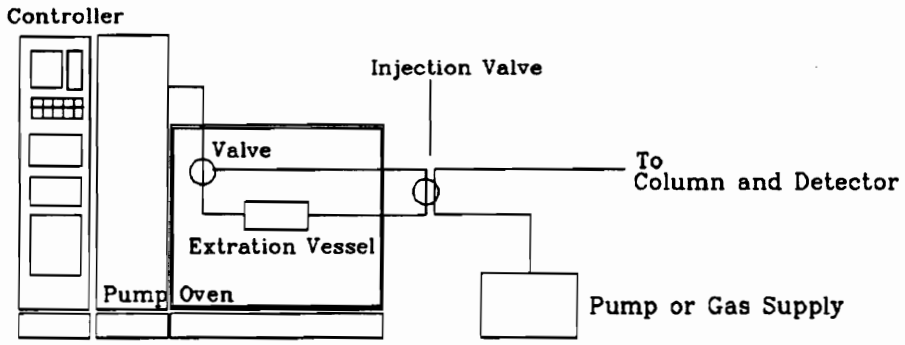


Figure 5

Static On-Line SFE/Chromatography

system. Because the system is always pressurized analyte does not fall out of solution until it exits the restrictor eliminating the major problem with strictly static extraction.

Extraction Profiles

Dynamic extraction is the most easily modeled of the two methods. Figure 6 shows the theoretical extraction profile of an analyte from a solid matrix using a dynamic system[35]. The y-axis represents the amount of analyte recovered from the system. The x-axis represents the amount of solvent or time used during the extraction. The extraction profile can be divided into three distinct regions. The initial extraction of material occurs relatively fast and is dependent upon the solubility of the bulk analyte in the SF (Region I). During this portion of the extraction the analyte is simply washed out of the extraction vessel. The limiting factors in region I are the solubility of the analyte in the SF, the rate at which SF passes through the system, and the amount of dead volume in the extraction vessel and associated tubing. Region II is an intermediate region where the extraction process is becoming diffusion limited and therefore shows a slower rate of extraction. Region III represents that portion of the extraction where the process is truly diffusion limited. The diffusion phenomenon is brought about by either the limited mobility of an analyte within a matrix (such as polymer additive in a polymer bead or a natural product within animal tissue) or by the slow rate of desorption from an active matrix surface.

Figure 6 can also describe the extraction profile for static or equilibrium extraction. The x-axis now represents time instead of volume of SF which is passed through the system. Region I, II, and III still represent a washing out, intermediate and diffusion limited process. As time increases, however, an equilibrium between the analyte in SF and analyte on (or in) the matrix is established. The diffusion process is no longer in one

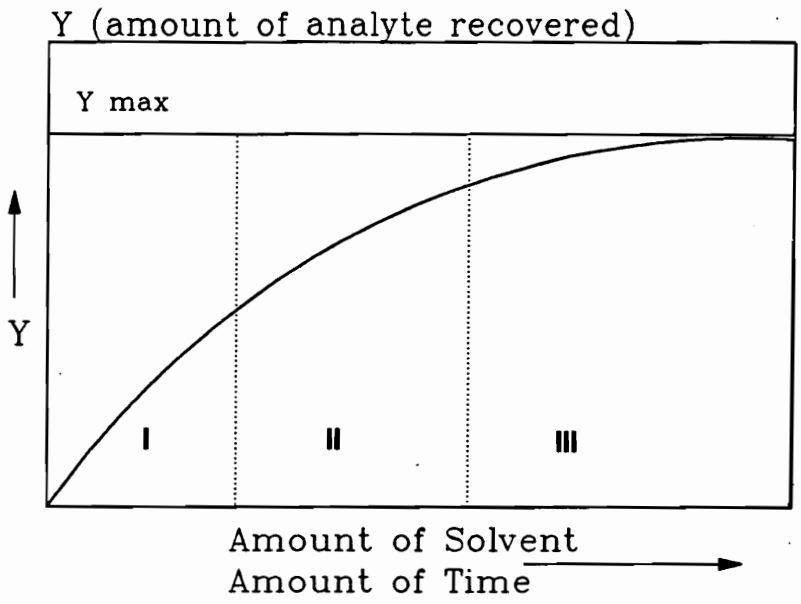


Figure 6

Extraction Profile for Dynamic Mode[35]

Region I - Solubility dependent region

Region II - Intermediate region (bulk analyte is almost removed)

Region III - Diffusion limited region

direction, as it is in the dynamic case. During a dynamic extraction fluid is constantly moving any extracted analyte away from the matrix so diffusion back into or onto the matrix does not occur. The static mode suffers from the fact that the equilibrium between the sample matrix and the supercritical fluid may not be favorable. Furthermore, exhaustive extraction can not be expected to occur in all cases. In practice the static mode is used most frequently in combination with a dynamic mode, as described previously.

Figure 7 shows the extraction profile of several static/dynamic cycles. Region I represents a true static extraction. There is no net flow through the system so the amount of analyte recovered (y-axis) is zero. Any analyte removed from the sample matrix is contained within the extraction vessel. Within the extraction vessel the concentration of the analyte increases with time as described in the previous extraction profile. Region II is a washing out phase. Valves have been switched so that there is now a net flow through the system. The analyte which has built up in the extraction vessel quickly washes out and a diffusion limited process follows. By returning the system to static mode (Region III) SF is conserved while the analyte concentration again continues to rise within the extraction vessel. Further dynamic/static extraction cycles are then carried out. The method can be seen to be advantageous if the SF is in limited quantity or costly. The time required to perform an exhaustive static/dynamic extraction, however, may be greater than that of a truly dynamic extraction.

In summary, the different modes by which SFE may be performed have been reviewed. SFE at the moment is a diverse technique. The techniques which have been mentioned herein are not meant to be all inclusive. Only a general review of some of the more popular methods was intended.

One aspect that was not addressed in this review is the actual time required for exhaustive extraction. The extraction profiles that were discussed were only

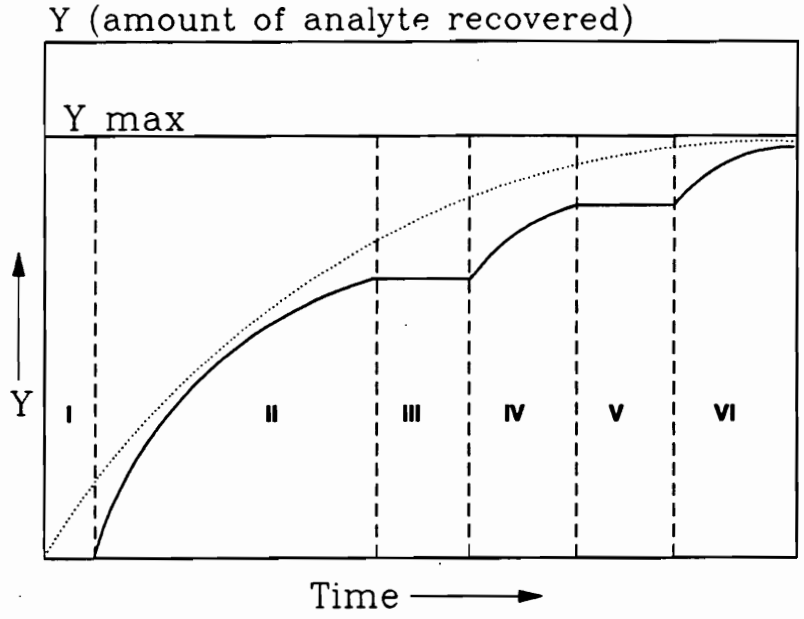


Figure 7

Extraction Profile for Static/Dynamic Mode

Region I - Initial static extraction (no movement of analyte through the system)

Region II - Dynamic extraction (analyte being removed from the system)

Region III - Second static extraction

Region IV - Dynamic extraction

Region V - Static extraction

Region VI - Dynamic Extraction

model systems. The exact time for complete extraction will be dependent upon the analyte and the specific matrix being studied[36].

Although the role of modifiers in extraction has not been discussed, modifier no doubt serves to alter the extraction profiles in contrast to the unmodified fluid. Both the bulk solubility of the analyte in the extraction fluid and the rate at which the analyte is desorbed from the matrix can be affected by the use of modifiers[37].

Chapter 3

Injection Considerations for Supercritical Fluid Chromatography

Introduction

Supercritical fluid chromatography has continued to mature over the past several years. In this regard, the reproducibility and manner of injection for both packed and capillary column SFC has been an area of active debate. When using capillary columns, the typical injection uses a splitting mechanism analogous to what occurs in standard split injection for gas chromatography. During the split injection process the organic solvent used for sample introduction is thoroughly mixed with the SF mobile phase so that a homogeneous phase is produced prior to the analyte entering the column. Injection onto microbore packed columns, however, is typically done in a manner analogous to injection onto a liquid chromatographic column. A sample loop is loaded with sample solution and then switched in-line with the mobile phase flow path. In liquid chromatography the injection presents little problem because the composition of the solvent used for injection is usually that of the mobile phase.

Supercritical fluid chromatography with both packed and capillary columns suffers in that the injected organic phase is never the same (disregarding SFE on-line techniques) as the mobile phase at the time of injection. If sufficient volume is not allowed for mixing of the organic sample solvent and the supercritical mobile phase, problems with column efficiency and reproducibility can occur. Split injection, as commonly performed with capillary column injection ($\cong 1$ μL), allows for sufficient mixing of organic and SF phases. Injection onto packed columns does not allow for adequate mixing for two reasons. The first is that there is no "mixing chamber" as there is for split injection. The second is that 10 to 100 times as much organic solvent is loaded onto the column (because the column is larger). Because the two types of injection are so different, injection of material into

capillary columns will not be discussed, although it is discussed in detail else where[38]. This is not to say that injection into capillary columns has been perfected. Injection into capillary columns has its own problems (most notably small volume introduction and reproducibility of the split ratio) which are not related to the following discussion.

A typical SFC analysis uses a pressure or density gradient to elute a wide range of analytes in a short amount of time. The density (pressure) gradient is analogous to a mobile phase gradient in HPLC or a temperature gradient in gas chromatography. Prior to the application of the gradient in SFC the pressure is held at a relatively low value (80-100 atm) to allow the sample solvent to separate from the analytes focusing the analytes at the head of the column. The low pressure hold is especially helpful when using flame ionization detection because many of the solvents used for injection respond well to a FID and can therefore mask the detector response of fast eluting analytes. The process can be thought of as microscale extraction where solvent is selectively extracted and removed from the system (through the column) while the analytes are trapped (focused) for subsequent chromatography by pressure programing.

The type of connecting tubing used between the injection valve and the column (1 mm packed) has been a point of argument in the past[39]. Conventional wisdom from HPLC suggests that the smallest amount of dead volume between the injection valve and column is prudent. The smaller the dead volume in the system the better the chromatographic performance. It has been suggested that for SFC this is not the case. Larger than necessary dead volumes between the injector and column may act as a mixing chamber so that the injected organic solvent will be less likely to interfere with the separation. It has been suggested that the result is an optimal dead volume between the injector and the column[42]. The optimum amount being a factor of the minimum amount of volume necessary for total mixing and the decrease in performance that will occur due to

extracolumn band broadening. The position and type of connecting tubing used with a standard injection valve has therefore been investigated.

Experimental

SFC Grade CO_2 was purchased from Scott Specialty Gas, (Plumsteadville, PA). Except where otherwise noted, the fluid was purchased with a 1500 psi headspace of helium to ensure that liquid CO_2 was delivered to the pump. All standards were purchased from Chem Service, Inc. (West Chester, PA). A Suprex SFC 200A (Suprex Corp.,Pittsburgh, PA) was modified to give better reproducibility of area counts of injected material. Figure 8a shows the standard configuration for the Valco injection valve, while Figure 8b shows the modified plumbing. The ideas for the modifications made to the stock valve came from several conversations with others[40,41] concerned with injection reproducibility in SFC as well as anecdotal evidence produced in our laboratory.

The injection valve has an internal loop (0.1 μL volume) which rotates from the load to inject position. In Figures 8a and 8b, the sample loop is designated by a thick solid arc. In the modified system, the injection and waste ports have been interchanged. The 0.05" i.d. Teflon line to waste used in the unmodified system was replaced with a section of 50 μm i.d. fused silica. The injection port itself was not modified. The only other modification to the system was the use of a section of 0.007" i.d. stainless steel tubing leading to the injector from the pump, as compared to the 0.05" i.d. stainless steel tubing commonly installed. The tubing connecting the valve to the column was of various i.d., length and composition (fused silica and stainless steel) and will be discussed later. A Micromeritics 725 autosampler (Norcross, GA) was used to deliver sample to the injection valve.

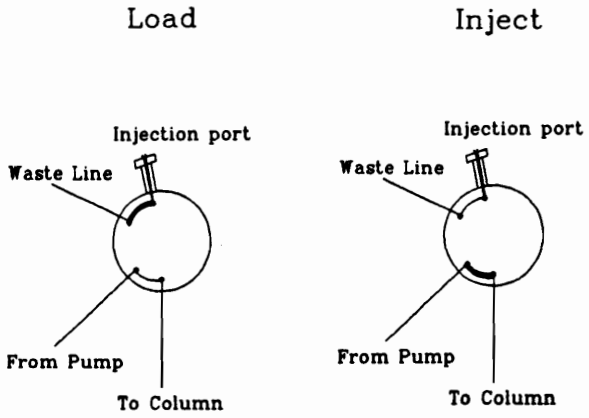


Figure 8a

Unmodified Injection Valve

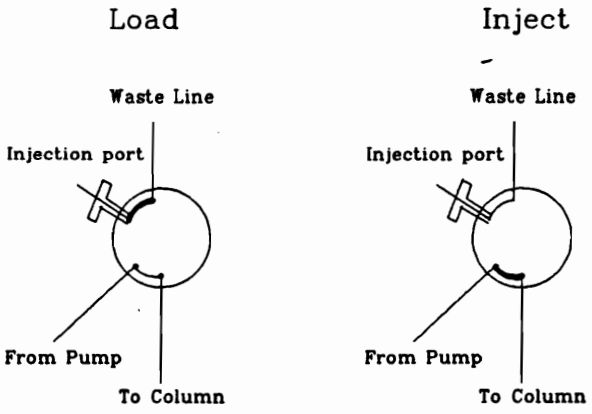


Figure 8b

Modified Injection Valve

Results and Discussion

The precision of injection in SFC is less than normally encountered with liquid chromatography for similar sample concentrations[42]. The precision of injection that is normally quoted in the literature for SFC with 1 mm packed columns and a 0.1 μL sample loop is on the order of 3-5% RSD[43] at analyte concentrations of 0.1-1 mg/mL. Increasing the precision to 1-3% RSD would be beneficial. The greater precision is especially necessary when a combination of techniques is being used, such as SFE followed by SFC. Greater precision of the SFC method allows for a more accurate interpretation of the extraction. The conventional plumbing of the valve was modified in hopes of increasing the precision of injection. The valve modification was discussed in the Experimental section of this work. The reversal of the inlet and waste ports had been shown to increase the reproducibility of injection for capillary columns[41] as did the use of a small bore capillary for the waste line. Presumably these changes allow for a more precise filling of the injection loop. The arrangement may also act to contain the sample as the sample loop is rotated. The use of small bore tubing leading from the pump to the injection port is novel. The idea here is that the small bore tubing will act as a slight restriction during injection so that flow of the injected solvent will be in one direction and not two (toward the column and toward the pump).

The test mix used to evaluate injection reproducibility was 0.1 mg/mL of 3,5-dinitrobenzamide dissolved in CH_2Cl_2. The results of the experiment (Table 1) represent five replicate injections of 0.1 μL for both the unmodified and modified injection valve. The chromatographic method used 100% CO2 as a mobile phase and a 1 X 100 mm DELTABOND CN packed column. The oven temperature and pressure were held constant throughout the run at 75°C and 200 atm. The

Table 1

Precision for Unmodified and Modified Injection Valve
100% CO_2
1 X 100 mm DELTABOND CN at 200 atm 75°C
restrictor = tapered
0.1 µL injection
0.1 µg/µL 3,5-dinitrobenzamide in CH_2Cl_2

	Unmodified		Modified	
	Tr (min)	Area	Tr (min)	Area
Average	5.93	6.2×10^5	6.56	5.62×10^5
s (n=5)	0.022	2.0×10^4	0.030	4.1×10^3
RSD	0.37%	3.2%	0.46%	0.74%

restrictor was tapered and flame ionization detection was utilized. Peak shape (as measured by peak asymmetry) of the analyte did not change with the valve modification.

The results of 5 replicate injections of 0.1 mg/mL of 3,5-dinitrobenzamide are shown in Table 1. The reproducibility of injection is substantially better for the modified valve. Relative standard deviation decreased to 0.74% compared to 3.24% for the unmodified valve. Although the goal of increasing the precision of injection was achieved fairly easily there are several other curious points which must be mentioned. Retention time for 3,5-dinitrobenzamide increased with the modification of the system. The increase in retention time along with an apparent increase in the RSD of retention time were completely unexpected. An F-test at the 95% confidence level revealed that the reproducibility of retention times for the two systems was not significantly different. In contrast, the retention times themselves were significantly different. The only modification to the flow path was the decrease in inner diameter of the connecting tubing which leads from the pump to the injection valve (0.05" to 0.007"). The increase in retention time of 3,5-dinitrobenzamide can only be explained by a restriction in the system caused by the smaller internal diameter tubing connecting the pump to the valve.

Area counts for 3,5-dinitrobenzamide decreased with modification of the system. The volume of the sample loop was not changed in any way so the reason for the decrease is puzzling. The retention time shift mentioned previously should not have affected the analyte response as the FID is a mass sensitive detector. The 50 μm i.d. capillary used as the waste outlet may have been the cause. The use of the 50 μm i.d. capillary, as opposed to the Teflon tubing for the waste outlet, was meant to provide a back pressure so that sample could not easily siphon to waste during the injection. Apparently, the exact opposite occurred for the unmodified system. The organic sample must have in some way compressed upon injection with the unmodified valve so that a larger amount (mass) of

sample was injected. The smaller mass of injected analyte giving a smaller signal in the FID. The decrease in signal of the analyte with the modified system was felt to be more than offset by the increase in precision obtained. The valve modification was therefore used in later studies.

Optimization of Injection Mixing Volume

In order to determine the effect of the connecting tubing between the injection valve and column three different types of tubing were investigated, (1) a 6.1 cm length of 0.01" (0.254 mm) i.d. stainless steel, (2) a 40 cm length of 0.100 mm i.d. fused silica and (3) a 20 cm length of 0.100 mm i.d. fused silica. The fused silica was methyl siloxane end capped. Fused silica was used for the smaller inner diameters because of its ease of use. The volume of both the 6.1 cm stainless steel and the 40 cm fused silica was 3.1 μL. The oven temperature was held constant while a pressure program was used. The pressure was held constant at 100 atm for 1 minute and then ramped to 400 atm in 20 minutes. The 20 cm fused silica was 1.6 μL in volume. A test mix of C_{12}, C_{18}, C_{24}, and C_{30} at 0.2 mg/mL per componant in CH_2Cl_2 was used. This particular mix was chosen in order to probe the effect that different analyte solubilities (in SF CO_2) would have on the injection. The lower chain length alkane (C_{12}) was the most soluble in CO_2. Five replicate injections were made with each length of connecting tubing. The modified plumbing scheme discussed earlier was used. The results are shown in Table 2.

In general the area count RSD increased with increasing carbon number for all three connecting tubes. This may be because peak width gets larger with increasing molecular weight (i.e. a greater capacity factor). The wider peaks may be more difficult for the integrator to measure precisely. Going from the stainless steel to the 40 cm length

Table 2

Influence of Connecting Tubing on Peak Width and Precision
100% CO_2
1 X 100 mm DELTABOND CN , 75°C
100 atm 1 minute
100 atm to 400 atm in 20 minutes
restrictor = tapered
0.1 µL injection

0.2 µg/µL C_{12}, C_{18}, C_{24}, C_{30} in CH_2Cl_2

Connecting Tubing		Analytes			
Stainless Steel		C_{12}	C_{18}	C_{24}	C_{30}
	Area RSD	2.0%	1.9%	2.0%	2.1%
	Peak Width (min)	0.144	0. 112	0.128	0.148
40 cm Fused Silica					
	Area RSD	0.73%	0.85%	1.1%	2.3%
	Peak Width (min)	0.118	0.116	0.132	0.152
20 cm Fused Silica					
	Area RSD	14 %	18 %	16 %	17 %
	Peak Width (min)	0.122	0.116	0.132	0.152

of fused silica the area count precision increases for the lighter alkanes but is actually higher for C_{30}. The fact that there is any difference at all between the two types of tubing is peculiar because the volume of the tubing is identical (i.e. only the internal diameter has changed). A possible explanation lies in the potential difference in flow characteristics of the two pieces of tubing. The larger diameter stainless steel may give rise to a mixing dead volume so that the volume of tubing must be cleared in much the same way as an extraction vessel must be cleared. The sample solvent and analyte would both follow an exponential dilution profile. The smaller diameter tubing may, on the other hand, act as a retention gap. The injected material would then flow in a discrete band to the column. Analyte would be deposited along a section of the capillary as the organic solvent dissolves into the CO_2 mobile phase. The action would be much the same as on column injection for gas chromatography. Retention gaps have been used in the past for capillary column SFC but not for packed columns. It is also possible that the smaller i.d. tubing simply imarts a more laminar flow to the "plug" of injected material. If the flow is laminar, an actual mixing void would not occur and efficiency of the syste would be expected to increase.

The use of peak widths here is to give an indication of efficiency of the system. The plate count of the column itself remains constant, no matter what connecting tubing is used. The loss, or gain, in efficiency due to extra-column effects is what is being probed. As a pressure gradient was used for this particular analysis plate height could not be calculated. Peak widths show a marked decrease for the lighter alkanes with the 40 cm fused silica as compared to the larger bore stainless steel. As the weight of the hydrocarbon increases the difference in peak width becomes smaller until there is no significant difference for C_{30}. Apparently the focusing effect due to the pressure program used for the analysis makes up for any loss in efficiency due to the wide bore stainless steel

connecting tubing. The use of a smaller section of fused silica (i.e. 20 cm) does not improve either the reproducibility of injection or peak shape. In fact the area reproducibility is far worse than either the stainless steel or the 40 cm fused silica cases. Although not reported in Table 2, decreasing the tubing length further to 10 cm decreases reproducibility even further. Peak widths however are the same as the values for the 40 cm fused silica case.

Band broadening with the 40 cm fused silica and the stainless steel tubing were also compared in an isobaric system. Plate counts for C_{30} could then be calculated . C_{30} was used as a probe because of the k' it exhibited under the test conditions. The lighter analytes exhibited k' well under 3 and also coeluted with the solvent front. The plate count would then give a direct measure of system efficiency. The chromatographic conditions were 100% CO_2, 1X 100 mm DELTABOND CN at 225 atm and 75°C. The restrictor was tapered and the injection volumewas 0.1 µL. The test mix was 0.2 µg/µL C_{12}, C_{18}, C_{24} and C_{30} in CH_2Cl_2. C_{30} displayed a k' of 3.3 for the fused silica system (1070 plates/column) and 3.0 for the stainless steel (873 plates/column). The difference in internal diameter apparently affected the plate count for the column by approximately 20%. This may explain why 1 mm columns display reduced plate counts with SFC as compared to the same columns used for HPLC[44].

The employment of fused silica tubing has a practical advantage in SFC, as is apparent in Figures 9a and 9b which show the separation of 0.2mg/mL C_{30}, C_{24}, C_{18}, and C_{12} in CH_2Cl_2. Figure 9a was obtained with the stainless steel (6.1 cm, 0.01" i.d.) transfer line Figure 9b was obtained with the 40 cm, 0.100 µm i.d. fused silica. The chromatographic conditions were the same as used for the plate count study with C_{30}. The fused silica clearly offers a reduced solvent front as compared to the stainless steel. The

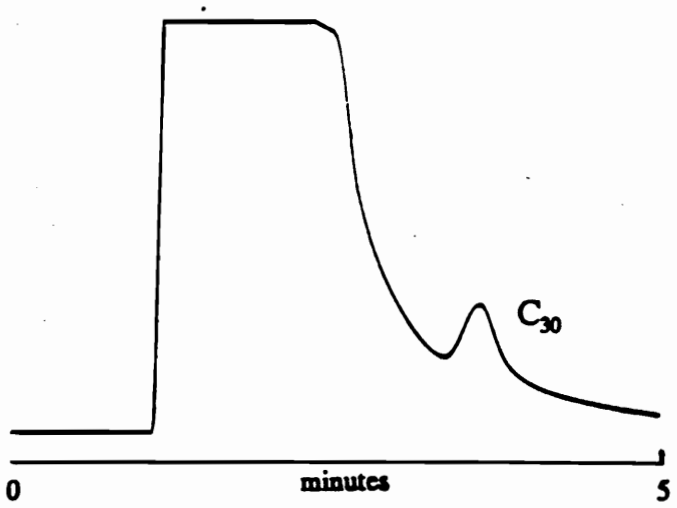


Figure 9a

Direct injection using 6.1 cm, 0.01" stainless steel transfer line.
100% CO_2
1 X 100 mm DELTABOND CN at 225 atm 75°C
restrictor = tapered
0.1 μL injection
0.2 μg/μL C_{12}, C_{18}, C_{24}, C_{30} in CH_2Cl_2

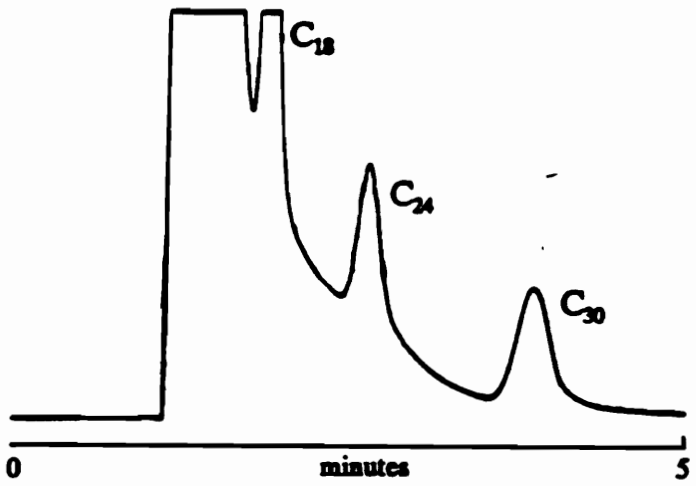


Figure 9b

Direct injection with 40 cm, 100 μm i.d. fused silica transfer line.
100% CO_2
1 X 100 mm DELTABOND CN at 225 atm 75°C
restrictor = tapered
0.1 μL injection
0.2 μg/μL C_{12}, C_{18}, C_{24}, C_{30} in CH_2Cl_2

amount of solvent injected is identical but the solvent front obtained with the 40 cm fused silica is much more compact. As a result C_{18} and C_{24} are observed with the fused silica tubing but not the 0.01" stainless steel. C_{12} is not observed in either case, while C_{30} can be observed in both.

Conclusion

The precision of conventional injection has been shown to be significantly improved by relatively minor modifications of the plumbing associated with the injection valve. The modifications do not limit the method nor do they in any way interfere with the normal operation of the instrument. The tubing that connects the injection valve to the column has been shown to be of impertance to the chromatographic efficiency of the system. Of the connection tubing that was studied, the best choice for connecting tubing is clearly the 40 cm length of fused silica. The 40 cm lenght of 100 μm i.d. fused silica provided increased system efficiency as well as a reduced solvent front. The smaller diameter connecting tubing, along with modifications to the injection valve have been shown to offer many advantages to a stock commercial system. The modifications to the system , however, do not interfere with its operation.

Chapter 4

Solvent Elimination Injection for SFC

Introduction

A topic related to standard chromatographic injection is solvent elimination injection. If standard injection of solvent and sample can be thought of as an extraction process followed by chromatography, it should be possible to perform the same sort of extraction on larger volumes of organic solutions. The ability to load larger solvent volumes (on the order of 10 to 100's of μL) onto 1 mm columns would drastically decrease the concentration of solutions that could be analyzed. This is especially attractive since flame ionization detection is fairly insensitive with packed columns due to the large amounts of CO_2 that pass through the flame. By increasing the size of the sample that can be injected by 10 to 100 times the resulting concentration limit of detection will be 10 to 100 times lower. The major problems with injecting large amounts of solvent into the chromatographic system are the dead volume created by the loop itself and the inability of large volumes of organic solvent to mix thoroughly with supercritical CO_2 in a small volume. Both problems would lead to loss in efficiency of the resulting separation.

A system has therefore been developed to remove the injection solvent in a short amount of time, prior to the chromatographic column, while retaining the analytes on a trap that can be backflushed so that on-line chromatography can be performed. Before the system was designed several goals for the system were established. (1) The system must be able to increase the sample injection size to 50 μL while maintaining chromatographic efficiency. (2) The time added to the analysis must be less than 10 minutes. (3) Precision of the system should be 5% RSD (relative standard deviation) at the 10 ppb level since other systems for solvent elimination[39,52] have been plagued by poor

reproducibility. The system developed in this laboratory will be referred to as solvent elimination injection for SFC (SEI for SFC).

Experimental: Solvent Elimination Injection for SFC

The ability to load large volumes of sample (10-100 μL) onto a 1 mm packed column can be beneficial in lowering the concentration of analyte that can be detected. The major problem with this approach is that large volumes of organic solvent are not necessarily compatible with supercritical CO_2 on small bore packed columns that are normally used for SFC. A method for removing the solvent while concentrating (focusing) the analyte was needed. Figures 10a and 10b show the plumbing scheme designed for SEI/SFC. The system consists of 2 six port valves (Rheodyne Inc., Cotati, CA) a three port valve (VICI, Houston, TX) and a decompression tee. Valve B and the decompression tee as well as the chromatographic column, a 1 X 100 mm DELTABOND cyano packed column (Keystone Scientific, Inc., Bellefonte, PA) are within the oven compartment of the Suprex 200A. Sample is loaded into a 50 μL injection loop (C). The loop is then placed in-line with the flow so that the fluid expands into a 0.5 mL dead volume (a 0.5 mL extraction vessel, Suprex Corp., Pittsburgh, PA) between valves "B" and "C". The sample (solvent and analyte), now in solution with supercritical CO_2, flows through associated plumbing to an outlet restrictor (liquid CO_2 flow set at 2 mL/min by pump displacement at 250 atm) that is located in a decompression tee (T). Several tee designs were evaluated (Figures 11a, 11b, and 11c). Analyte is trapped within the tee while solvent, having a greater vapor pressure, as well as gaseous CO_2 are vented to the atmosphere (W). After sufficient CO_2 has passed through the system to clear the dead volume of analyte, the trap is then backflushed with SF (by rotating valve B) and conventional SFC is performed (Figure 10b).

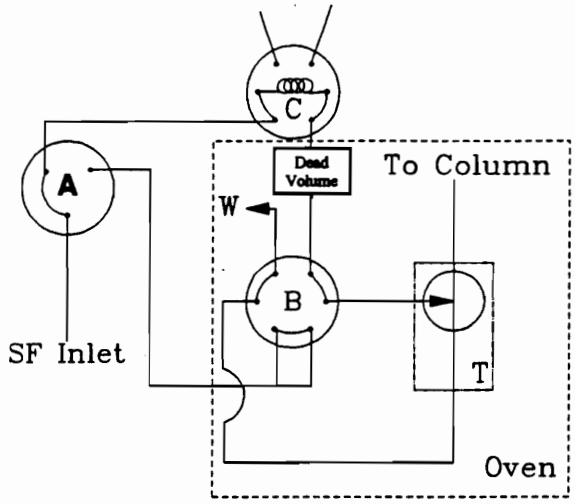


Figure 10a

Inject Mode for SEI/SFC

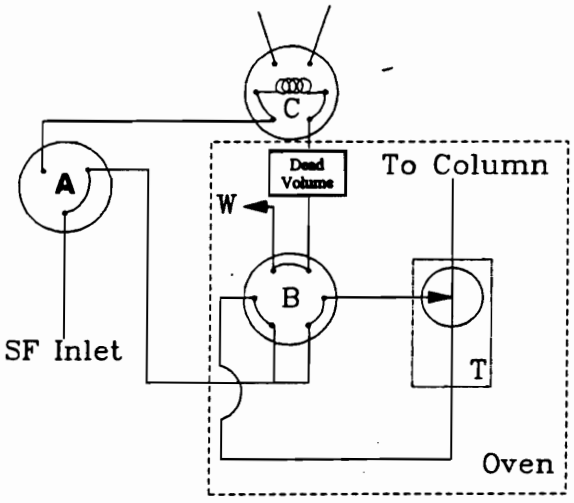


Figure 10b

Chromatography Mode for SEI/SFE

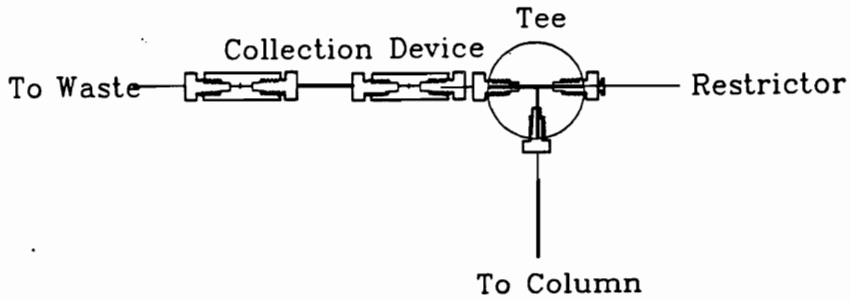


Figure 11a
On-Line SFE/SFC Trap A

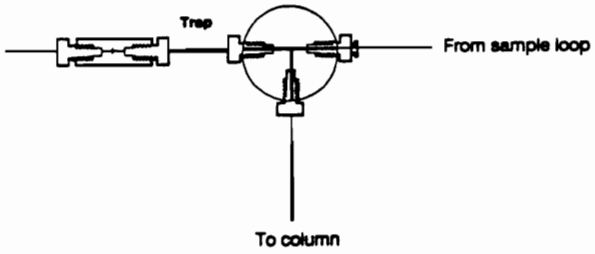


Figure 11b
On-Line SFE/SFC Trap B

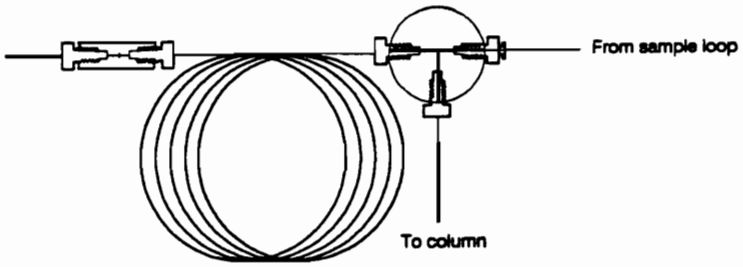


Figure 11c
On-Line SFE/SFC Trap C

A novel plumbing arrangement was designed to allow solvent to pass through both the restrictor and the trap during the backflushing portion of the analyses. This scheme differs from the plumbing found on commercial SFE/SFC systems where SF is only passed through the trap and not the extraction restrictor. Initial development of the SEI system as well as work performed on SFE/SFC systems suggested that SF flow through only the trap during backflushing of the system leads to restrictor plugging. By allowing fluid flow through the restrictor during "extraction" it is felt that restrictor lifetime was significantly lengthened. The restrictor of the SEI system did not plug at all during the four month period of time the experiments were being done.

Chromatographic response as a function of "extraction" time was performed by extracting separate samples for different amounts of time. Between each extraction and subsequent chromatography a blank extraction was performed to remove any analyte which had not been extracted in the prior experiment. An incomplete extraction, therefore would not interfere with a subsequent analysis.

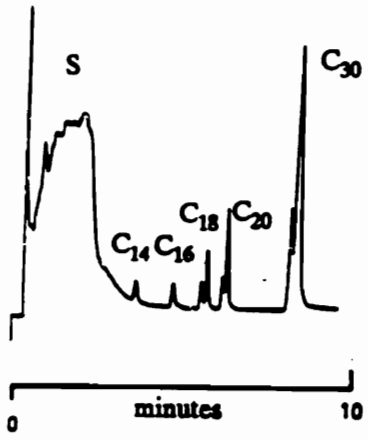
Results and Discussion

The valving system used in this work was described in the Experimental section. The heart of the system is the trapping scheme. Several different factors of the SEI/SFC system were studied. The first was the design of the trap itself. The decompression and trapping system is similar to that used in some commercial instruments(most notably the Suprex MPS-225). The main difference between SEI/SFC and SFE/SFC being that the trap design for SFE/SFC is designed to collect all of the componants that are extracted and then load the extracted material onto a column for chromatography. Solvent elimination has the added requirement of allowing volatile solvent to exit the system while maintaining good trapping efficiency for the analytes of interest.

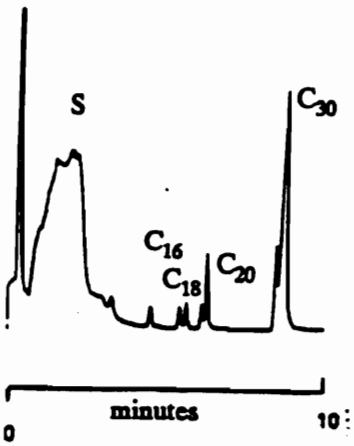
Three different trapping systems were evaluated. The traps are shown in Figure 11a, 11b and 11c (p 39). Traps 11a and 11b contain a section of 0.05" internal diameter stainless steel filled with 5 μm DELTABOND CN chromatographic packing material. On Figures 11a and 11b the section filled with solid phase is labeled "collection device" and "trap". The difference between trap 11a and trap 11b is the presence in trap 11a of a connecting section of tubing between the decompression tee and the solid sorbent. It was hoped that the packing material would actively trap analytes but allow solvent to pass through unhindered. In addition, the cryogenic cooling (from Joule-Thompson cooling of the expanding CO_2) of the packing material should also contribute to the trapping process.

Figure 12 shows the effect of "extraction time" on SEI/SFC for a series of alkanes (C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{30} at 50 ppm per component in CH_2Cl_2) with trap 11a. The injection volume was 50 μL. The four different traces are the result of four different sets of SEI/SFC conditions. In all cases the SFC portion of the experiment was identical (1 X 100 mm DELTABOND cyano, 75° C, 1 minute hold at 100 atm then ramped linearly to 300 atm in 15 minutes). The SEI portion of the experiment was carried out at 300 atm and 75°C for different lengths of time. Clearly the longer the extraction is carried out the smaller the solvent peak. Unfortunately, the later eluting analyte peaks are split for all extraction times. As the time of extraction increases the splitting of the lighter hydrocarbons (C_{16} and C_{18}) becomes less apparent until at an extraction time of 16 minutes C_{16} and C_{18} do not appear to be split. This splitting is thought to occur due to the two different trapping regions that exist in trap 10a. Specifically, cryogenic trapping can occur on both the connecting tubing between the tee and trap as well as on the solid sorbent itself. The solid DELTABOND CN sorbent also acts to actively trap analyte due to adsorption. The reduction in peak splitting for the lighter hydrocarbons with increased extraction time is probably due to a reduction of material trapped solely by adsorption (the

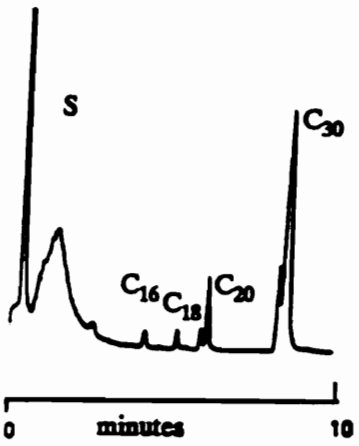
2 Minute Extraction



4 Minute Extraction



8 Minute Extraction



16 Minute Extraction

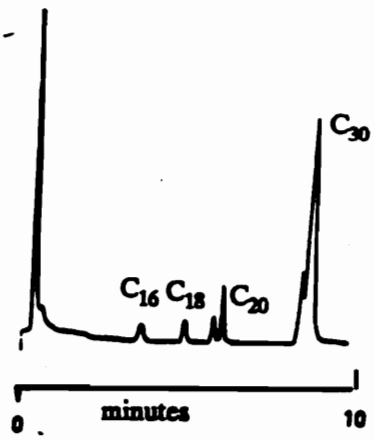


Figure 12

Effect of extraction time on peak areas

Trap Type 10a (SEI/SFC conditions are given on the previous page)

volatile analyte is vented with the solvent). The loss is attributed to the solid sorbent region and not the cryogenic trapping region because it is the later eluting of the two peaks which decreases in area. Since the cryogenic region precedes the solid sorbent (i.e., it is the closest to the chromatographic column), it would be expected to be accountable for the earlier peak. Trap type 11b also gave rise to peak splitting similar to trap 11a. Apparently enough dead volume still existed in the system to trap analyte in two distinct areas, even though the tubing connecting the solid sorbent and the decompression tee had been removed. The splitting exhibited for trap 11 b strongly suffests that the majority of analyte trapping occurs in the region directly after SF CO_2 decompression.

Trap 11c has no solid packing material. The only mechanism by which analytes can become accumualted by the system is by cryogenic trapping. Because there is only one trapping mode splitting is not a problem. This can be seen in Figure 13. Trap type 11c was used for all further work to avoid peak splitting of analytes.

The effect of different internal diameter tubes at the exit of the decompression tee (shown in Figure 11C) was then studied. It was hoped that smaller internal diameter tubing would both increase the trapping and chromatographic efficiency of the system. The tubing used in the study was one of the following: 30 cm of either 0.02" (500μm) or 0.005" (125 μm) internal diameter stainless steel, or 1 meter lengths of 110 μm and 200 μm internal diameter fused silica. Chromatographic peak area for a series of hydrocarbons (C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30} at 50 ppm per component in CH_2Cl_2) was plotted against "extraction time" to determine the optimum time of extraction (SEI) as well as to give an indication of the trapping efficiency of the system. The results are shown in Figures 14-17. A horizontal line indicates that the amount of trapped analyte remains constant with extraction time. A curve sloping upward indicates that the extraction has not gone to completion and the amount of trapped analyte increases with time. A

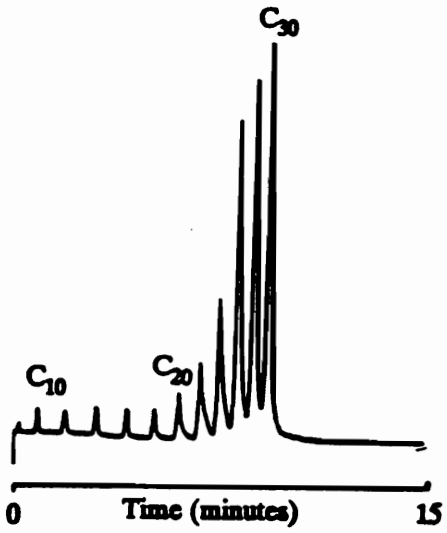


Figure 13

Chromatography using Trap 11c with 0.02" Stainless Steel

Oven Temperature = 50°C
Column = DELTABOND CN
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes
50 ppm C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30} in $MeCl_2$

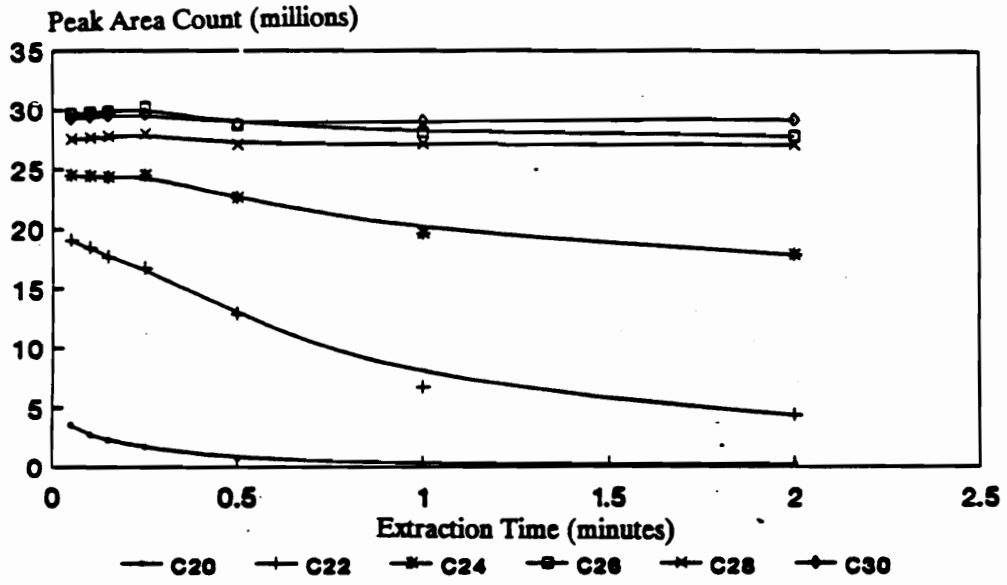


Figure 14

Peak Area vs Extraction Time using Trap 11c with 0.02" i.d. Stainless Steel Tubing

Oven Temperature = 90°C
Column = DELTABOND CN
Variable SEI time
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes

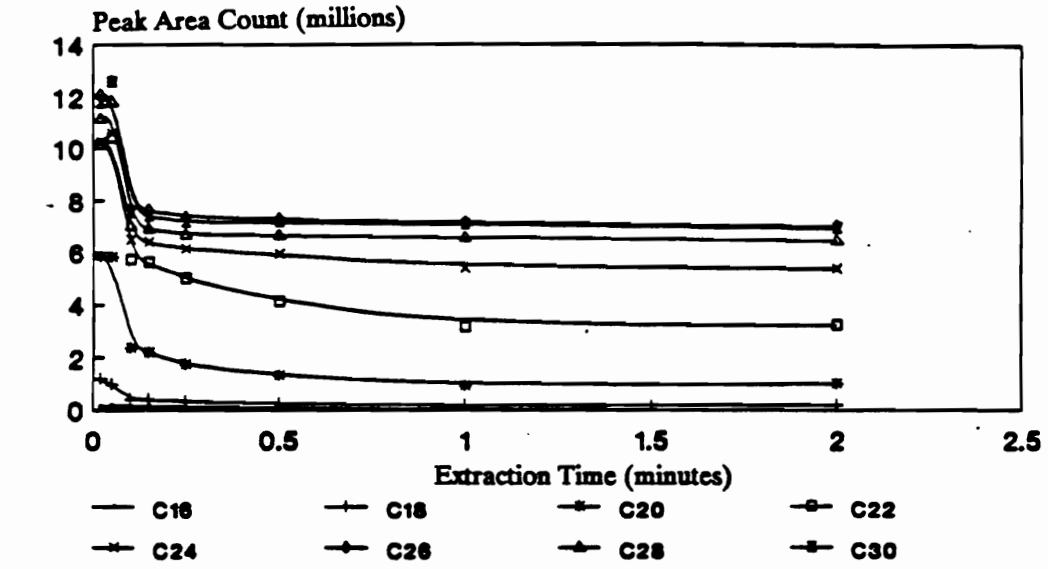


Figure 15

Peak Area vs Extraction Time using Trap 11c with 0.005" Stainless Steel Tubing

Oven Temperature = 75°C
Column = DELTABOND CN
Variable SEI time
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes

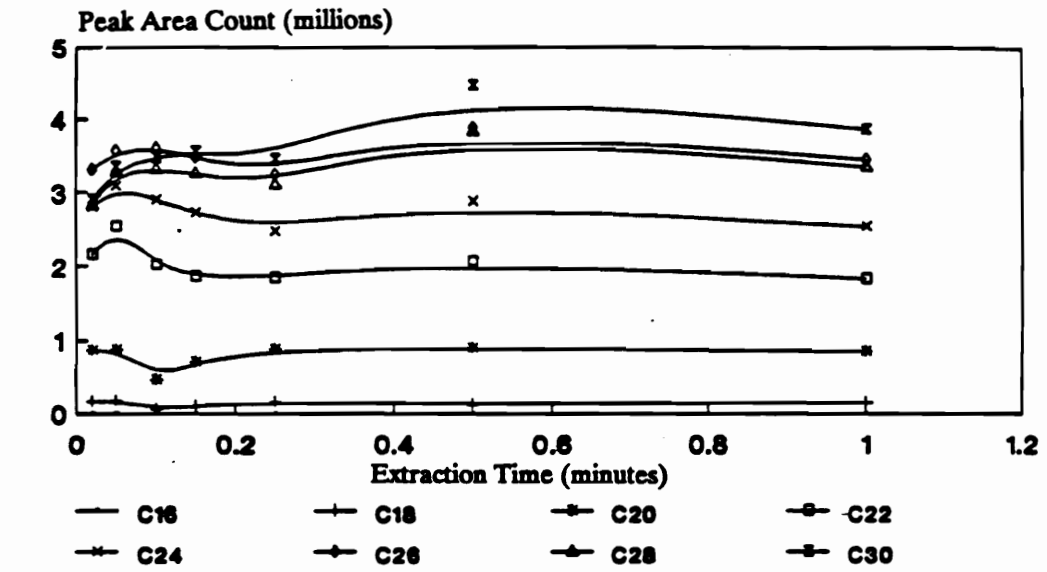


Figure 16

Peak Area vs Extraction Time using Trap 11c with 200 µm Fused Silica Tubing

Oven Temperature = 75°C
Column = DELTABOND CN
Variable SEI time
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes

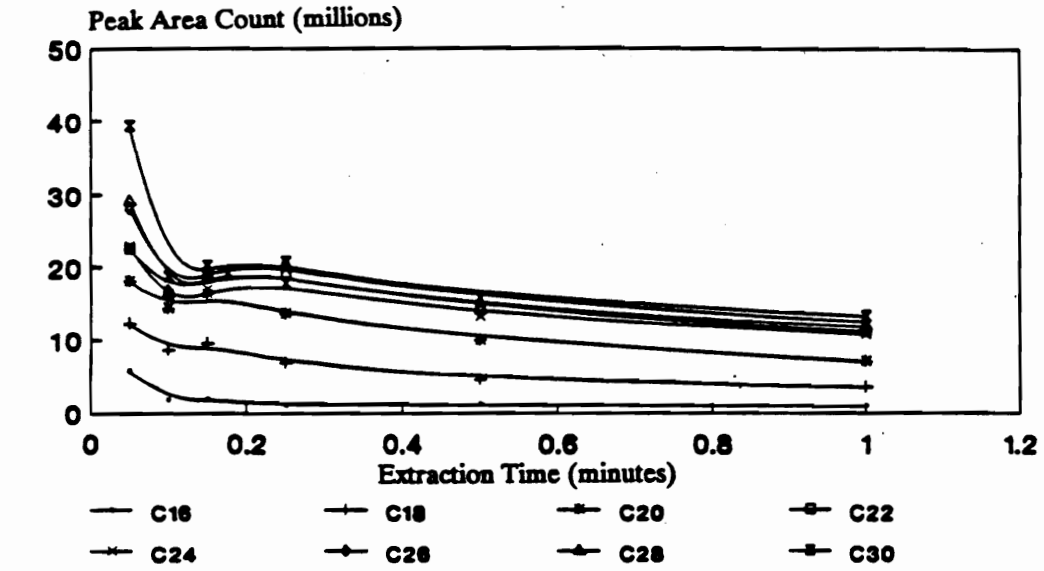


Figure 17

Peak Area vs Extraction Time using Trap 11c with110 µm Fused Silica Tubing

Oven Temperature = 75°C
Column = DELTABOND CN
Variable SEI time
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes

downward sloping curve, indicates that the trap is functioning less than perfectly and analyte is not being trapped at 100% efficiency. The 0.02" i.d. stainless steel tubing (Trap 11c) shows good trapping for alkanes with vapor pressures less than that of C_{22} at an oven temperature of 50°C. Decreased trapping efficiency of the lighter alkanes was observed when the trapping temperature was 90°C (note the gradual decrease in peak area vs time for C_{20} - C_{24}). A system of this type would provide adequate trapping of analytes with fairly low vapor pressures but inadequate trapping for more volatile components.

The smaller (0.005" i.d.) stainless steel tubing (trap 11c) produces interesting results. There is an initial decrease of analyte trapped with extraction time, followed by constant trapping of all compounds (Figure 15). Increasing the oven temperature from 75° C to 90°C had the same effect as in the 0.02" i.d. tubing case. The initial decrease in analyte trapping may be due to sample solvent condensing in the trap, thereby dissolving some analyte. The analyte, now redissolved in the injection solvent, could then be mechanically removed from the system (fluid pushed by the force of the gas flow).

The trap tubing was then changed to fused silica (200 µm i.d.) in order to provide more even heat transfer and hopefully eliminate the loss of analyte due to condensation of solvent. Figure 16 shows the results. Trapping efficiency is good (relatively constant) for all analytes down to C_{20} although an initial loss still occurs. The trapping is also discriminative for the heavier compounds. The 200 µm tubing provides efficient removal of solvent and provides good trapping characteristics. Decreasing the internal diameter of the fused silica used as the trap to 100 µm (Figure 17) produces the same results as the 0.005" diameter stainless steel tubing. An initial decrease of material is observed followed by a slow loss of analyte with increasing extraction time.

Retention time precision for the hydrocarbons is worse with SEI/SFC than that of a standard injection with or without modification of the injection valve (Table 3). The use

Table 3

Precision for 200 µm trap

Oven Temperature=75°C
Column= DELTABOND CN
1 minute extraction
1 minute hold at 100 atm
100 atm to 400 atm in 20 minutes

Alkane	RSD Rt	RSD Area
C20	0.22	5.9%
C22	0.25	2.8%
C24	0.22	1.3%
C26	0.27	0.87%
C28	0.34	1.7%
C30	0.34	1.4%

of several manually operated valves in the SEI mode to control the various flow paths may be the cause of the poor reproducibility. Automatic switching of these valves is possible with the appropriate hardware, and this automation may decrease the RSD of retention times.

Peak area precision, however, is quite good for C_{22} and above when using 1 meter of 200 μm i.d. fused silica as the trap tubing (Table 3) although it is not as good as was obtained with the modified direct injection system as discussed in Chapter 3. The concentration of the analytes studied, however, was ten times less (50 ppm) in the SEI/SFC case than the conventional analysis case (500ppm) (see Table 1, p28). C_{20} is the lightest hydrocarbon that appears reproducibly (< 6% RSD). Clearly the technique would not be appropriate for analytes with a vapor pressure greater than C_{20}. The difference between an analyte being trapped precisely and not being trapped precisely is quite sharp. The fact that the SEI system has difficulty in trapping materials with vapor pressures greater than C_{20} is a short coming of the system. Obviously, many compounds of interest, will have significant vapor pressures. From a practical point of view, compounds with vapor pressures greater than C20 should probably be analyzed by gas chromatography and not SFC.

Applications of SEI for SFC

The utility of SEI/SFC is apparent in Figures 18a and 18b. The sample is a CH_2Cl_2 extract of a single base propellant. Figure 18a is the FID trace of the extract employing conventional injection, while Figure 18b is the same sample performed by SEI/SFC. Chromatography was performed on a 1x250 mm DELTABOND CN column at 75°C. The pressure was held at 100 atm for 1 minute and then ramped to 400 atm in 40 minutes. The SEI step was performed at 200 atm for 2 minutes. The injection volume was 50 μL

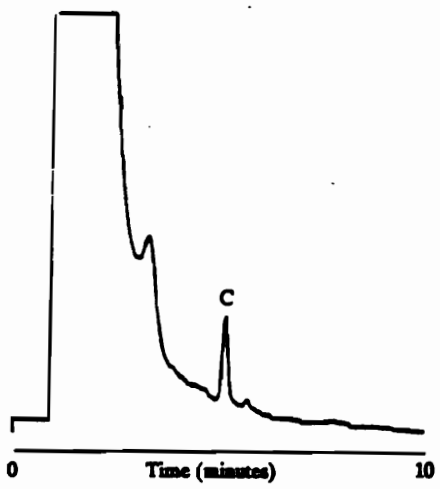


Figure 18a

Conventional Injection of Single Base Propellant

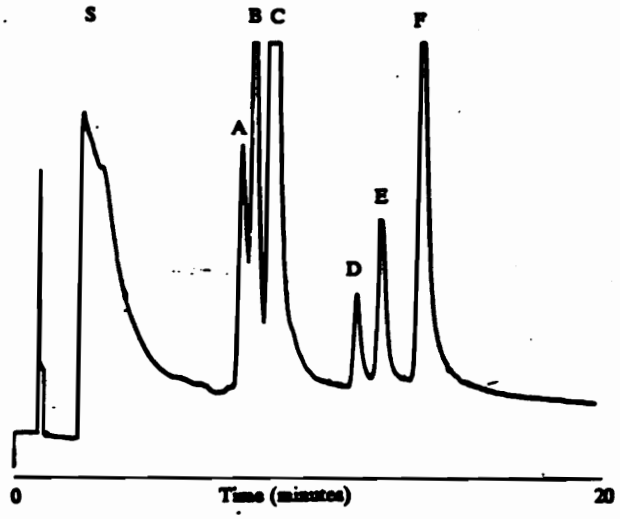


Figure 18b

SEI/SFC of Single Base Propellant

A,B,C dinitro-toluene (different positional isomers)
D diphenylamine
E 4-nitrodiphenylamine
F 2,4-dinitrodiphenylamine

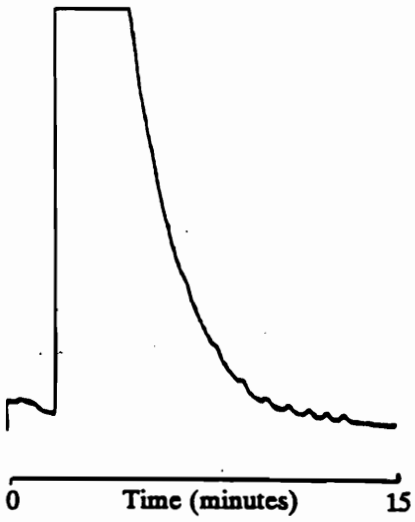


Figure 19a

Conventional Injection of Alkane Series

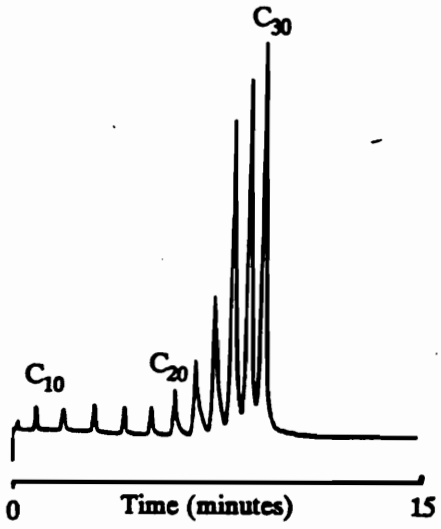
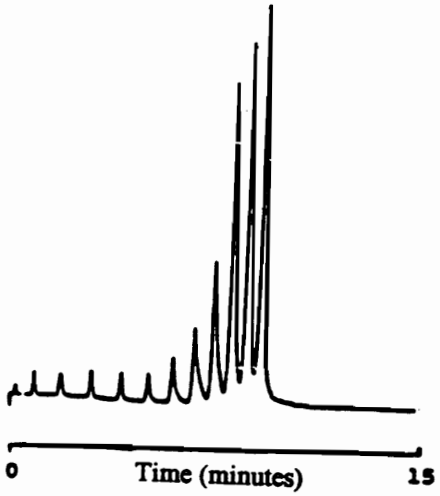


Figure 19b

SEI /SFC of Alkane Series



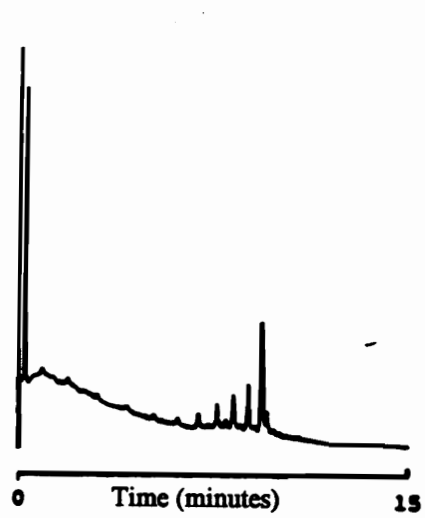


Figure 20

SEI Analysis of Concentrated Hydrocarbons

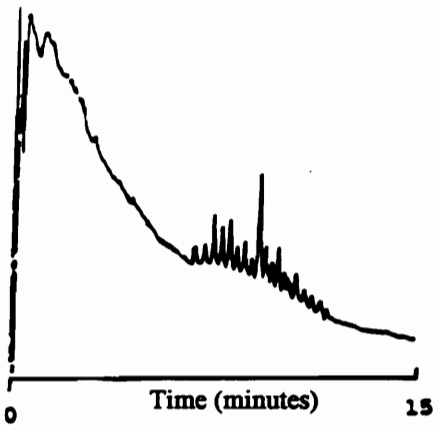
Extraction

- 225 atm
- 75°C
- 10 minutes
- 50 μL loop

Chromatography

- 100 atm for 1 minute
- 100 atm to 400 atm in 20 minutes
- 75°C
- 250 X 1 mm DELTABOND CN

30 ppb



Blank

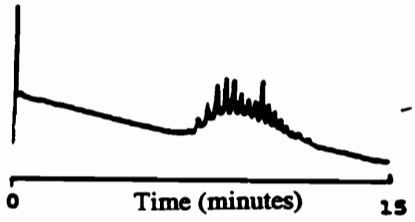


Figure 21

SEI of Dilute Hydrocarbons

Extraction

- 225 atm
- 75°C
- 10 minutes
- 50 μL loop

Chromatography

- 100 atm for 1 minute
- 100 atm to 400 atm in 20 minutes
- 75°C
- 250 X 1 mm DELTABOND CN

while the trap that was used was 11c with 200 μm fused silica tubing. Clearly the amount of information delivered is much greater when SEI/SFC is performed. Concentration of the CH_2Cl_2 extract by rotary evaporation would probably allow the sample to be analyzed by conventional SFC, but would increase the chance for impurities to be incorporated into the sample and for labile analytes to decompose. The peak identifications are listed below the chromatogram. For the standard injection only the dinitrotoluenes can be observed.

Figures 19a and 19b compare the different SFC/FID responses of SEI and conventional injection (0.1 μL injection) for an alkane series (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30}) at 50 ppm per component in hexane. The SEI injection was 50 μL and again used Trap 11c with 200 μm fused silica tubing. The figure shows the advantage of SEI/SFC as well as the selective trapping that occurs (i.e. lighter components are not trapped as efficiently as the heavier ones). Figures 20 and 21 show the chromatographic traces for the same hydrocarbon series at 50 ppm, 1.5 ppm, 30 ppb and a blank analysis. The 30 ppb level trace indicates a major problem with SEI/SFC. Contaminants in the CO_2 can build up and interfere with the detection of analytes. The contamination levels in the CO_2 are probably less than 1 ppb per component but because several mL's of CO_2 are passed through the system they can account for fairly large peaks that can mask the elution of the analytes of interest.

Conclusion

SEI/SFC has been shown to be a valuable technique for the analysis of solutions that are too dilute to be easily analyzed by conventional SFC. The design that has been discussed does suffer from the fact that a good deal of operator involvement is necessary in order to switch valves manually. As a consequence the reproducibility of retention time

is less than that of a fully automated system. With the proper automatic valving however the system would easily lend itself to automation.

SEI also suffers from the loss of analytes during the solvent elimination process. Analytes with a significant vapor pressure (greater than that of C_{20}) are vented along with the solvent. The loss of these compoents is of minor consequence as the majority of compounds that are analyzed by SFC are non-volatile. Volatile compouunds are more readily analytzed by GC. The fact that volatile componants are removed may also be an advantage for two reasons. The first being that a wide range of solvents could be used (here only CH_2Cl_2 and hexane wwere used). The second advantage is that volatile components that interfere with detection are removed from the analysis.

CHAPTER 5

Solid Phase Trapping for SFE

Introduction

During the course of off-line supercritical fluid extraction there comes a point when the analytes which have been extracted must be collected and the SF decompressed and vented from the system. Some of the more popular methods of trapping are discussed in Chapter 2. The use of a solid phase sorbent has certain advantages over direct collection in a liquid solvent. Trapping onto a solid sorbent after extraction from aqueous systems was chosen for several reasons. The first reason was purely practical. Past experience from SFE of high water content samples suggested that restrictor plugging would be a problem if direct collection into a solvent was used. Another related problem was that, after extraction of high water content samples, the fused silica restrictor was found to become brittle and break with excessive movement. Along with these reasons the more general advantages of solid phase trapping (discussed in Chapter 2) were also a factor in the decision to use solid phase trapping. Unfortunately, during the time when SFE was being performed in our laboratory a commercial instrument for depositing analytes which have been extracted by SFE onto a solid sorbent did not exist. Since that time, two instruments of this kind have been introduced.

The design and evaluation of a solid sorbent trapping system for use with on-line SFE follows. There were five design goals that were hoped to be achieved in the system. (1) The flow of SF through the system was to be between 1 mL to 3mL/min. Slower flow rates were deemed impractical as extractions would be flow dependent and faster flows were thought to produce too large a volume of gaseous CO_2 to vent easily. (2) The temperature of the trap had to have some degree of freedom. That is, that the temperature of the trap should be below ambient and be able to be set with some measure of freedom.

(3) A variety of solid phase packins should be available. A variety of packings would give more freedom to any sample preparation that needed to be performed after the extraction.
(4) The system should be easy to use. The use of multivalved systems was to be avoided.
(5) The restrictor tip should be mounted in such a way as to increase its life time.

Experimental

Figure 22 shows the solid phase trapping apparatus used for the collection of analyte from both aqueous and solid matrices in the dynamic off-line extraction mode. The pump, oven and controller of the system were an unmodified SFE 50 (Suprex Corp., Pittsburgh, PA). Supercritical fluid is passed through the extraction vessel in the usual manner to an outlet restrictor. The flame ionization detector heating block from a Suprex SFE 50 (Suprex Corp., Pittsburgh, PA) was modified so that a standard solid phase extraction (SPE) tube (Supelco, Bellefonte, PA) could be mounted on what normally would have been the flame jet. An adapter used to join two SPE tubes in series was drilled out so that it would fit over the outside of the "flame jet". The adapter fit snugly on the "flame jet" with the end of the jet extending 5 mm from the end of the adapter. The adapter allowed for a variety of SPE tube sizes to be used as collection devices. The larger the amount of material being extracted the larger the SPE tube used. By matching the amount of material extracted with the correct size SPE tube efficent trapping was hoped to be attained as well as minimizing the amount of solvent used to elute the analytes off the SPE tube. The SPE tubes had 40 μm silica particles of differing bonded phases. Traps from other vendors with smaller diameter particles could not be used as a back pressure would build up inside the SPE tube and ultimately disengage the tube from the heating block. The restrictor tip extended approximately 1 mm from the end of the jet tip. Nitrogen was plumbed in through the hydrogen inlet to provide a stream of heated gas

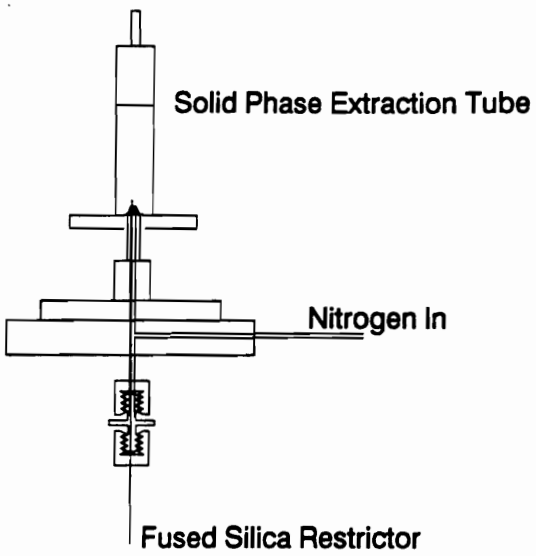


Figure 22

Solid Phase Trapping Scheme

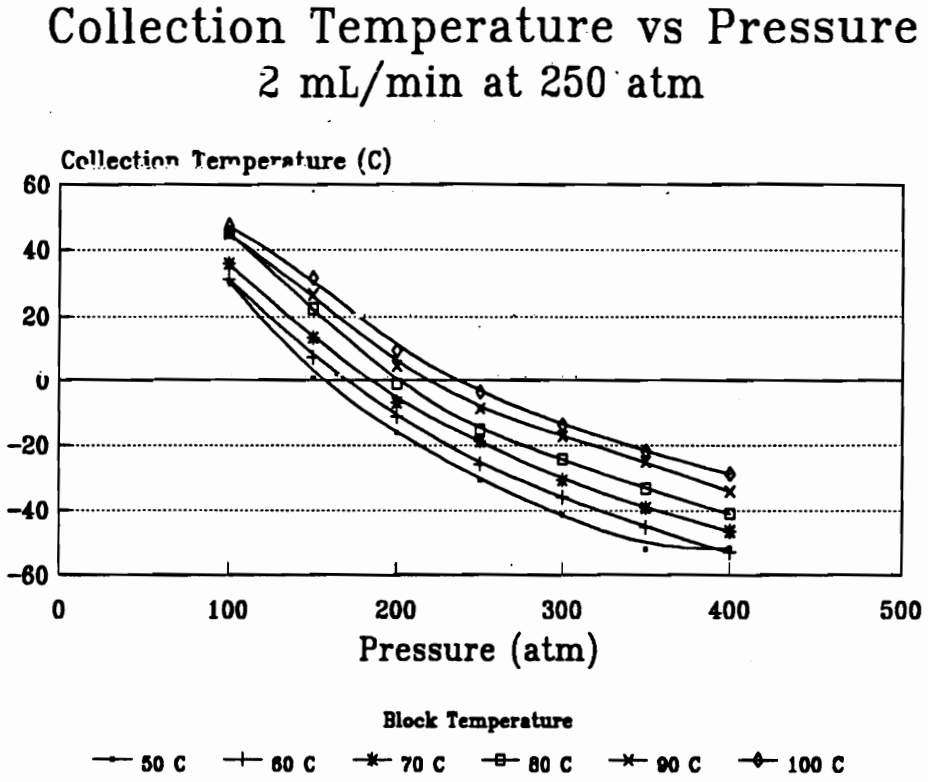


Figure 23

Collection Temperature as a Function of Extraction Pressure and Heating Block Temperature

(600 mL/min). The heated nitrogen ensured that the pulled restrictor tip would not ice up and possibly foul, due to cooling of the supercritical fluid upon decompression. The restrictor was 50 μm internal diameter deactivated fused silica tapered at the end to provide a restriction. The taper of the restrictor was such that at an extraction pressure of 250 atm a flow of 2 mL/min of supercritical fluid through the extraction vessel was realized. Restriction tapers were made in house by heating a fused silica capillary with a butane/nitrous oxide torch and pulling. The taper created was then trimmed until the desired flow was achieved. Once mounted within the "flame jet" the restrictor was protected from any abuse that could cause breakage.

Decompression of CO_2 at the restrictor caused a fair amount of cooling. The final temperature of the trap would be a function of flow rate, pressure of the fluid, and the temperature of the heating block. To calibrate the system, temperature readings in the SPE tube were made by placing a thermocouple into the space of the trap not occupied by packing material. Temperature equilibrium was reached in less than one minute for all of the extraction pressures that were monitored. Figure 23 shows the effect of different extraction pressures (flows) and heating block temperatures on collection temperature once thermal equilibrium had been established. It is evident from Figure 23 that the collection temperature can be "tuned" for various extraction pressures by varying the temperature of the heating block.

Collection Efficiency

Prior to using the trapping system previously described on actual samples, several experiments were performed to determine the trapping efficiency of various packing materials for analytes of interest. The scheme shown in Figure 24 allowed for a reproducible amount of material to be presented to the trap in a form which would mimic

Solid Phase Trap
Recovery Scheme

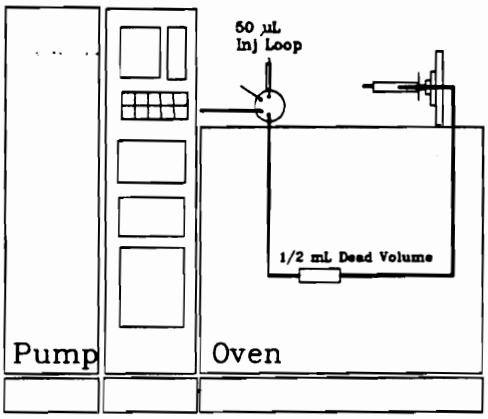


Figure 24

an extraction. More specifically, by loading a sample loop with a desired concentration of analyte in solution form, the contents of the sample loop could then be injected into the "extraction" system. Alternatively, the sample loop could also be rinsed into a vial with organic solvent and then analyzed (by GC) to provide a reliable 100% recovery value. Five replicate injections of this type were used to determine the 100% recovery value for each component. The sample loop was approximately 50 μL in volume. A 0.5 mL dead volume was introduced between the injection valve and the collection device to ensure that the organic solution was not mechanically moved through the system but was solvated by the supercritical fluid. The dead volume was an empty 0.5 mL extraction vessel (Keystone Scientific, Bellefonte, PA).

Two test mixtures were studied. A mixture of n-alkanes (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28} and C_{32}) was dissolved in CH_2Cl_2, while another more polar mixture of phenols (phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 3-methyl,4-chlorophenol, 2,4-dimethylphenol and 4-nitrophenol) was dissolved in MeOH at approximately 2 mg/mL per component. The n-alkane series was chosen to act as a vapor pressure probe. The smaller n-alkanes have a greater vapor pressure than the larger ones yet any adsorptive interactions are the same regardless of size (although the strength of the interaction may differ). The effect of vapor pressure of the analyte on collection efficiency could be isolated within a certain type of bonded phase trap. By comparing collection efficiencies from different chemically bonded phases the effect of the adsorptive interactions (hydrocarbon/bonded phase) could also be studied.

The phenol mix was chosen because the aqueous solution that is discussed in Chapter 6 was the ultimate goal of the design of the system. By studying the collection behavior of the phenols, the trapping and rinsing portions of any actual extraction performed could be isolated from other extraction parameters.

Therefore, assuming a 50 µL loop and a concentration of 2mg/mL, 100 µg of each component was "extracted" and should have been loaded onto the solid trap. The analytes were transferred to the traps by putting the filled sample loop in-line with the flow of CO_2 for a period of 10 minutes at 350 atmospheres and 50°C. Approximately 25 mL (50 "cell" volumes) of supercritical CO_2 passed through the system during this period. The temperature of the collection traps was -20°C (the heating block temperature was 100°C). The traps were rinsed with 1 mL of CCl_4 in the case of the alkanes and 3 mL of MeOH for the phenols. For each different solid phase studied, five separate injections of each test mix were performed. An internal standard (anthracene) was added to both the recovered alkanes and phenols. The analysis of recovered analytes was then performed by gas chromatography. The GC was a HP 5890 with an HP 7673 auto injector and HP 3365 Chemstation (Hewlett Packard Company, Avondale, PA). The column used was a 25 meter X 0.200 mm id, HP 5 (Hewlett Packard Company, Avondale, PA). The injection volume of each solution was 1µL and was performed in the purged splitless mode.

Results and Discussion

Collection Temperature

Figure 23 shows that the cooling provided by the expansion of CO_2 at the restrictor provides a fair degree of cooling to the SPE collection tube. By varying the temperature of the heating block the temperature of the SPE collection tube can be controlled over a range of extraction pressures. This requirement is necessary so that extraction conditions over a range of pressures can be studied while the trapping portion of the experiment remains constant.

Hydrocarbon and Phenol Recovery

The effect of different packing materials on the collection and release of a series of hydrocarbons and phenols was studied. Figures 25 and 26 show the trapping and rinsing efficiency of normal phase and reverse phase packing material for the hydrocarbon series. Table 4 shows the same data but in numerical form. As expected the phenyl, C_8 and C_{18} traps performed the best. The C_8 and C_{18} phases behave vary similar in both recovery and precision. The phenyl phase does not trap C_{10} and C_{12} as efficiently as the C_8 and C_{18} phases but the precision of the trapping is much better for each hydrocarbon. The reason for the better precision of the phenyl packing material may be a more efficient rinse with CCl_4. This notion is backed up by better recoveries of the higher molecular weight alkanes (> C_{16}) on the phenyl trap. The interaction between the alkane analytes and the C_8 and C_{18} is certainly a strong one. Both phases have little difficulty in trapping C_{10} very efficiently. Trapping the analyte is, however, only part of the experiment. The ability to rinse cleanly with organic solvent must also be accomplished. A trade off is apparent. A phase which is able to interact with the analyte in order to provide efficient trapping will not be the most efficient phase at releasing the analyte once trapped. On the other hand, a phase which rinses cleanly (such as alkanes from the phenyl phase with CCl_4) may not be "sticky" enough to trap the more volatile components.

The more polar traps performed poorly, for the alkanes especially with the more volatile analytes. The data indicate, however, that for n-alkanes above C_{18}, all traps performed well (give high recovery), with the exception of the amino bonded phase. The precision of the polar phases is, at best, marginal with the exception of bare silica. The SiO_2 packed trap exhibited poor recovery (only 94% for the best case) yet the precision of recovery was quite high. The precision increased with the molecular weight (vapor pressure) of the analyte.

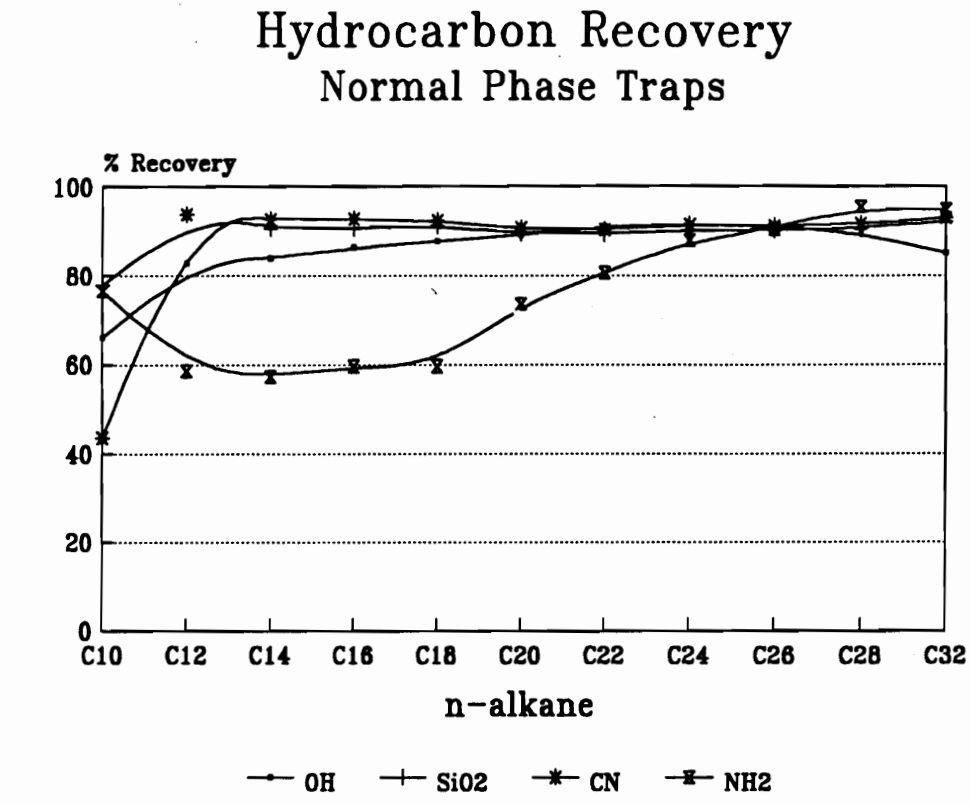


Figure 25

Recovery for Alkane Series with Normal Phase Packing Material

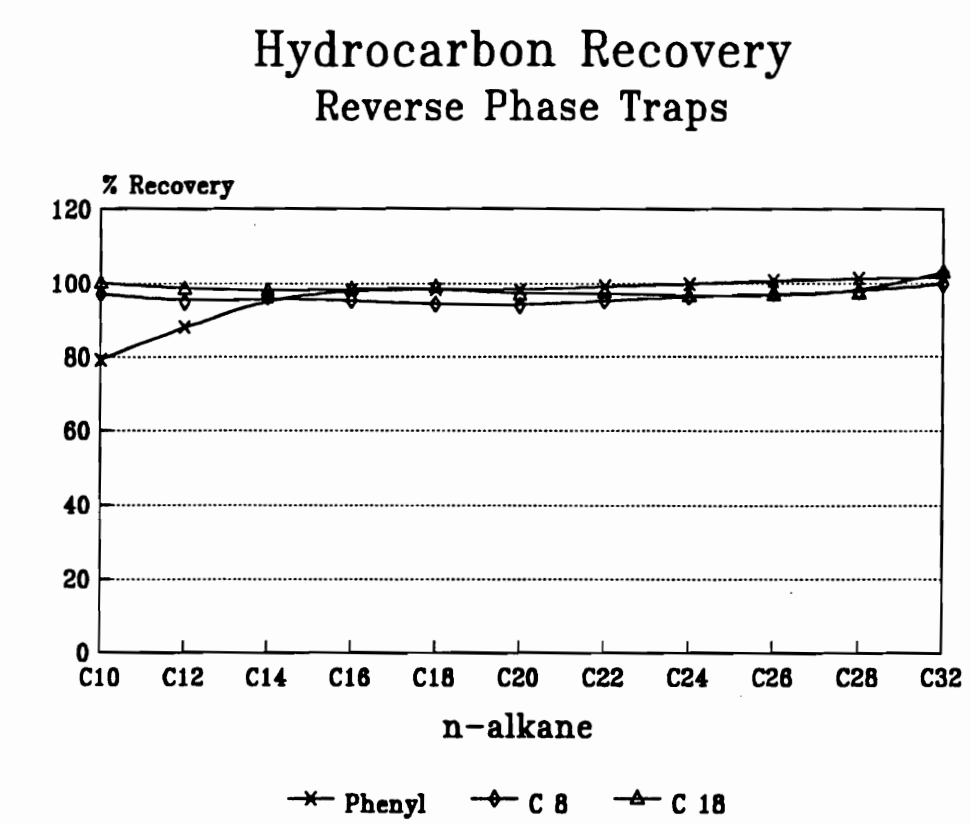


Figure 26

Recovery of Alkane Series from Reverse Phase Trapping Material

Table 4

Recovery of Alkanes from Traps of Various Packing Materials (n=5)

	CN Trap		OH Trap		SiO_2 Trap	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
C10	44	42	66	23	78	15
C12	94	2.3	83	13	94	6
C14	93	1.6	84	10	91	4.5
C16	93	1.7	86	10	91	3.6
C18	92	2.1	87	9	91	2.9
C20	91	2.6	89	9	90	2.3
C22	90	2.6	91	8	89	2.4
C24	91	3.9	91	8	90	2.2
C26	91	2.6	91	8	90	2.6
C28	92	2.6	90	8	90	1.6
C32	93	3.3	85	10	92	1.8

	NH2 Trap		Phenyl Trap		C_8 Trap	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
C10	77	5.0	79	3	97	5.0
C12	59	3.9	88	7	95	11
C14	57	6	96	3.3	96	8
C16	60	9	98	3.0	95	9
C18	60	14	98	2.4	94	8
C20	74	7	98	1.7	94	6
C22	81	7	99	1.5	95	3.0
C24	88	5.0	99	1.5	96	25
C26	90	6	100	1.5	97	2
C28	96	5	100	1.6	98	2.5
C32	95	7	100	2.0	100	2.8

At a trapping temperature of -20°C each packing material appears to act as a filter for any material with melting points well above -20°C. Recovery of the less volatile compounds is fairly high (greater than 85%) for all the traps studied with the exception of the NH_2 phase. The precision of the recovery appears to be dependent upon two factors. The first is the ability of the packing to efficiently trap the analyte. The best example of this is the SiO_2 and C_8 act as traps for decane. Silica is a poor trap for decane recovering only 78%. The precision of the recovery is also quite low (15%). Decane recovered from a C_8 trap gives a much higher recovery and better precision. The C_8 trap is more efficient at trapping and gives predictably good recovery of decane. The second apparent factor in the precision is the ability of the organic solvent to rinse the analyte from the trap completely. Lower collection temperatures may extend the range of polar sorbents applicable to n-alkanes less than octadecane. By lowering the collection temperature of the polar phased the trapping of the higher vapor pressure hydrocarbons will be improve while maintaining good rinsability once the trapping is complete.

Figures 27 and 28 show the trapping and rinsing efficiency for normal and reverse phase trapping material for phenols as the extracted analyte. Table 5 shows the data in numerical form. The analytes were rinsed off the trapping material with 2 mL of MeOH. The phenolic series is not as well behaved as the simple hydrocarbon series. That is to say that recovery and precision of recovery does not appear to be as straight forward as the hydrocarbon case.

The recovery of phenols during solid phase extraction is a common problem. Pentachlorophenol in particular is difficult to remove from SPE cartriges during normal solid phase extraction. The same problem seems to exist for the trapping and rinsing after SFE. The diol trap performed the best (showed the highest recoveries) but failed to

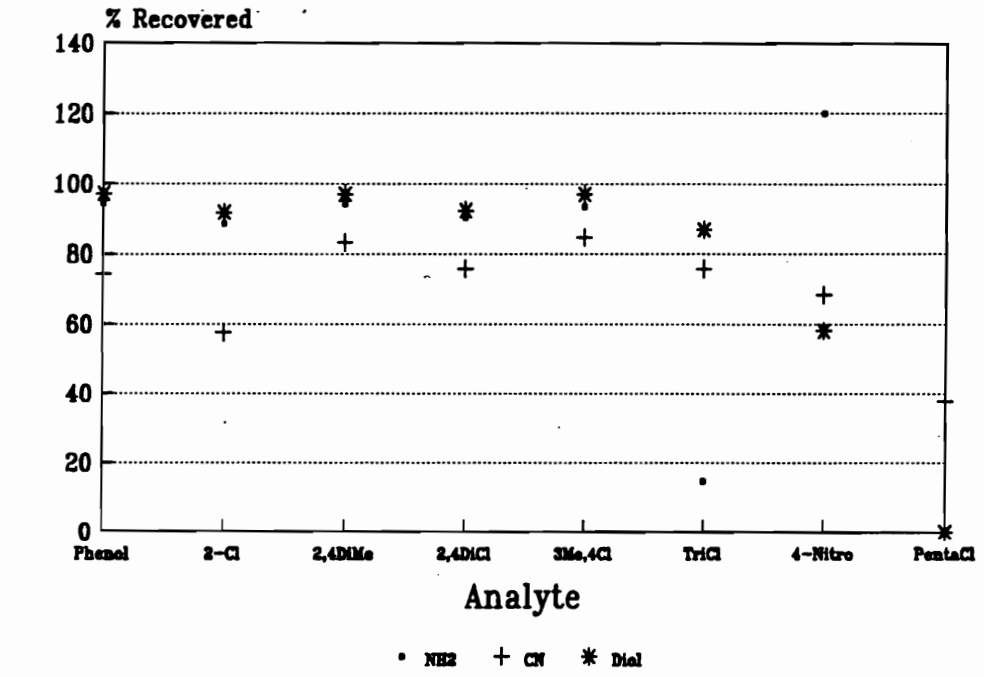


Figure 27

Recovery of Phenols from Traps of Normal Phase Packing Material

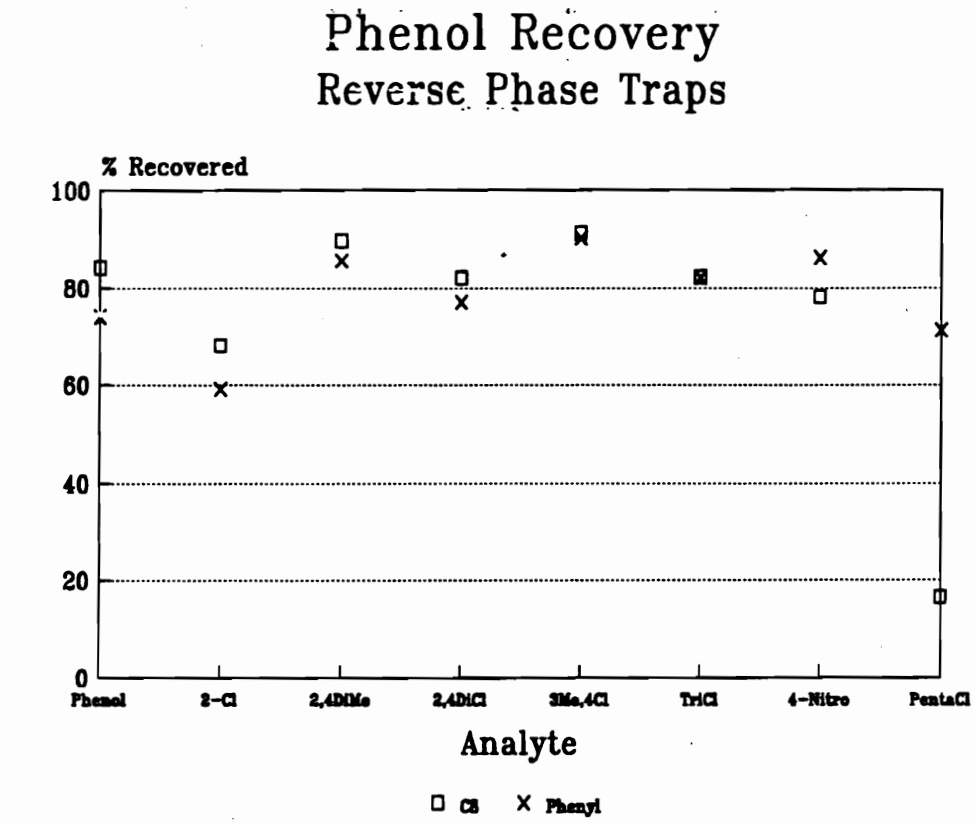


Figure 28

Recovery of Phenols from Traps of Reverse Phase Trapping Material

Table 5a

Recovery of Phenols from Traps of Normal Phase Packing Materials (n=5)

Trap	Compound	Recovery (%)	RSD (%)
NH2	phenol	95	6
	2-chlorophenol	89	12
	2,4-dimethylphenol	94	2.6
	2,4-dichlorophenol	90	4.0
	3-methyl-4-chlorophenol	94	2.5
	2,4,6-trichlorophenol	15	90
	4-nitrophenol	120	24
	pentachlorophenol	-	-
CN	phenol	74	6
	2-chlorophenol	58	10
	2,4-dimethylphenol	83	2.2
	2,4-dichlorophenol	76	3.8
	3-methyl-4-chlorophenol	85	2.9
	2,4,6-trichlorophenol	76	3.6
	4-nitrophenol	68	12
	pentachlorophenol	38	7.9
Diol	phenol	97	13
	2-chlorophenol	92	19
	2,4-dimethylphenol	97	11
	2,4-dichlorophenol	92	13
	3-methyl-4-chlorophenol	97	10
	2,4,6-trichlorophenol	87	14
	4-nitrophenol	58	2.4
	pentachlorophenol	-	-

Table 5B

Recovery of Phenols from Traps of Reverse Phase Trapping Materials

Trap	Compound	Recovery	RSD
C8	phenol	84	4.7
	2-chlorophenol	68	6.3
	2,4-dimethylphenol	90	4.8
	2,4-dichlorophenol	82	6.3
	3-methyl-4-chlorophenol	91	6.5
	2,4,6-trichlorophenol	82	8.2
	4-nitrophenol	78	20
	pentachlorophenol	17	200
Phenyl	phenol	74	10
	2-chlorophenol	59	12
	2,4-dimethylphenol	86	14
	2,4-dichlorophenol	77	10
	3-methyl-4-chlorophenol	90	14
	2,4,6-trichlorophenol	82	18
	4-nitrophenol	86	29
	pentachlorophenol	71	81

release 2,4,6-trichlorophenol, pentachlorophenol and 4-nitrophenol completely as did most of the other traps.

Conclusion

The assumption is made that because of the relatively high melting point of these compounds trapping was not the cause of poor recovery. The use of greater rinse volume or rinsing at elevated temperature may solve some of the low recovery problems. It is doubtful , however, that these changes could overcome all the problems of a trapped analyte that was not released at all using solvent at room temperature.

It is evident from both the hydrocarbon and phenol recovery data that both the choice of trap and rinse solvent are important for reasonable recovery of analytes with solid phase traps.

Chapter 6

Supercritical Fluid Extraction of Phenols From Aqueous Matrices

Introduction

Many different methods have been employed in order to concentrate trace organics from aqueous solution such as liquid-liquid extraction, purge and trap analysis[45], adsorption on solid adsorbents and passage through capillary polymeric columns. Desirable analyte features for accomplishing this task are low water solubility, low polarity and, for purge and trap analysis, high volatility. The presence of solids or particulates as well as alcohols in the aqueous matrix can cause problems with many of the techniques just mentioned. The pH of the solution is also important as it can influence the trapping behavior of the analyte onto solid sorbents as well as inhibit the transfer of material during liquid-liquid extraction.

The use of SF's to extract analytes from fluids is somewhat analogous to headspace sampling of liquids done by purge and trap GC[46]. Headspace sampling relies on the vapor pressure of the analyte being substantially large so that simple purging with an inert gas will remove the analyte from the system. The technique is limited to compounds which are sparingly water soluble. The molecular weight of the analytes is also limited because of the need for a high vapor pressure. Practically speaking the use of purge and trap methods are limited to small halocarbons[47,48].

The supercritical fluid extraction (SFE) of analytes from aqueous samples has received little attention as compared to SFE of solid matrices. The mechanical difficulty in retaining the liquid matrix in the extraction vessel probably accounts for this fact. However, there are several desirable aspects of working with liquid samples. One of the most attractive aspects is that the conventional organic solvents used in liquid/liquid

extraction can be replaced with a SF such as CO_2. In addition the use of SF's could extend the range of the headspace sampling process by taking advantage of the solvating power of SF's. Larger molecular weight compounds and truly water soluble compounds, such as phenols, might be extractable from water using SF's.

SFE of aqueous systems has been performed on the process scale, with two purposes: 1) extract a desired material from water with the hope of isolating the material for use, and 2) extract toxic materials from water so that the water can be released into the environment. Because of the large scale involved, both process operations use forms of counter current extraction[22]. Counter current extraction takes advantage of the density difference of the two fluids so that the more dense fluid can be transported in one direction (down), while the other can be transported in the opposite direction (up), within the extraction compartment. The extraction cell is usually filled with various beads and other proprietary mixing apparati in order to increase the efficiency of contact between the two fluids. Counter current extraction of small quantities of water (<100 mL) is currently not feasible because of the lack of appropriate technology. Consequently, SFE of liquids on what would be considered an analytical scale is in its infancy. Most of the limited experimentation that has been performed has dealt with determining distribution coefficients of various materials in order to support the process scale extractions. Ideally, the distribution coefficient of an analyte in a two phase system will determine the amount of SF needed to perform an exhaustive extraction.

The first reported work with a SF/water system dealt with the vapor phase composition of SF-CO_2/H_2O. The subject was studied extensively by Wiebe and Gaddy[49,50] in the late 1930's and early 1940's. Under practical operating conditions water was found to be soluble in CO_2 at a mole fraction of 0.005. The solubility behavior was influenced, however, by pressure as well as the temperature of the SF (Figure 29). Up to

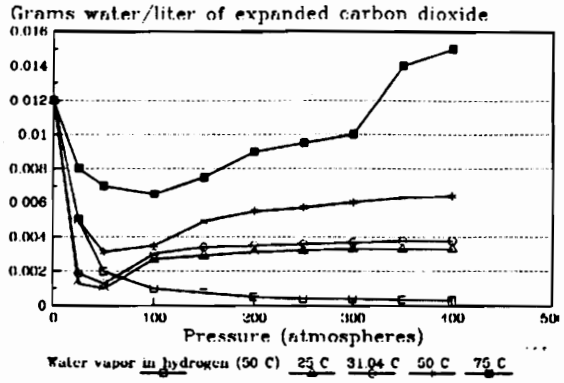


Figure 29

Solubility of H_2O in supercritical CO_2 (Data replotted from Weibe[49])

approximately 100 atm the solubility of water in CO_2 drops dramatically with increasing pressure. At these low pressures water has very little solubility in CO_2. Subsequently, any water present in the SF phase is due primarily to the partial pressure of water vapor in the system. Above 100 atm the solubility of water in CO_2 increases dramatically, but beyond about 200 atm the water content of the system becomes constant.

The solubility behavior of water in CO_2 when performing CO_2/H_2O extractions is important for two reasons. First water in the system may present problems, especially during the isolation of analytes. Many isolation steps require the use of a solvent which is not compatible with water (i.e.: hexane, CCl_4, etc.). The amount of water transported should therefore be minimized. Second, water also acts to modify the SF and alter the solvating power of the fluid.

Elgin and Weinstock[51] reported the first extraction of various organics from water employing supercritical ethylene. Their work, along with much of the literature reported in the next two decades, dealt with process scale extraction of aroma and flavor components from oils and liquors derived from natural products[52]. The mid-1970's showed a revived interest in the supercritical fluid extraction of aqueous systems. The interest was driven by both environmental and economic concerns. On the environmental side, CO_2 was seen as a desirable solvent for the removal of hazardous compounds from waste streams. At the same time, the current energy shortage was responsible for research involved in finding a more cost effective method (as compared to distillation) of removing fuel stocks, particularly MeOH, EtOH, and acetone, from aqueous streams.

Ehntholt et al.[53] reported the extraction of 23 organics from 400 mL of water at the ppm and ppb level with varying success. The extraction scheme is shown in Figure 30. The main parts of the system are a compressor which feeds off a gas cylinder. The compressed fluid is then passed through a heating element which raises the fluid to

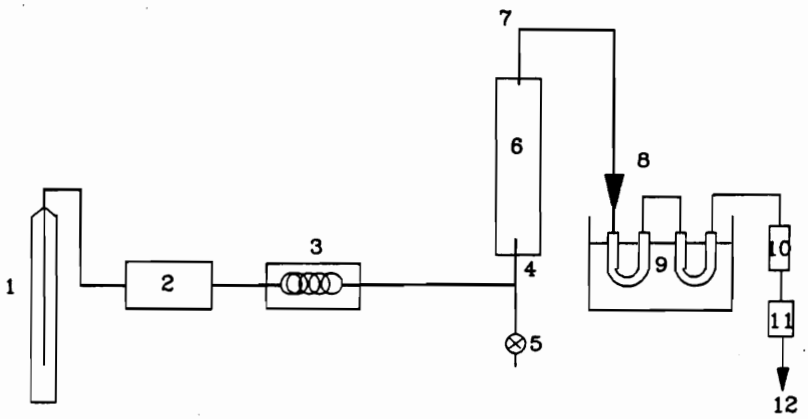


Figure 30

Extraction Scheme from Entholt et al.[59]

1) Gas Cylinder

2) Compressor

3) Heating Element

4) High Pressure Fluid Inlet

5) Liquid Take-off Valve

6) Extraction Vessel

7) Thermocouple

8) Pressure Reduction Valve

9) Trap

10) Rotameter

11) Gas Meter

12) Vent

supercritical conditions. The fluid enters the bottom of the extraction vessel which contains the aqueous solution of interest. The supercritical phase passes through the top of the extraction vessel to a pressure reduction valve. Trapping was performed by a series of u-tubes in a refrigerated liquid. Flow through the system was measured by both a gas meter and a rotameter.

Recoveries of material varied widely with the analyte being observed. Reproducibility of the extraction process varied from 10-50% RSD. Clearly the precision needed for analytical work was not achieved. The lack of reproducibility may have been caused by the relatively crude way in which the analytes were trapped after extraction.

Roop et al.[54] demonstrated the extraction of phenol from 150 mL of water using supercritical CO_2. The system used is shown in Figure 31. The system was charged with CO_2, mechanically mixed for one hour and then allowed to sit for two hours in order to allow the two phases to separate. The water phase was subsequently drawn off and analyzed. The goal of the experiment was to determine the distribution coefficient (K_d) of phenol at 298 K and 323 K. Results showed a linear increase in K_d with increasing pressure at fixed temperature. There was a good deal of scatter at the higher temperature although RSD's of 1.5% were quoted. In another paper Roop et al.[55] reported the extraction of creosote from water using

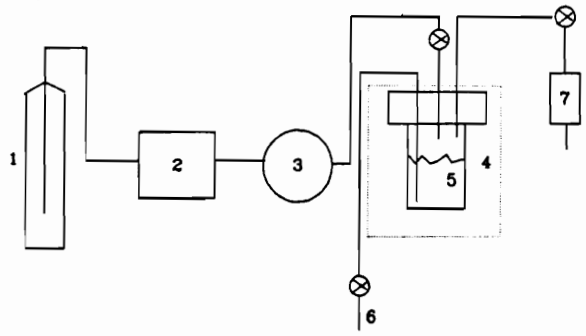


Figure 31

Extraction Scheme from Roop et al.[60]

A) Gas Supply

B) Compression Cylinder

C) Valves

E) Metering Pump

H) Extraction Vessel

K) Tubing for Water Retrieval

L) Wet Test Meter

M) Exit Tubing

the same system. Distribution coefficients for creosote were reported at three different temperatures. The creosote data indicated a reversal in the distribution coefficient at high temperature and fixed pressure. That is, K_d increased with increasing temperature initially, but beyond 35°C K_d decreased with a temperature rise for the same pressure.

Ong et al.[6] were successful in extracting cholesterol from both solid and liquid matrices. The volume of the liquid sample, however, was not reported. The extraction cell design is shown in Figure 32. Ong reported a steady increase in extraction efficiency for the solid matrix with an increase in pressure and temperature but no data for the liquid system were reported. The volume of CO_2 passed through the system also was not reported.

Departing from the conventional extraction cell design, Theibolt et al.[56] used a novel phase segmentor and subsequent phase separator in order to extract 4-chlorophenol and phenol from water with supercritical CO_2 (Figure 33). Two high pressure pumps one pumping water and the other CO_2 are joined at a T junction. This results in a segmented system. The segmented flow passes through a section of tubing where the extraction takes place. The phase then passes through a phase separator, a hydrophobic membrane. Water is repelled by the membrane and goes to waste, by way of a restrictor. The carbon dioxide phase passes through the membrane and to an injection loop and finally to waste by way of a restriction. On-line SFC was used for analysis once the SF phase was removed from the water. Recoveries and reproducibilities for the system were not reported, although with the single pass system 100% recovery would not be expected.

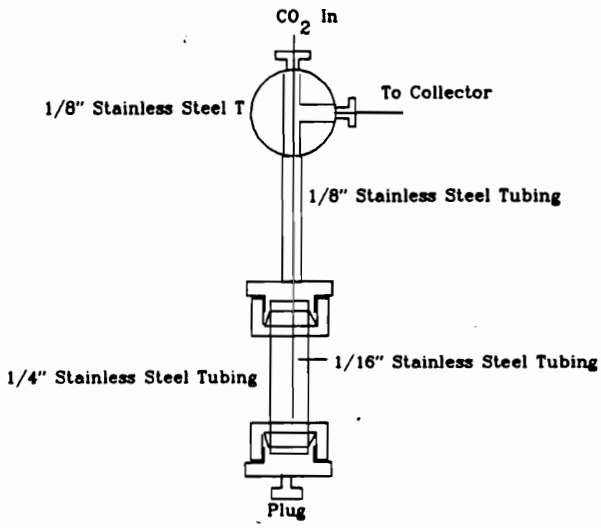


Figure 32

Extraction Cell from Ong et al.[6]

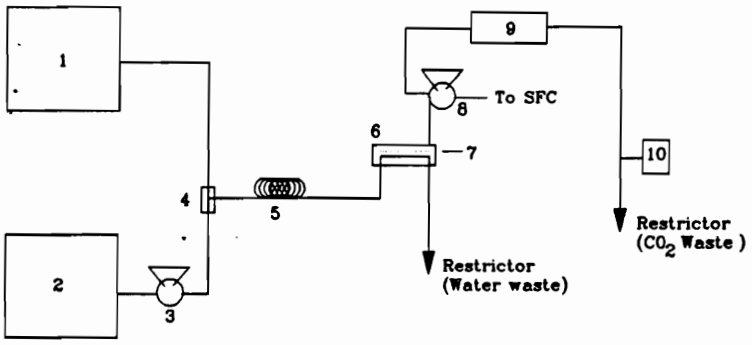


Figure 33

Segmented Extraction Scheme from Theibolt et al.[64]

1) CO_2 Pump

2) H_2O Pump

3) Sample Injection Valve

4) Segmenting Tee

5) Extraction Coil

6) Hydrophobic Membrane

7) Phase Seperator

8) Injection Valve to SFC

9) Flow Gauge

10) Pressure Gauge

The previous works cited represent most of the process scale and all of the analytical scale work that has been reported to date. SFE of aqueous systems is clearly still in its infancy but no doubt will continue to grow as technology and techniques continue to become available.

Concurrent with much of the work cited on aqueous extraction, work in our laboratory has focused on the development of practical analytical methods for the extraction of aqueous system with supercritical fluids. The goals for practical SFE of aqueous systems were as follows:

1) Purified CO_2 extraction solvent

2) Reasonable sample size (1 - 25 mL)

3) Applicability to a wide range of analytes

4) Fast and exhaustive extraction

5) Efficient and easily rinsed trapping system

(off-line mode)

6) Compatible with GC and SFC for on-line analysis -

Carbon dioxide was deemed to be the most desirable extraction solvent because of its low toxicity and its ability to solvate a wide range of analytes. Carbon dioxide is also relatively inexpensive and available in high purity. The critical parameters of supercritical CO_2 are such that neither boiling or freezing of the aqueous system would be a problem.

Sample sizes less than 1 mL afford a sampling problem as accurate and precise introduction of small liquid volumes is inherently more difficult than the introduction of larger volumes. Sample volumes greater than 25 mL present problems of containment as the larger diameter vessel needed to enclose the sample becomes difficult to seal. Volumes in the 1 to 25 mL range are easily contained in modified commercially available extraction vessels.

The ability to extract the sample fast (25-30 minutes) is strictly pragmatic. The faster the extraction can be performed, the faster the total analysis can be performed. Ultimately, a fast extraction will result in a rapid total method. The extraction efficiency should not be compromised, however, by the desire for a fast analysis. Greater than 95% recoveries should be obtained in all cases.

The extraction behavior of various phenols was explored in depth. Phenols were chosen because of their presence in many industrial waste streams (making their study practical) and the extraction of phenol from aqueous solution is mentioned although briefly in the literature[59,61]. Other compounds studied included various nitrogenous bases and alkyl phosphonates.

Experimental

Aqueous Extraction Vessel

The extraction vessel (Figure 34) used for liquid-fluid extractions was acquired from Keystone Scientific (Bellefonte, PA) and measured 10.0 cm in length with an internal diameter of 0.94 cm (6.94 mL volume). This vessel was subsequently modified for use with liquid samples in the following manner. The zero dead volume channel at either end of the stock vessel was drilled out to a diameter of 1/16". The stainless steel frits that were contained as an integral part of the fitting seal were punched out so that 1/16" stainless steel could pass freely through either end of the vessel. The stainless steel inlet and outlet tubes were 0.01" internal diameter and extended to within 1 cm of the top and bottom of the vessel. The locking ferrule of a Kel-F finger tight fitting (Keystone Scientific) was swaged manually, once the inlet and outlet tubings were in the proper position. Finger tight fittings, with Kel-F ferrules, were used instead of a standard stainless steel nut and ferrule at both ends of the vessel in order to provide a more reliable

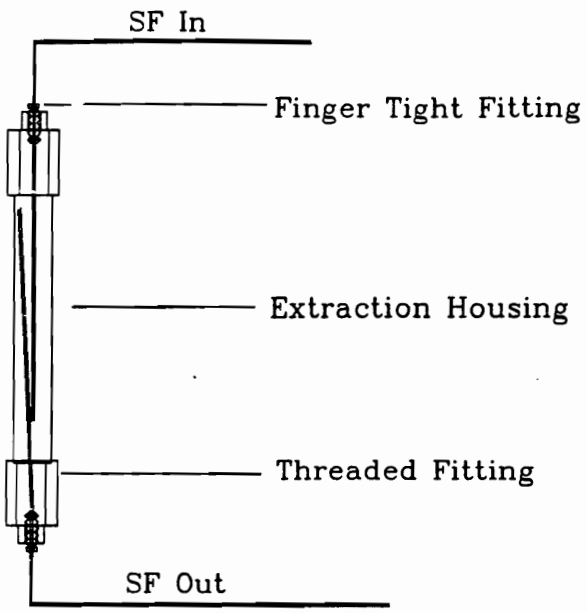


Figure 34

Aqueous Extraction Vessel

seal that could be broken and re-sealed numerous times. Screens (0.5 μm, VICI, Houston, TX) were placed at either end of the vessel so that solid material would not be transported through the system and possibly foul valves and restrictions.

The vessel is conceptually the same as one reported by Ong et al.[6] (i.e. SF is introduced from the bottom by passing the tubing in from the top) with a few major differences. The Ong vessel requires many different seals, most of which are stainless steel. The repeated swaging and unswaging of stainless steel seals can be done a limited number of times before a high pressure seal can not be maintained. More importantly the sample volume of the Ong vessel is extremely limited in that a sample size of 0.5 mL would be quite large. The vessel designed in this lab has no stainless steel/stainless steel swaged seals, greatly improving vessel lifetime. The volume of the cell is such that 3-5 mL samples may be used. Small liquid samples (<0.5 mL) can be readily analyzed by immobilizing the solution on filter paper,celite of molecular sieve.

Extraction Profiles of Phenols

Extraction of phenols from water was performed on 3 mL of sample. Each sample was 160 ppm per component of phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 3-methyl-4-chlorophenol and 2,4-dimethylphenol. In order to obtain the extraction time profile a number of SPE tubes were used sequentially. Each collection tube was used for a specific volume of CO_2. The placement of the valve after the extraction vessel allowed for flow from the extraction chamber to be stopped momentarily for the replacement of collection tubes. Figure 35 shows the plumbing scheme used. The fluid, after exiting the extraction vessel, enters a six port valve. By switching the valve position, the fluid from the extraction vessel either traveled on-line to the collection device or was stopped by a plug in the valve. The rate of flow from the pump was monitored and

Time Study Extraction Scheme

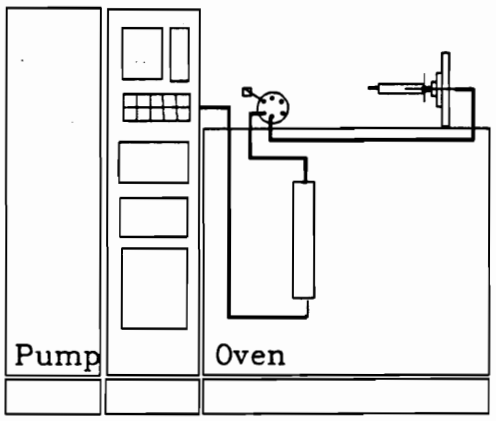


Figure 35

Extraction Profile Plumbing Scheme

corrected for differences in density between the oven (supercritical fluid), where the extraction took place, and the pump head (liquid). The density of the CO_2 at the pump was interpolated from density tables taken from a LSI 501 SFC (Salt Lake City, Utah). The density at the extraction vessel was taken from the density algorithm on the Suprex SFE-50 pump controller. Once the SFE was complete, the analyte was removed from each SPE tube with 4 mL of a MeOH. The rinse solutions were then diluted to volume in 10 mL volumetric flasks. Extraction profiles were generated for all phenols at 100, 150, 200, 250, 300 and 350 atm at 50°C. Analysis of the analytes was done by gas chromatography.

Gas chromatography was performed on a HP 5890 equipped with 7673 auto-sampler and DOS series chemstation (Hewlett Packard Co., Avondale, PA). A 25m HP-5 column with 200 μm internal diameter and 0.33 μm film thickness was used for all separations reported herein. The injection mode was purged splitless (1 μL injection) and the temperature ramp used for all separations was 60°C for 1 minute ramping then to 300°C at 20°C/minute.

Equilibrium Extraction System

An equilibrium extraction system was used to determine distribution coefficients of a phenol/water/CO_2 system, as well as to perform static SFE/SFC of various analytes. The system used, shown in Figures 36a and 36b, consists of three six-port valves, A,B, and C (Rheodyne Inc., Cotati, CA), a recirculating pump, R, (Micropump, Inc., Concord, CA), extraction vessel, E, and associated plumping in a temperature controlled oven. A three-port switching valve, S, (VICI, Houston, TX) was used to allow for easy conversion of the instrument (Suprex 200A SFC or HP1084B) back to a conventional SFC. A one meter length of 100 μm i.d. deactivated fused silica was used to interface the extraction

apparatus to a 1 X 250 mm DELTABOND CN packed column (Keystone Scientific, Inc., Bellefonte, PA) for chromatography done on a Suprex 200A , or a 4.6 X 250 mm in the case of chromatography done on a HP 1084B. The total volume of the system was calculated to be 8.84 mL, and included all tubing, dead volume associated with the recirculation pump and a 6.94 mL extraction vessel.

Figure 36a shows the supercritical fluid flow path during charging of the system. Fluid enters the system from the pump (SF in) and travels in a direction consistent with that which will be induced by the recirculating pump. The system is closed so that no net flow through the system (i.e.: no dynamic extraction) occurs during pressurization (notice the plug on valve "B". Once the system has reached the desired pressure, switching valve "B" to its other position (Figure 36b) creates a closed loop system. The recirculation pump moves fluid through the system at a rate of 3-5 mL/min. The flow induced by the recirculating pump was measured for methanol. The exact rate of flow is difficult to determine as the efficiency of the pump with supercritical fluids was not determined.

The results stated herein were obtained with a 20 µL sampling loop (found on valve "C"). Since each 20 µL injection represented only 0.3% of the extracted phase (5.84 mL with a 3 mL sample), multiple injections could be run on the sample without significantly depleting the extracted phase of analyte.

For determination of phenol distribution coefficients the extraction proceeded for 15 minutes in order to reach equilibrium. The time required to reach equilibrium was determined by making five replicate injections of the headspace, the first after the extraction had proceeded for 15 minutes at 50°C and 100 atm. The later injections were made at 10 minute intervals. No change in the amount of phenol was detected.The variability in the amount of phenol detected was less than 3% RSD. Had the system not been at equilibrium a trend in area count either increasing or decreasing in value would

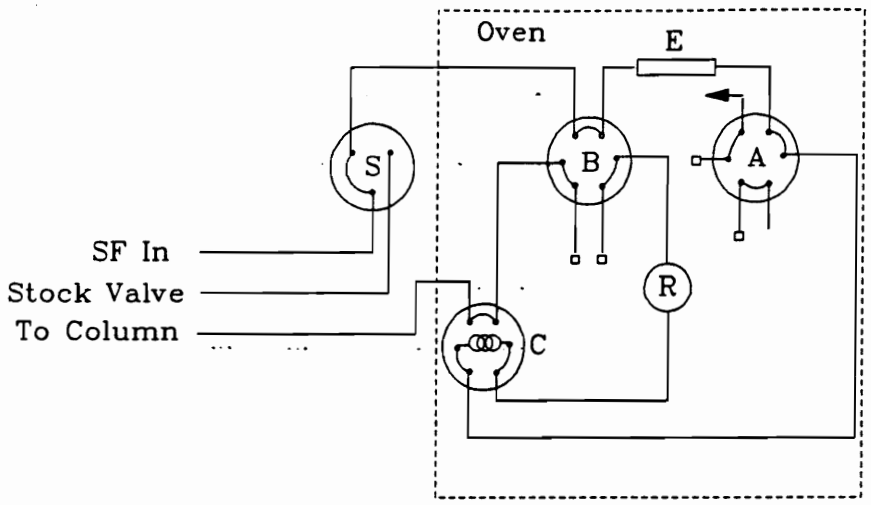


Figure 36a

Pressurization of Static Extraction Apparatus

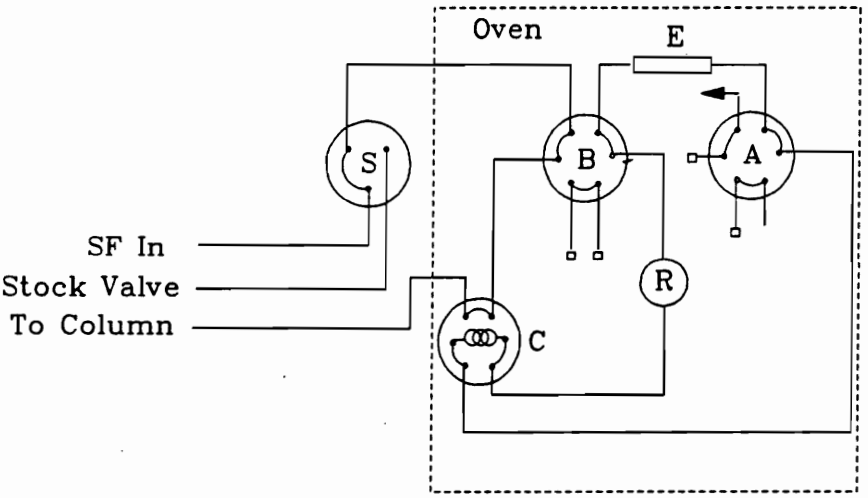


Figure 36b

Equilibration of Static Extraction Apparatus

have been observed as the analysis was performed at different times of extraction. Following the equilibration time, three injections onto a SFC system were made. At the end of the third injection the pressure inside the extraction apparatus was increased (by opening valve "B" to pump flow) and the system was again allowed to equilibrate. The injection process was then repeated. The amount of phenol in each injection was then calculated by the external standard method from a conventional SFC injection using a 0.1 μL loop. As the total volume of the system was known, the amount of phenol in the SF could be calculated. The amount of phenol in the aqueous phase could then be calculated by mass balance.

Results and Discussion

Extraction of Phenols

The extraction behavior of various substituted phenols (160 ppm) from water was investigated. Table 6 lists the phenols with their respective boiling point and pKa. Extractions were performed under various pressures (densities) at 50° C. Rather than noting the time of extraction, the volume of liquid CO_2 delivered by the pump to the extraction vessel was monitored. A correction was made for differences in density between the oven (supercritical fluid) and the pump head (liquid). The extraction vessel was approximately 7 mL in volume which in each case was filled with 3 mL of aqueous solution. Consequently one vessel volume of SF CO_2 would equal 4 mL in our experiments. Extracted phenols were trapped after CO_2 decompression onto solid phase extraction (SPE) tubes packed with 40 μm particle size bonded phase diol silica as described in Chapter 4. The extractant was removed from the SPE tube by rinsing with 4-mL of methanol, for analysis by gas chromatography as described in the Experimental.

Extraction profiles for the five phenols at 100, 150, 200 and 250 atm are shown in

Figures 37-40. At low pressure two extraction vessel volumes of CO_2 (100 atm, 50°C) gave less than 40% recovery for each phenol. The lowest recovery was for phenol (<10%) followed by 3-methyl-4-chloro-, 2,4-dimethyl-, 2-chloro- and 2,4-dichlorophenol. For pressures below 250 atm the extraction behaves as expected with greater recoveries occurring for all components at increasing pressures (densities). Above 250 atm a pronounced decrease in extraction efficiency is observed for each phenol. Figures 37 and 38 illustrate the greater extractability/recovery found for the higher pressures at fixed volumes of CO_2 (e.g.phenol: 5% (100 atm)vs 15% (150 atm) for approximately two vessel volumes (8 mL)).

The strongest acid of the group is 2,4,6-triichlorophenol. Since CO_2 saturated water is known to have a pH ≅ 3.8[57], each phenol should be fully protonated under these conditions and extractability should not correlate to acidity. In other words, if any phenol were deprotonated in part,only the protonated form would be extracted (assuming the ionic form was not soluble in CO_2). The relative rates of extraction would then be partially dependent upon the concentration of neutral species in the aqueous system. The data appears to support that pH is not a major factor in determining extractability in this particular system.

If one excludes phenol and considers only data at 100 atm, volatility appears to correlate with extractability since the lowest boiling phenols (2-chloro-phenol and 2,4-dichloro-phenol) exhibit the highest recovery. At 200/250 atm, the lowwwest boiling (phenol) and highest boiling (3-methyl-4-chloro-phenol) yield recoveries between 60% and 80%; wheras, the remaining three phenols cluster near 100% recovered. It is interesting to note that phenol is at least four times more soluble in water than the other phenols, probably because of hydrogen bonding. The slope of the phenol extraction profile plot at fixed pressure appears to be single valued. Upon increasing pressure, the

Table 6

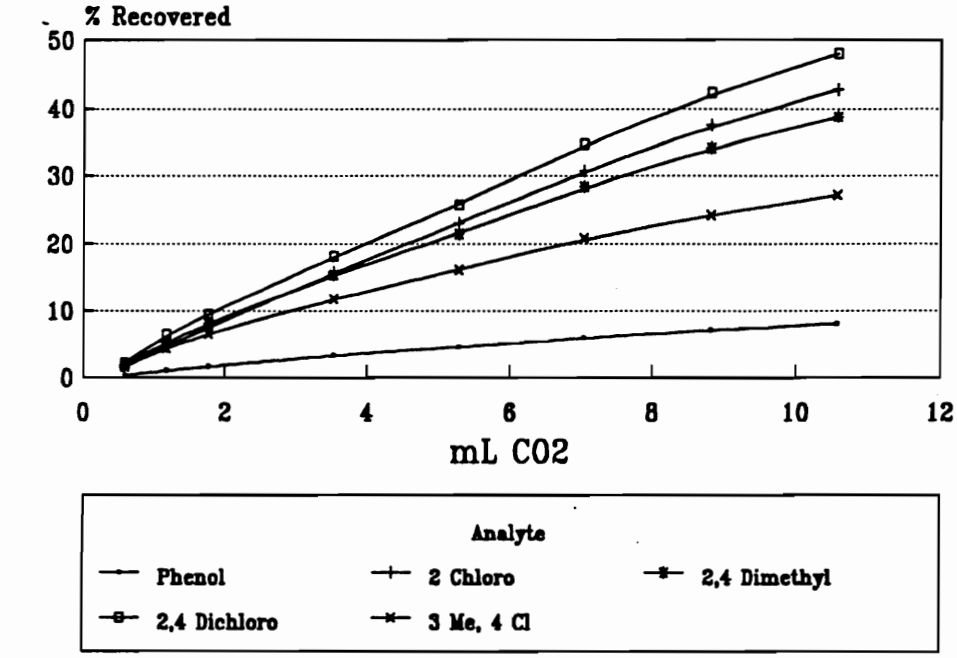
Phenols Subjected to SFE

Analyte	M.W.[57]	Melting Point (°C)[57]	Boiling Point (°C)[57]	pKa[58]	Sol.[58]
phenol	94.11	43	182	10.0	1g/15g H_2O
2-chlorophenol	128.56	33.5	214	8.48	slightly
2,4-dichlorophenol	163.01	45	209	7.85	
2,4,6-trichlorophenol	177.46	69	246	6.0	<0.1g/100g H_2O
4-chloro-3-methyl phenol	142.58	55.5	235		1g/260g H_2O
2,4-dimethylphenol	122.16	25.4	211.5	10.58	slightly

phenol profile slope also increases. At 200/250 atm the other phenols give rise to plots of varying slope (e.g. relatively high initially, but after approximately 10 mL of SF the profile slope decreases). Above 250 atm a pronounced decreases in extraction dfficiency is observed for each phenol.

The order of extractability is altered upon increasing the pressure. The 2-chlorophenol and 3-methyl-4-chloro-phenol reverse; while the 2,4-dichlorophenol and 2,4-dimethylphenol coincide. At 250 atm (Figure 40), where fluid flow is higher and more vessel volumes are employed for a fixed extraction time, the profiles for all of the phenols studied except phenol and 3-methyl-4-chlorophenol are similar. That is the extraction rates are the same and recoveries for 2-chlorophenol, 2,4-dimethylphenol and 2,4-dichlorophenol all approach 100%. The solvating power of the SF has become the dominant factor in determining extractability under these conditions as opposed to the vapor pressure of the analyte at lower pressures. As a consequence at 350 atm the order of extractability follows the solubility of analytes in CO_2 (i.e. 3-methyl-4-chloro is the most soluble) rather than vapor pressure of the analyte.

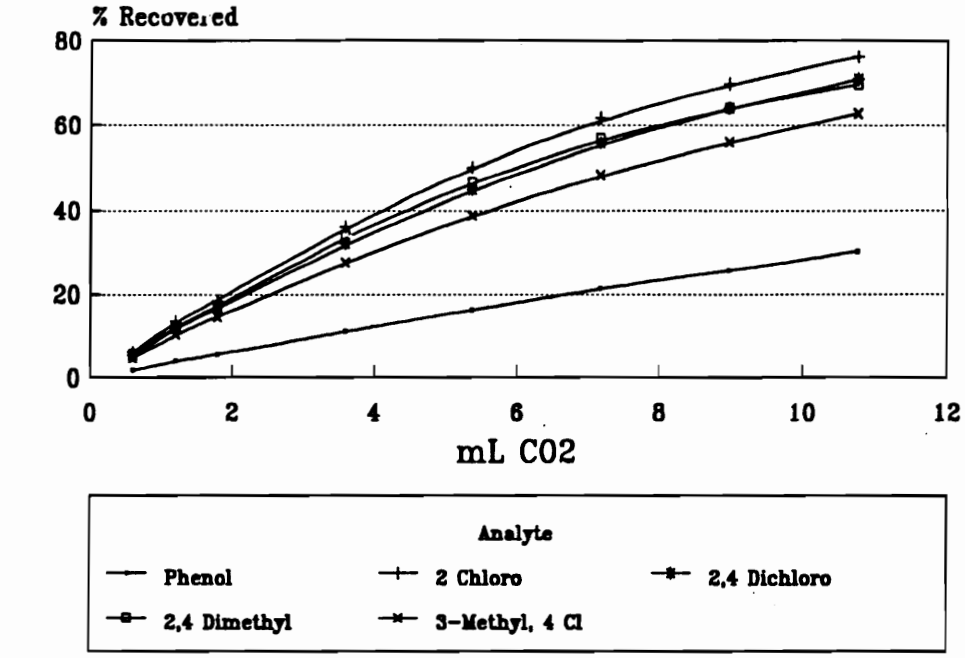
As noted earlier for fixed vessel volumes and here with extraction profiles, percent recovery decreases at 300 and 350 atm at 50°C (Figures 41 and 42). The profile for a fixed phenol at various pressures is shown in Figures 43-47. The shape of the extraction profile for each phenol at various pressures is fairly interesting. At low pressure (100 atm) the extraction profile is almost linear. A flat curve such as this suggests that the extraction kinetics are being limited by something other than flow through the vessel. As the pressure increases the shape of the profile becomes more curved. At 250 atm the curve is very near what one would expect for a simple first order extraction. That is for each dead volume put through the system 50% of the remaining material is removed. Beyond 250



density corrected

Figure 37

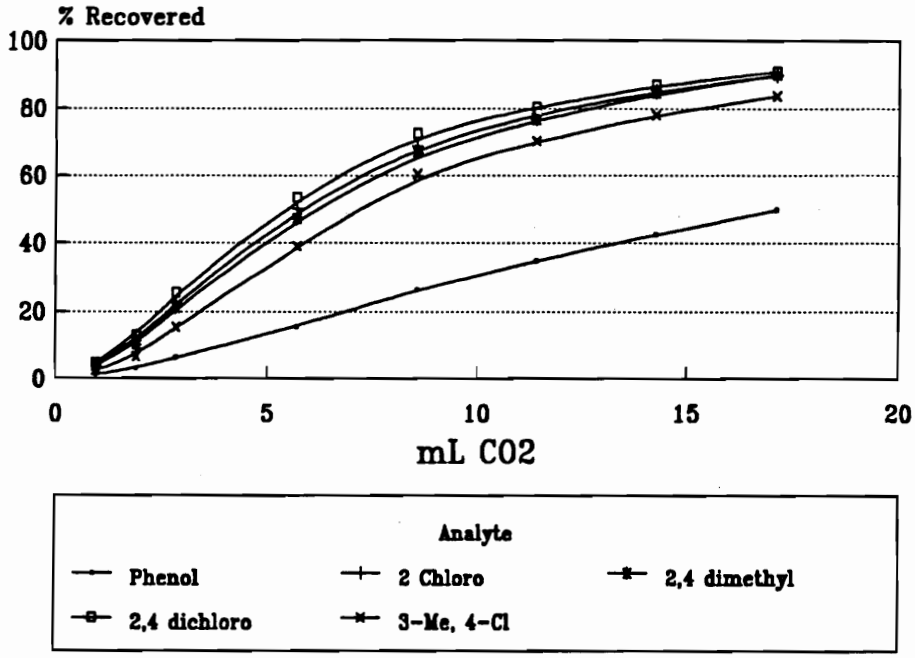
Extraction Profile of Phenols At 100 atm and 50°C



density corrected

Figure 38

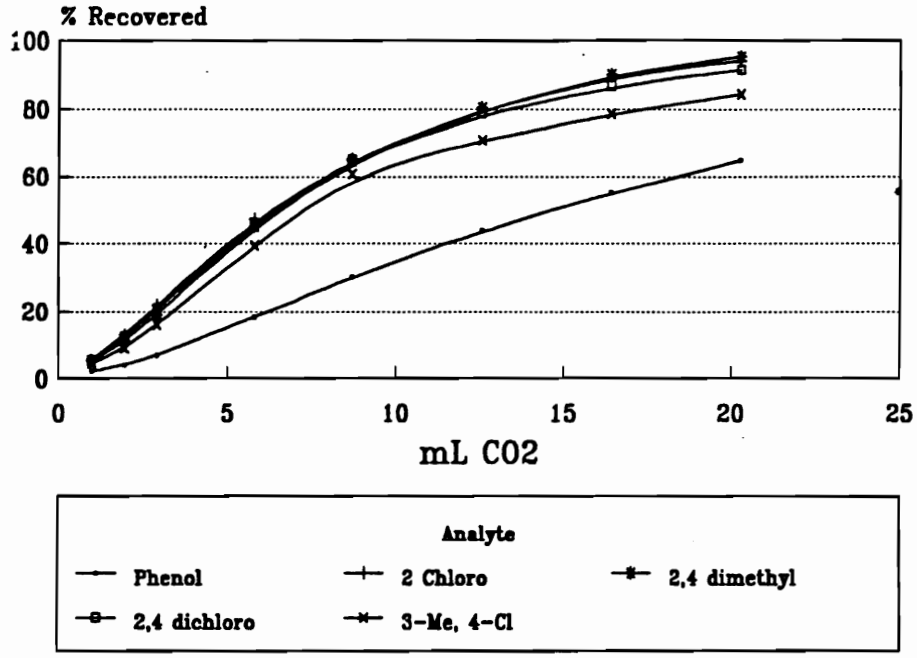
Extraction Profile of Phenols At 150 atm and 50°C



density corrected

Figure 39

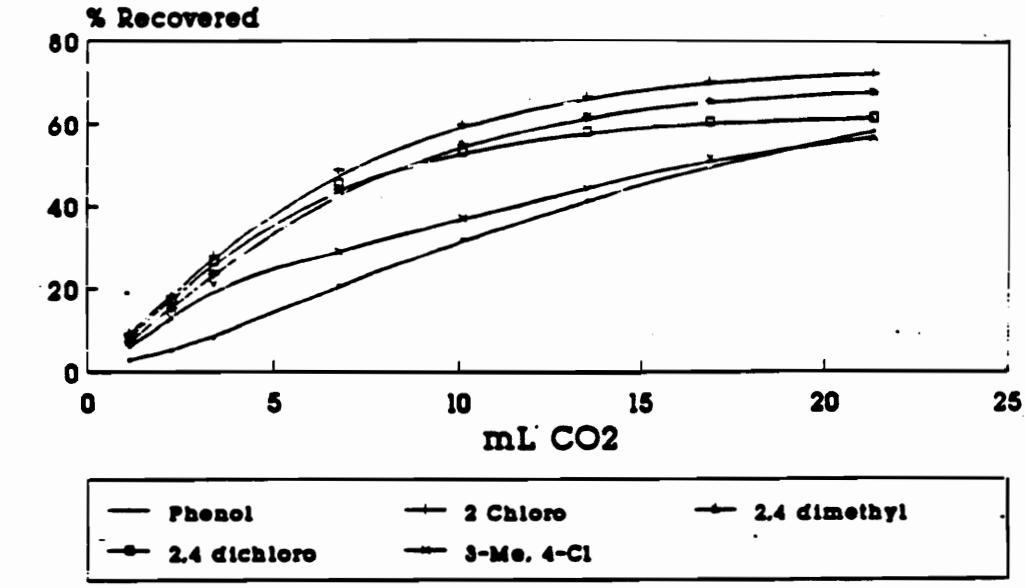
Extraction Profile of Phenols At 200 atm and 50°C



density corrected

Figure 40

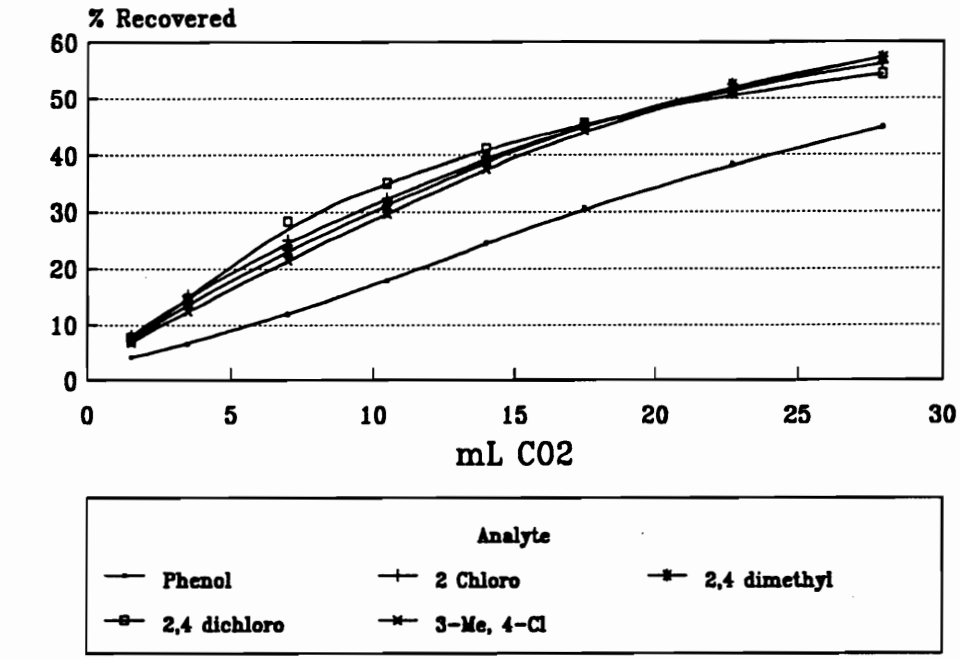
Extraction Profile of Phenols At 250 atm and 50°C



density corrected

Figure 41

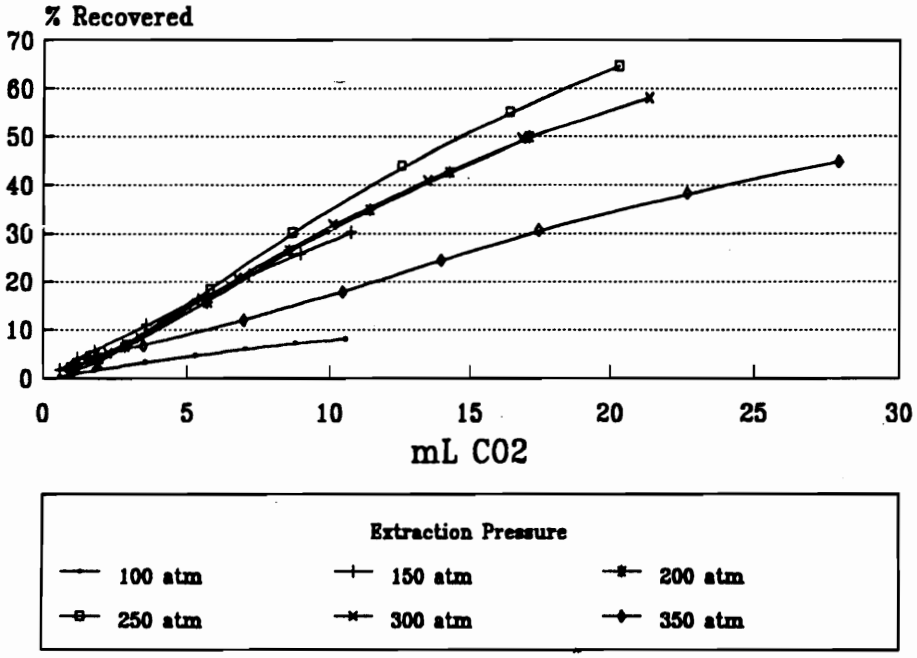
Extraction Profile of Phenols At 300 atm and 50°C



density corrected

Figure 42

Extraction Profile of Phenols At 350 atm and 50°C



density corrected

Figure 43

Extraction Behavior of Phenol at Different Pressures

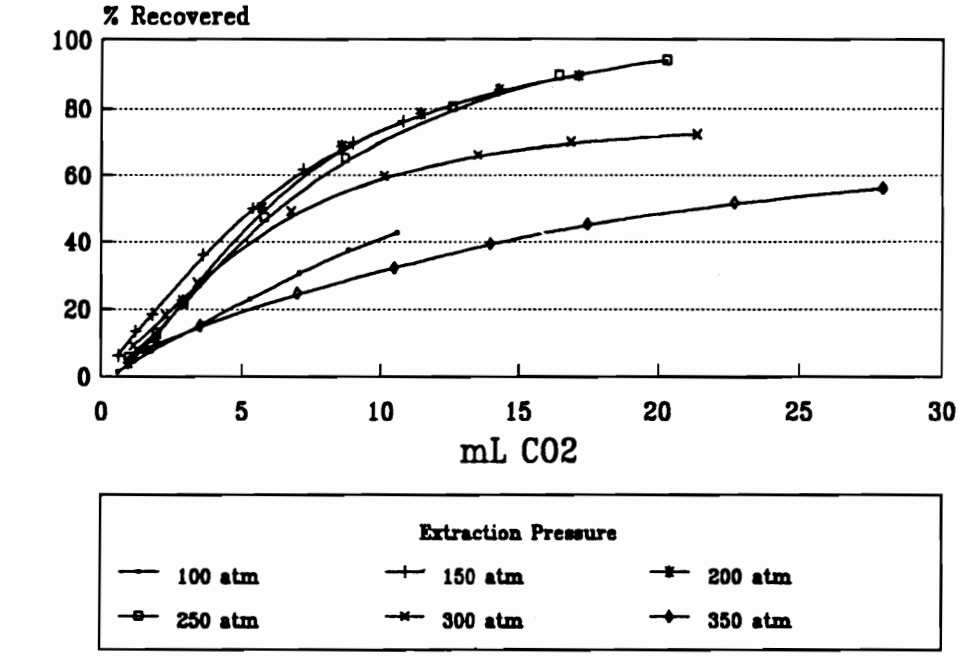
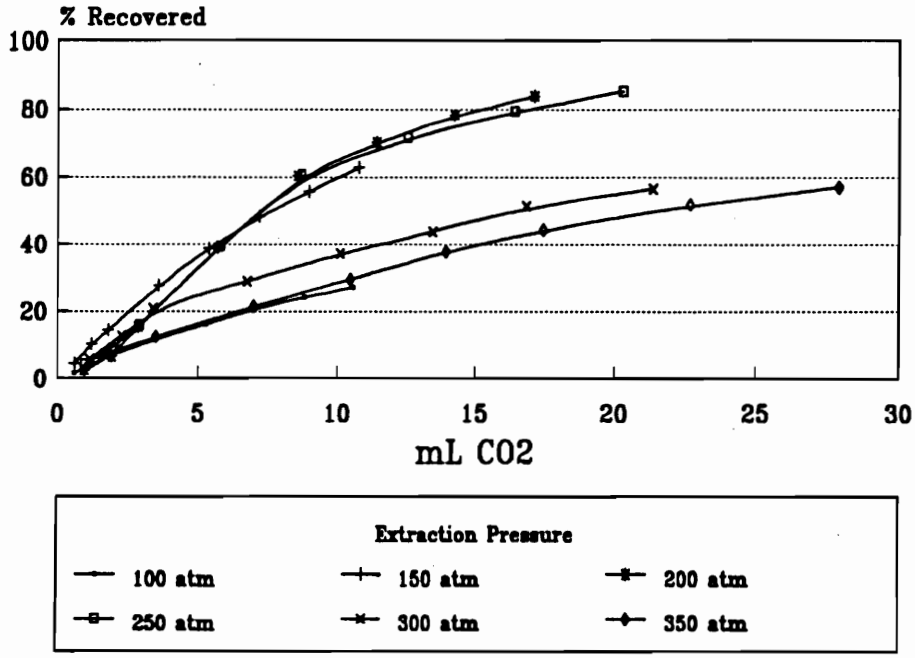


Figure 44

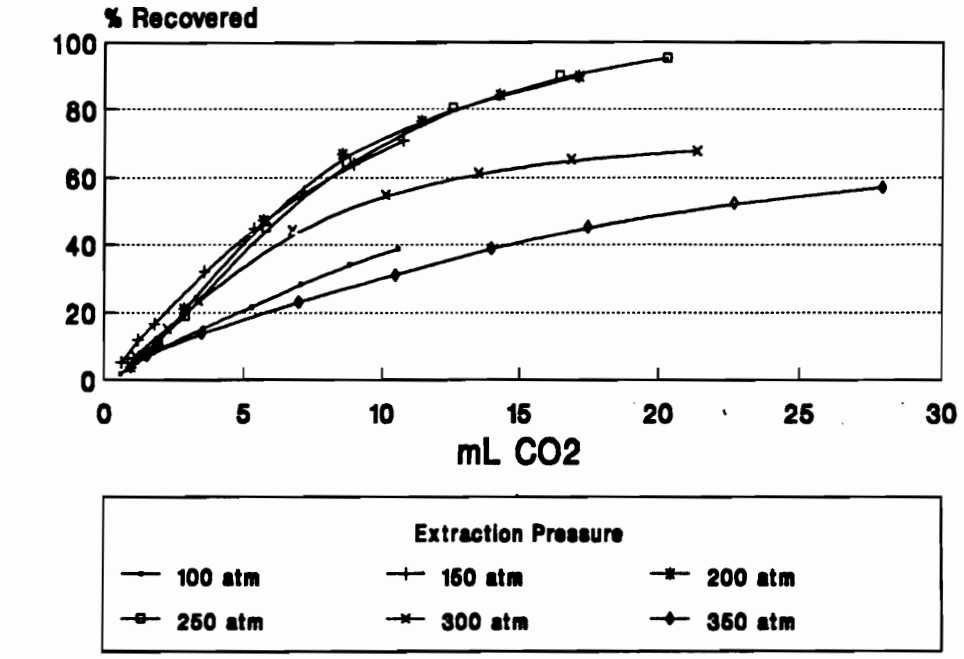
Extraction Behavior of 2-Chlorophenolat Different Pressures



density corrected

Figure 45

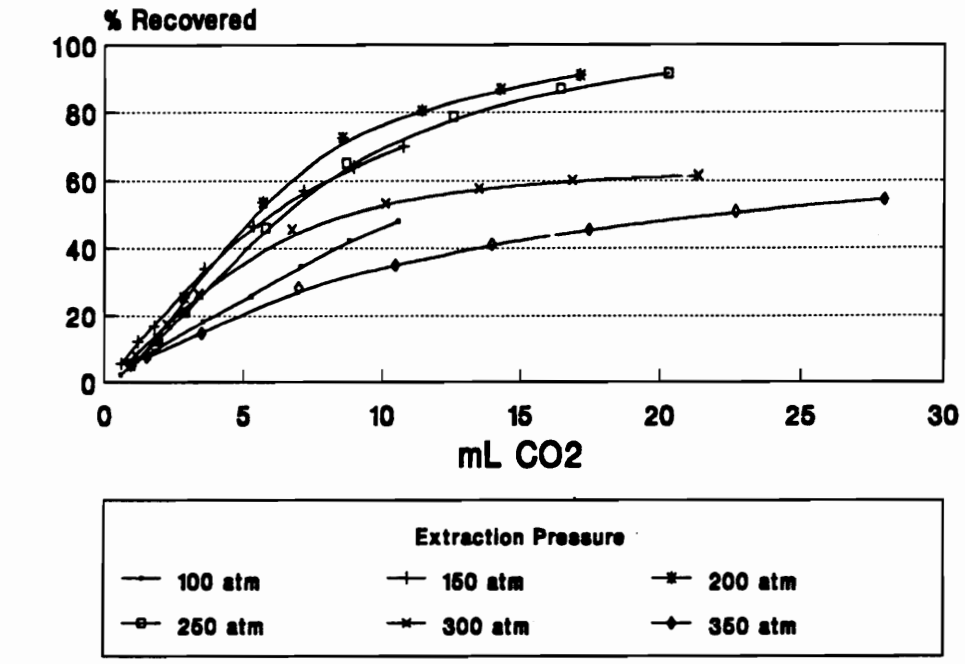
Extraction Behavior of 3-methyl-4-chlorophenol at Different Pressures



density corrected

Figure 46

Extraction Behavior of 2,4-dimethylphenol at Different Pressures



density corrected

Figure 47

Extraction Behavior of 2,4-dichlorophenol at Different Pressures

atm the extraction profiles become more linear. Again, suggesting that the extraction kinetics are becoming limited by another factor.

Phenol Distribution Coefficient

The observation of an optimized extraction pressure in this study was surprising. To aid in understanding this phenomenon, which was observed for all five phenols, static extraction of a phenol solution with headspace sampling was performed to determine the distribution coefficient of phenol in the SF-CO_2/water system. The static extraction apparatus described in the Experimental was used. Three mL of 100 ppm phenol was allowed to come to equilibrium at various pressures and temperatures.

A portion of the CO_2 was then sampled onto an SFC column and the amount of phenol in the CO_2 phase was determined by external standard. The amount of phenol in the aqueous phase was determined by mass balance and the distribution coefficient calculated. It was assumed that both H_2O and CO_2 did not change appreciably in density or volume. This assumption was thought to be valid because of the extremely limited solubility of H_2O in CO_2 and the incompressibility of water. Figure 48 and Figure 49 show the effect of pressure and density, respectively, on K_d at constant temperature. The distribution coefficient was found to increase (more phenol in the CO_2 phase) as pressure increased. K_d as a function of pressure was found to be somewhat linear which is surprising. Solubility of analytes is usually thought of as linear with density, not pressure[42].

K_d as a function of temperature at constant pressure (Figure 50) decreases with increasing temperature. This is easily explained by the fact that the density of the SF phase decreases with increasing temperature leading to a decrease in solubility of phenol at the higher temperatures. K_d as a function of temperature at constant density (Figure 51)

Table 7

Experimentally Determined Average Kd Values at Various Temperature and Pressures

Pressure	K_{34}	K_{48}	K_{54}	K_{76}
100	1.8E-01	3.5E-02	3.1E-02	1.3E-02
125	1.8E-01	6.2E-02	4.2E-02	2.1E-02
150	2.6E-01	1.3E-01	8.3E-02	4.5E-02
175	2.9E-01		1.3E-01	7.8E-02
200	3.4E-01	2.2E-01	1.6E-01	1.1E-01
225	3.8E-01		1.6E-01	1.4E-01
250	4.2E-01	2.7E-01	1.8E-01	1.7E-01
275	4.1E-01		1.9E-01	2.0E-01
300	4.4E-01	2.8E-01	2.2E-01	2.1E-01
325	4.6E-01		2.3E-01	2.2E-01
350	5.6E-01		2.1E-01	2.4E-01

For all measurements n=3 and RSD </= 5%

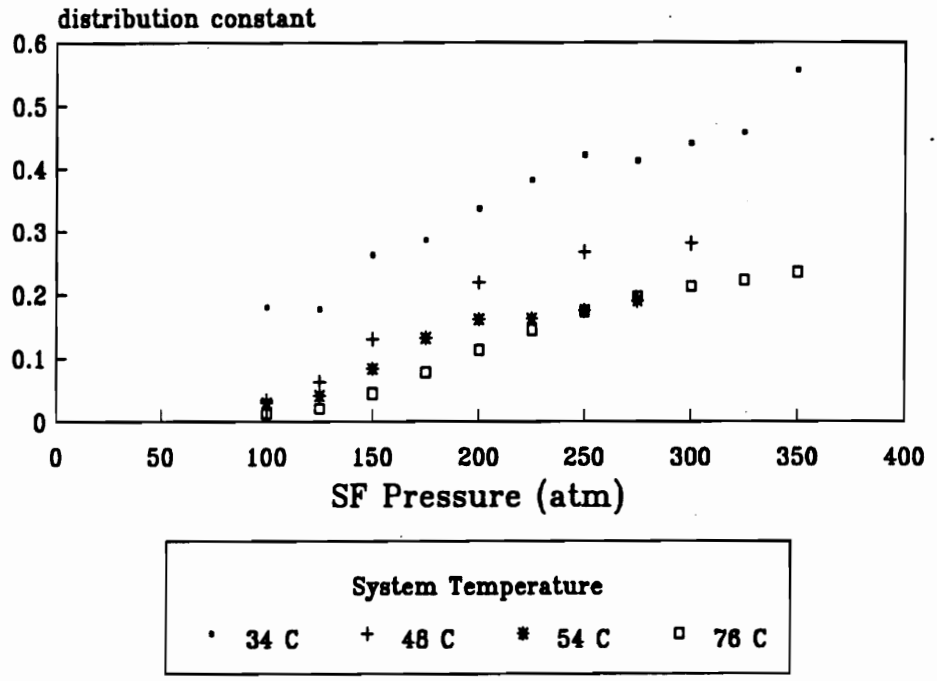


Figure 48

Distribution Coefficient vs Pressure

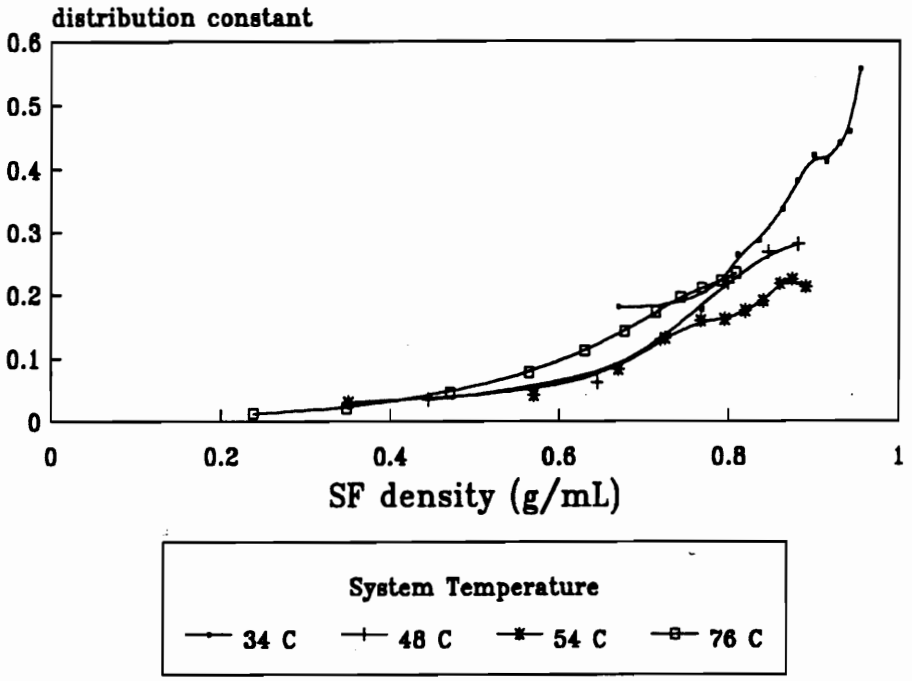


Figure 49

Distribution Coefficient of Phenol vs Density

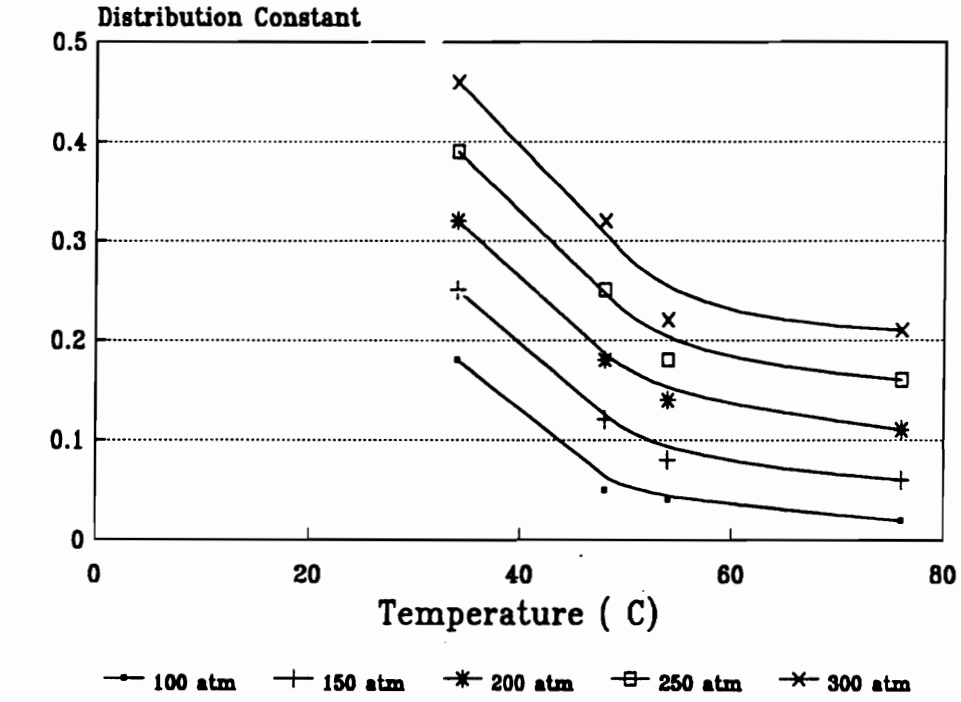


Figure 50

K_d as a Function of Temperature (Pressure Constant)

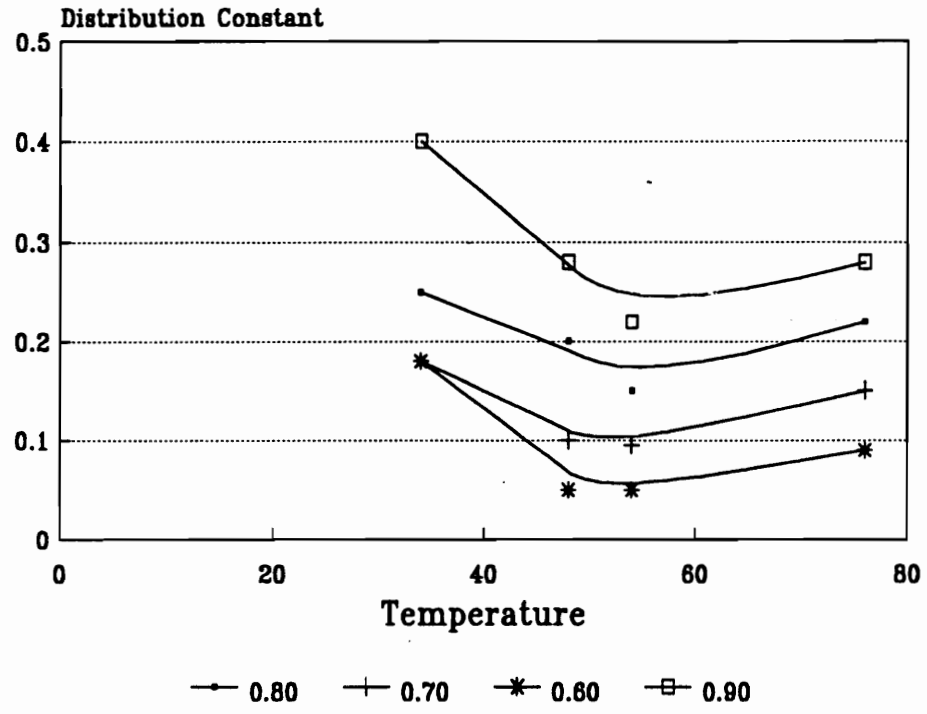


Figure 51

K_d of Phenol as a Function of Temperature (Density Constant)

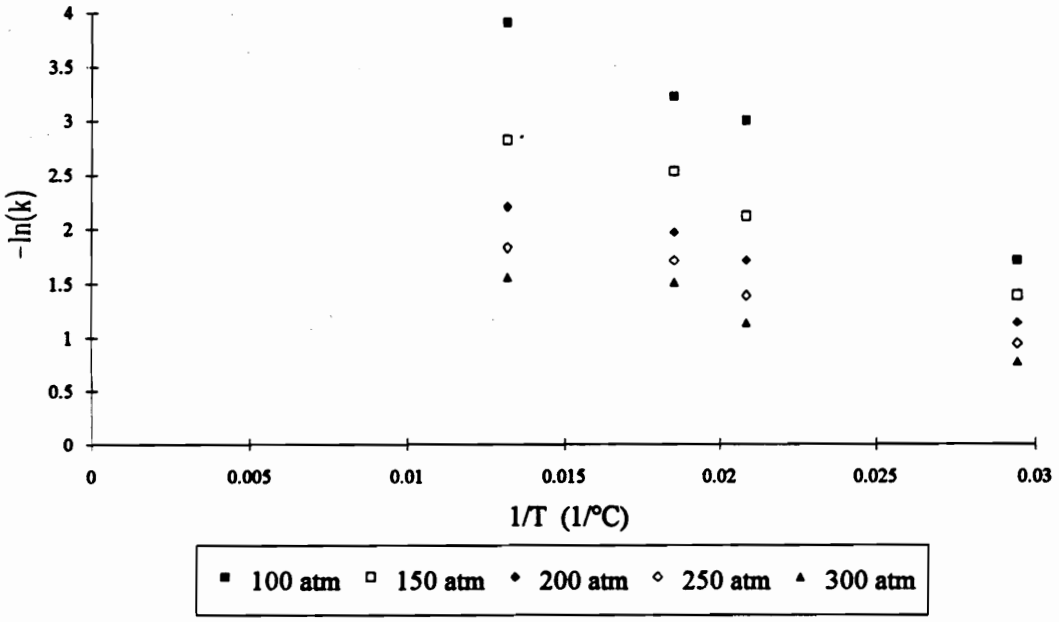


Figure 52

-ln(k) vs 1/T at Constant Pressure for Phenol in a H_2O/CO_2 system

shows a minimum in the curve at approximately 50°C. Figure 51 can be interpreted as showing the two factors that determine K_d, solubility in the aqueous phase and solubility in the SF phase. The downward trend seen initially corresponds to an increase in the solubility of phenol in the aqueous phase. The solubility of phenol in the SF phase is also increasing but does not overtake the increase in solubility in the aqueous phase. Past 50° C, however the increase in solubility in the SF phase is greater than that for the aqueous phase and the distribution constant increases.

Figure 52 is plot of the same data in another form (1/T vs -ln(K_d)). In this form the data appears to become linear at all pressures. K_d also appears to be less dependent upon temperature at increasing pressure (the slope of the line becomes less). A complete statistical workup of the data was not, however, performed.

Conclusion

The ability to extract phenols from water has been demonstrated. The extraction behavior for the materials studied has been shown to be rather complex. Clearly much more work is needed, particularly in the area of extraction kinetics, before SFE can be used routinely for the isolation of analytes from aqueous systems. The fact that extractability of analytes appears to decrease with increasing pressure must be a factor of extraction kinetics. The distribution constant for phenol was shown to increase with increasing pressure, leaving only the kinetic realm to account for the behavior. The most reasonable explanation for the decrease extraction rate deals with the size of the SF "bubbles" migrating through the aqueous phase. As pressure increases it seems reasonable to assume that the size of the SF bubbles increases while their number decreases. The effect would be a decrease in the over all surface area between the SF and the aqueous phases. This would account for the decrease in extraction rate.

Another plausible account for the decrease in extraction rate is a reaction of phenol with CO_2 at high pressures to form a carboxyl ester. The product would presumably be less soluble (or show slower mass transfer rates) in the SF phase than the phenol. The equilibrium studies do not provide information that discounts this possibility. Any change in the system (such as injection onto a SFC) might decompose the carboxyl ester so that only the phenol would be present in the chromatography.

Chapter 7

Applications of SFE from Aqueous Matrices

INTRODUCTION

As reported in Chapter 6, the use of supercritical fluids to extract organic material from aqueous solutions is a recent development. Currently there are only a few references which deal with the subject on the process, preparative or analytical scale[53-56]. In the previous chapter, the extraction of phenols from aqueous solution was described. Phenols were an appropriate choice for evaluating the extraction system since other workers were concurrently studying the extraction of phenols from aqueous solution[54,55]. Some comparison to the work of others could therefore take place.

The extraction of analytes other than phenols from aqueous matrices was also studied. Based on the instrumentation developed in previous chapters, the following classes of compounds were investigated:

Alkyl Phosphonates

Alkyl phosphonates of the general structure RR'POOR" (where R,R' and R" are alkyl groups of varying length and saturation) are of interest to various government agencies because of their chemical similarity to some nerve agents. A common method for sampling nerve agents in air is to remove them from the atmosphere (they are present as an aerosol) with a stream of water. The water is then collected and analyzed for the analytes of interest.

Hydrochloride Salts of Basic Drugs

Many basic drugs exist as salts, because the formation of a salt aids in their isolation as well as increases stability of the compound. The fact that they exist as salts can make analysis by HPLC and SFC problematic. Two common non prescription drugs, triprolidine and pseudoephedrine, were analyzed by equilibration extraction but no quantitative work was performed. Both triprolidine and pseudoephedrine were originally in the form of hydrochloride salts; as salts they were expected and found to be insoluble in supercritical CO_2.

Phenol from an Acidic Medium

Waste streams from production environments are often hostile to many forms of sample preparation and analysis. One such environment is a concentrated solution of sulfuric acid. Traditional sample preparation could involve solid phase extraction. Before solid phase extraction could be done on such a medium, the solution would first have to be neutralized with base. Neutralizing in this way takes time and could add substantial volume to the sample in question. Diluting the sample may also make trace analysis more difficult. By extracting the analyte (in this case phenol) directly from solution, time is saved and the sample is not diluted.

Sulfamethazine from Milk

It is common practice among cattle and dairy ranchers to administer sulfa drugs to their herds. The drugs are doped into feed prior to ingestion by the animal. There is concern that traces of the drug can be found in milk, animal tissue and organs[59]. Sulfamethazine is a typical sulfa drug administered to dairy cattle. Isolation of the drug from milk is difficult by traditional methods because of the high fat content of milk.

Glycerides have been shown to have limited solubility in SF CO_2 at low densities[60]. Selective extraction of sulfamethazine from milk (primarily water and glycerides) should therefore be possible.

EXPERIMENTAL

The extraction vessel used was of the same design as the aqueous vessel discussed in Chapter 5. The volume of the vessel and the connecting tubing that was used was the same. However, finger tight fittings (Supelco Inc, Bellfonte, PA) were tested as the fittings for the 1/16" stainless steel tubing, and were found not to alter the extraction characteristics of the extraction vessel. The finger tight fittings from Supelco were replaced by another design of finger tight from Keystone Scientific (Keystone Scientific, Bellfonte, PA). The Keystone fittings were superior to the Supelco fittings because they incorporated a locking ferrule which held the 1/16" tubing securely in place at high pressure even if the sealing ferrule slipped. Single ferrule systems such as those from Supelco tended to release the tubing under pressure. It should be noted that although the single ferrule systems were rated to 7000 psi they were intended for use with liquids, not a compressible gas such as CO_2.

Supercritical grade CO_2 (Scott Specialty Gas, Plumbsteadville, PA) was delivered to the extractor and any subsequent supercritical fluid chromatography was done by either a Suprex SFC 200 (Suprex Corp.,Pittsburgh, PA) or a Hewlett Packard 1082 liquid chromatograph modified for use as a SFC (Hewlett Packard Corp., Avondale, PA). The equilibration system described on pp 90-93 was employed for the equilibrium extractions.

Liquid chromatography was performed with a LC/9560 ternary pump (Nicolet, Madison, WI) equipped with a TriDet UV 254 detector (Perkin-Elmer). Fourier

transform infrared (FTIR) data were acquired from a Nicolet (Madison, WI) supercritical fluid chromatography/infrared (SFC/IR) interface.

Experimental Conditions

Alkyl Phosphonates

Diisopropyl methylphosphonate was extracted from aqueous solution by the equilibrium method. The equilibration system is the same as described on page 92. Supercritical CO_2 was delivered to the system by the pump and control module from a Suprex 200A SFC. Chromatography was performed by the same Suprex 200A. The extraction was performed with 100% CO_2 at a pressure of 250 atmospheres and a temperature of 75°C. A DELTABOND CN column was used to perform the separation. When FTIR detection was used, the pressure during chromatography was held constant at 100 atmospheres for 10 minutes after injection and then ramped linearly to 400 atmospheres in 15 minutes. For work not employing FTIR detection the pressure was held at 100 atmospheres for 30 seconds and then ramped linearly to 400 atmospheres in 15 minutes. For the study a range of DIMP concentrations were studied form 800 ppm to 800 ppb.

Hydrochloride salts of Basic Drugs

The hydrochloride salts of triprolidine and pseudoephedrine were extracted by the equilibrium system described on page 92. Supercritical CO_2 was delivered to the system by a Hewlett-Packard 1084 liquid chromatograph modified for supercritical fluid use. Chromatography was performed by the same HP 1084. The extraction was performed at a pressure of 340 bar and a temperature of 50°C. A 250 x 4mm i.d. DELTABOND CN column was used to perform the separation. A mobile phase gradient was used to elute

both compounds. The mobile phase consisted of 100% CO_2 flowing at 2 mL/min and 150 μL/min of a 0.01 M solution of tetrabutyl ammonium hydroxide (TBAOH) in MeOH. The initial mobile phase concentration was held constant for one minute after injection. The MeOH/TBAOH modifier was then ramped from 150 μL/min to 450 μL/min in three minutes and then held constant. The TBAOH solution was delivered to the system by a syringe pump from a SUPREX 200A SFC. UV detection at 290 nm was employed.

Phenol from sulfuric acid

Extractions of phenol from 6 M H_2SO_4 were done using the dynamic scheme outlined in Chapter 4. The extraction was carried out using 100% CO_2 at 100 atmospheres and 50°C. Thirty milliliters of SF CO_2 were passed through the system. Trapping of the analyte was carried out in two ways. Specifically, the decompression was carried out in a solvent (50/50 MeOH/water) so that the analyte was put directly into solution or the analyte was deposited onto a solid support (C18) as described in Chapter 4. After the extraction the solid support was then rinsed with 3 mL of a 50/50 MeOH/water solution. In both cases the extracted phenol was then analyzed by liquid chromatography. A range of concentrations from 400 ppm to 20 ppm for phenol from aqueous solution were explored. Liquid chromatography was performed using a 250 x 4.6 mm Nucleosil C8 column (Keystone Scientific, Bellfonte PA) and a 50/50 MeOH/water mobile phase at 1 mL/minute.

Sulfamethazine from milk

Sulfamethazine was extracted from milk using the dynamic mode, with trapping onto a solid support. A 3 mL sample of whole milk was fortified with sulfamethazine at the 60 ppm level and used as the sample. The extraction was done at 250 atmospheres

and 50°C. Thirty milliliters of SF CO_2 were passed through the system. Trapping was done on a phenyl SPE tube (Supelco Inc.) which was rinsed after the extraction with 3 mL of MeOH. Liquid chromatography was used to detect extracted sulfamethazine. A 150 x 4.6 mm Nucleosil phenyl column (Keystone Scientific) was used as well as a 30/70 MeOH/water mobile phase. UV detection was carried out at 250 nm.

RESULTS AND DISCUSSION

Alkyl Phosphonates

Figure 53 shows a comparison of the flame ionization and Gram-Schmidt Reconstruction (GSR) (i.e. essentially total infrared absorbance) of a SFE/SFC run on 834 ppm diisopropyl methyl methylphosphonate (DIMP) in water (the equilibration apparatus was used). FTIR detection was used to provide positive identification of the eluting peak as DIMP as it provides structural information and the spectrum obtained from SFE/SFC can be compared to that of a standard. The large peak at the beginning of the GSR is due to a relatively small amount of water (solvated by the SF CO_2 phase) being injected onto the column along with the DIMP. The relatively long hold time allowed the water to completely elute from the column prior to DIMP. Figure 54 compares the spectrum of the major eluting peak from the SFE/SFC with the spectrum of DIMP standard run in a traditional manner under the same chromatographic conditions. Clearly, the two peaks result from the same compound.

In order to be a useful extraction technique the method must be fast (less than 30 minutes). A time study was done to determine the length of time required for the system to reach equilibrium was studied. As the time required to reach equilibrium was assumed to be independent of analyte concentration a fairly concetrated solution of DIMP in water, 800 ppm, was examined. In order to produce reproducable The extraction conditions

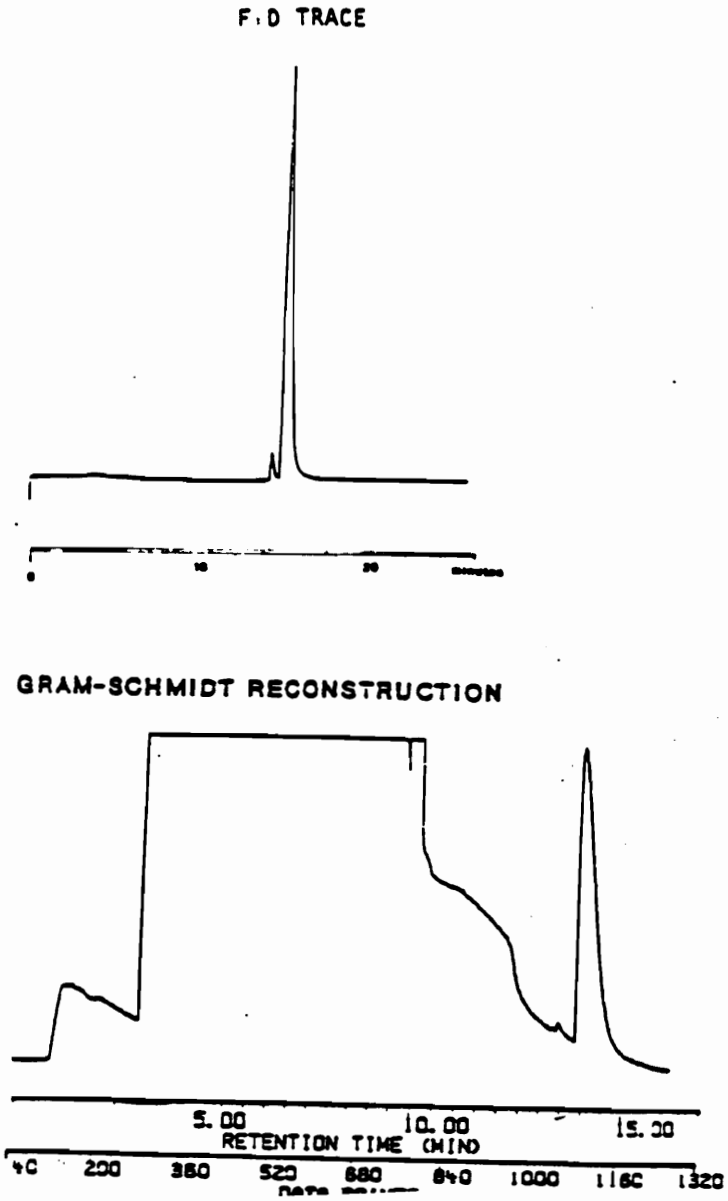


Figure 53

FID and GSR Traces From SFE/SFC of 800 ppm Diisopropyl methylphosphonate

The extraction conditions were: 100% CO_2 at 250 atm and 75°C. Chromatography was done with a 25 cm DELTABOND CN. The initial pressure was 100 atm and it was held for 10 minutes. The pressure was then ramped linearly to 400 atm in 15 minutes.

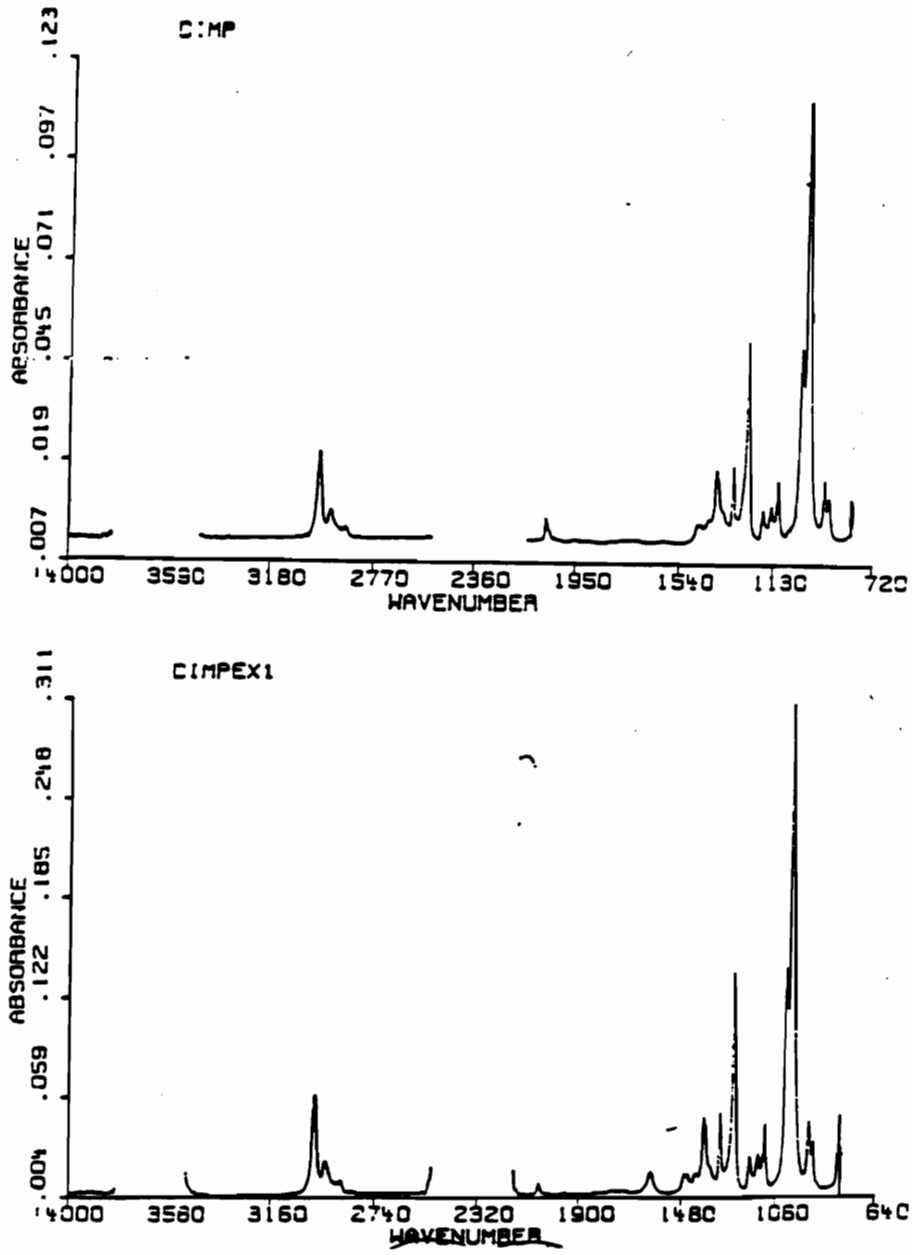


Figure 54

Comparison of standard to spectra from major eluting peak from SFE/SFC

were: 100% CO_2 at 250 atm and 75°C. Chromatography was done with a 25 cm DELTABOND CN. The initial pressure was 100 atm and it was held for 10 minutes. The pressure was then ramped linearly to 400 atm in 15 minutes.results, the system has to be at equilibrium. If the system is not at equilibrium, then it would be necessary to sample the system at exactly the same time for all extractions. The results are shown in Figure 55. As expected, there is an initial equilibration time where there is a slow increase in the amount of analyte extracted with time. After 1.5 hours the system reaches equilibrium and there is no change in the SFC/FID area response (trace A). However, when 0.1 mg of NaCl was added to the sample before extraction, the equilibration time was reduced to less than 5 minutes, as shown in trace B of Figure 55. The addition of salt to facilitate the precipitation of polar organics from solution is a common practice in synthetic organic labs. Increasing the ionic strength of the aqueous phase decreases the solubility of the organic analytes in the salt solution.

The major advantage of the static SFE/SFC system is speed. Analysis times for diisopropyl methylphosphonate (DIMP) were typically less than 45 minutes. This time includes all sample preparation, extraction, equilibration, and chromatography (3 injections). Off-line SFE followed by chromatography would have certainly taken longer for the same number of analyses. The major limitation of the system was its relatively high limit of detection with flame ionization. A calibration curve for DIMP constructed by the equilibration method was found to be linear over three orders of magnitude. Relative standard deviation was found to be less than 1.5% for concentrations above 1 ppm and between 1% and 10% for concentrations less than 1 ppm but greater than 500 ppb for DIMP. Analysis with this system much below 500 ppb is thought to be impossible while maintaining chromatographic efficiency, since a larger sample loop would be required. A

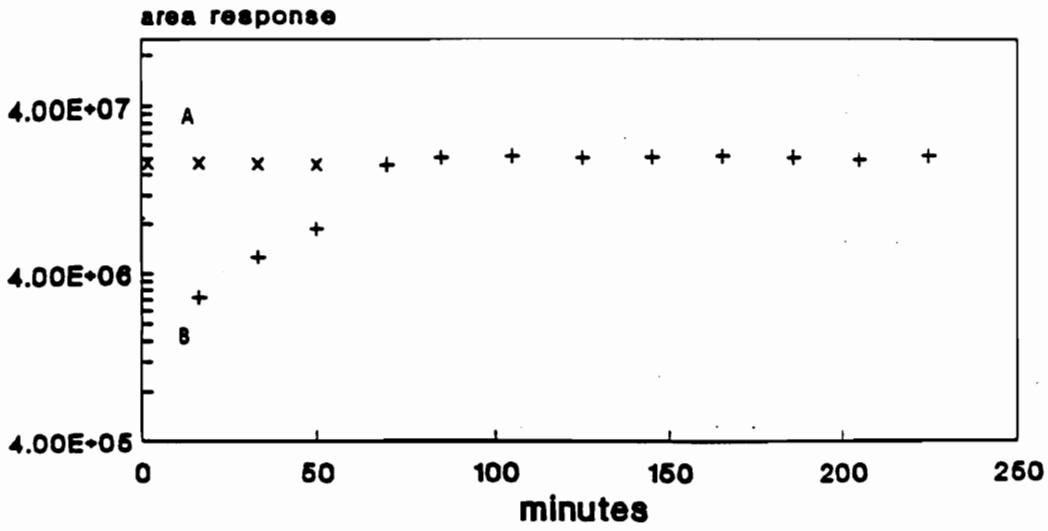


Figure 55

Peak Area vs Time for the extraction of DIMP from water.

Trace B is that for a simple water/DIMP solution. Trace A is from a water/DIMP/NaCl solution.

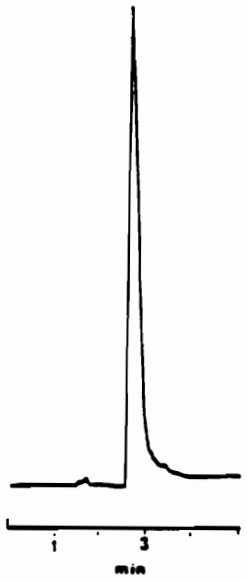
sample loop larger than 500 µL would increase the amount of sample loaded onto the column but would result in unacceptably large peak widths when using a 1 mm I.D. column. The use of a 2 mm i.d. column would allow for a larger sample loop to be used and would not preclude the use of flame ionization detection.

Hydrochloride salts of Basic Drugs

Initially, an attempt was made to elute the hydrochloride salts of triprolidine and pseudoephedrine. Even at extremely high (i.e.> 20%) MeOH concentrations in the mobile phase the compounds would not elute. In hopes of first forming and then extracting the free bases, a molar excess of tetrabutylammonium hydroxide was added to 3 mL of 1 mg/mL solutions of both compounds. Static extractions of the type described earlier were then performed. SFC traces of the analysis for both extracted compounds are shown in Figures 56 and 57. Retention times of the peaks from extraction match those of the free bases standards done by the same chromatographic method. The chromatographic traces show that the free bases were extractable from water with CO_2 as well as chromatographable under supercritical conditions.

Phenol from Sulfuric acid

Many of the problems encountered with the static SFE/SFC (as discussed in the previous applications) arose from poor elution behavior of the extracted analytes using SF CO_2 as the mobile phase for the SFC portion of the experiment. An obvious way around this problem is to use off-line SFE. Off-line SFE may be slower than on-line SFE/SFC on a strict one sample per amount of time basis but it does allow for any analytic technique to be used after the extraction. In addition the experience gained from off line extraction can



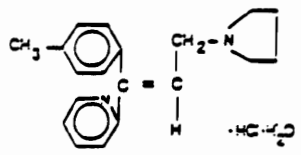


Figure 56

SFE/SFC of triprolidene

The extraction conditions were: 340 bar and 50°C for 20 minutes. The chromatographic conditions were: 25 cm DELTABOND CN column, mobile phase was 2 mL/minute CO_2 and 150 µL/minute methanol/TBAOH for one minute, the modifier was then ramped from 150-450 µL/minute in 3 minutes, the temperature was 60°C , detection was done by UV at 290 nm.

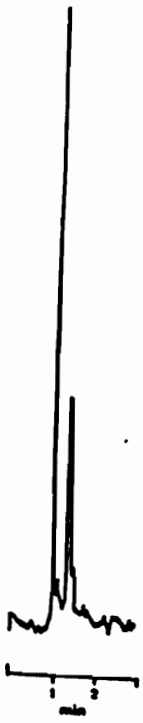


Figure 57

SFE/SFC of pseudoephedrine

The extraction conditions were: 340 bar and 50°C for 20 minutes. The chromatographic conditions were: 25 cm DELTABOND CN column, mobile phase was 2 mL/minute CO_2 and 150 µL/minute methanol/TBAOH for one minute, the modifier was then ramped from 150-450 µL/minute in 3 minutes, the temperature was 60°C , detection was done by UV at 290 nm.

be more directly applied to the design of depostition type SFE/chromatograpy on-line systems. From a more pratcical point of view, the use of off-line SFE allows for traditional chromatographic or spectroscopic techniques to be used which may be superior to SFC for practical as well as legal reasons.

The different types of off-line SFE were discussed in Chapter 2. One of the major problems associated with the isolation of the extracted analytes is the large volume of gas produced on the low pressure end of the restriction. A compressed flow of 1 mL/min corresponds to 100-500 mL/min of decompressed gas, depending upon the density and nature of the SF used for the extraction. Collection of the analyte into a liquid solvent can be especially difficult. Violent bubbling of the liquid solvent by the gas can cause sporadic loss of analyte. Both liquid collection and solid phase collection were used for the extraction of the same phenol solution. The performance of the two different types of collection could then be compared.

Using liquid collection sample loss was evident, as the extraction efficiency for phenol out of water (a 3 mL sample at 150 atm, 50°C with 30 mL of CO_2 used) was found to be 60% with 15% RSD at the 400 ppm level (n=5). Flow rate of SF CO_2 through the system was 1 mL/min. The large RSD was thought to result from the sample loss created by violent bubbling. Flow rates faster than 1 ml/min were not used as they tended to lead to "gross" loss of collection solvent and analyte. At the faster flows, solution would "bump" out of the collection vessel leading to obvious error. Faster flow rates are desirable as they allow for faster extractions to be run, because a greater volume of fluid can be put through the extraction vessel in a given amount of time. A final problem with collection into a liquid solvent was that while the analyte is soluble in the collection solvent, so was CO_2. It was necessary to let the solution warm slowly for about half an hour in order to allow the dissolved CO_2 to evolve. This warming step was followed by

sonication to remove any remaining CO_2 in solution. Failure to perform these steps resulted in sporadic "bumping" of the solution. In some cases the cap to the collection container was blown off the vessel by the sudden evolution of CO_2.

Deposition of the analyte onto a solid support was intended to remove the problem of loss of the analyte due to violent bubbling solvent. The trapping system described in Chapter 4 provided a significant improvement over collection of analyte into a solvent. At the 20 ppm level phenol was found to be extractable to 80% (16 mg) with 30 mL of supercritical CO_2 (at 150 atm and 50°C with 30 mL CO_2 used). RSD of the entire method, including the chromatography was determined to be 9% (n=10), about half of that obtained by collecting a much more concentrated solution into a liquid solvent. The extractable amount of phenol was confirmed by observing the amount of phenol left in the raffinate (4 mg with an RSD of 20%). The recovery of phenol may have been improved by passing more SF CO_2 through the system. At the time this study was performed the extraction behavior of phenol discussed in Chapter 5 was not yet known. The recovery agrees well with the predicted recovery from Figure 38 (p10θ).

SFE of 42 ppm phenol in 6 M sulfuric acid with collection in methanol was carried out. (This work was performed prior to the study which compared efficiencies of solid and liquid trapping schemes. The advantages of using a solid sorbent were clearly shown in the previous discussion.) This represents the case of an analyte in an aqueous environment which is hostile to many forms of analysis. A representative trace for the separation of the extracted phenol is shown in Figure 58. The work was not continued further because it was found that the concentrated sulfuric acid solution etched the stainless steel extraction vessel. The raffinate was green indicating that nickel had been removed from the stainless steel. Further work into the extraction of analytes from such stronly acidic solutions will require an extraction vessel an inert lining.

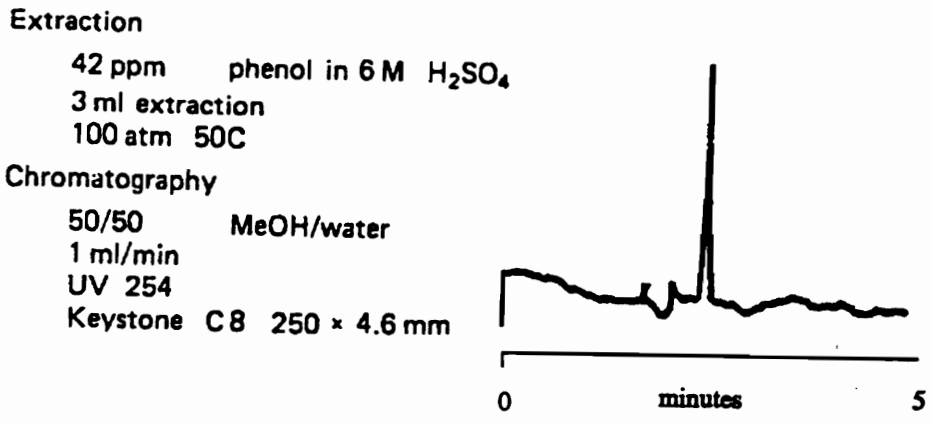


Figure 58

Extraction of phenol from 6M sulfuric acid

The extraction conditions were: 100 atm, 50°C. Chromatography was done with a Nucleosil C8 column (250 X 4.6 mm), 50/50 MeOH/H_2O mobile phase at 1 mL/minute and UV 254 detection.

Sulfamethazine from Milk

Figure 59 shows the reverse phase chromatographic traces of a sulfamethazine standard and sulfamethazine which had been extracted from whole milk at 250 atm, 50°C. Thirty milliliters of liquid CO_2 was passed through the system, about 60 mL of supercritical CO_2. The sulfamethazine was collected on a diol trap at -20°C and rinsed with 3 mL of MeOH/H_2O (50/50). Recovery of sulfamethazine was greater than 95%. No salt or base was added to the milk prior to extraction. Analysis of the raffinate afterwards showed no detectable amount of sulfamethazine.

CONCLUSION

It has been shown that many different classes of analytes can be extracted from water using supercritical CO_2. The initial results here indicate that the technique has promise for a wide range of analytes which can be difficult to analyze by other methods. Analytes such as phenols are extremely soluble in H_2O and are therefore difficult to analyze by purge and trap methods. The alkyl phosphonates studied, as well as the drugs, have little vapor pressure so are not candidates for purge and trap methods. In both cases, a simple on-line chromatographic technique would be uselful to lower the concentration limit which could be easily analyzed as well as to speed up the total analyses time. Currently liquid/liquid extraction or solid phase extrction is necessary to first remove the analytes from aqueous solution and to remove unwanted interferences from the sample. The SFE methods developed provide a useful alternative to current methods.

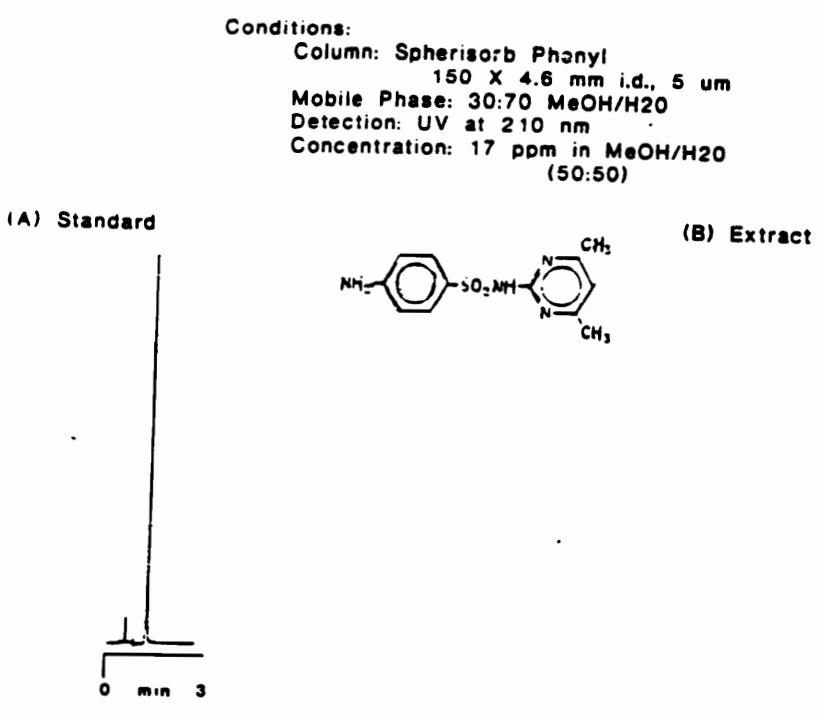


Figure 59

Extraction of sulfamethazine from whole milk

The extraction conditions were: 250 atm, 50°C. Chromatography was performed with: a phenyl column (150x4.6 mm), 30/70 MeOH:H_2O mobile phase at 1 mL/minute. The standard was at 60 ppm as was the original sulfamethazine spiked whole milk.

Chapter 8

Conclusions and Further Work

The work in this thesis began when commercial instrumentation available to perform SFE was extremely limited. Commercial off-line SFE equipment basically consisted of a syringe pump and and an oven system. Restrictor and collection apparatus were constructed by the user. At the time a simple fused silica restrictor and beaker of solvent were the most popular modes of collection. Since that time commercially available instrumentation has advanced considerably. Of the four major SFE vendors (Dionex, Hewlett Packard, Co., ISCO, and Suprex) two now offer solid collection apparatus while the others offer advanced liquid collection apparatus. None of the vendors offer routine apparatus for the extraction of "large" volumes of liquid samples.

There is not a consensus of how to perform SFE. The instruments currently on the market are all very different and embrace very different extraction philosophies. The variation in extraction and extractor types is not limited to commercial instrumentation. The literature is full of different extraction schemes, each having its own merits and problems.

In this thesis, many different types of extraction apparatus were developed. The goal of the development was to make a convenient and rugged system for the extraction of analytes from aqueous solution using supercritical carbon dioxide. Much of the work was the first of its kind. Three systems stand out as being quite unique; a simple extraction vessel for use with extracting aqueous solutions, a rugged trapping system which allowed for efficient trapping of analytes after supercritical fluid extraction and a reliable system for measuring the distribution coefficient of an analyte between water and a supercritical fluid. Without these systems the extraction of analytes from aqueous

solutions using supercritical fluids would still be possible, although much more difficult. The systems developed here are not perfect systems and will undoubtedly be improved if they prove to be of value.

There is, obviously, room for further development. The extraction vessel developed for use with aqueous solutions is straight forward but could be improved. The vessel is of limited volume and is awkward to fill and empty. It also may prove to have poor mixing between the supercritical fluid and the aqueous solution. A high pressure vessel more like the low pressure vessels used for purge and trap GC would have advantages over the current design. Employing such a vessel would give better mixing and allow for easy and quick sample introduction and removal.

The trapping system, while sufficient for the needs of the work stated herein, needs further investigation. To be useful for more general use the effect of various trapping materials on different classes of analytes must be noted. Some of this work has already been reported[61].

The equilibration extracton system worked well. It could easily be expanded for use in determining the solubility of materials in supercritical fluids. Neet samples, either liquid or solid could be used with the vessel developed for aqueous samples. The effect of modifiers on solubility could also be probed as long as the modifier/SF mixture could be mixed prior to introduction.

Supercritical fluid extraction is far from a mature technique. The variation in the types and ways different people perform SFE is evidence of the very "young" nature of SFE. As the technique matures the variation in extraction techniques will certainly diminish. It is the authors opinion that solid phase collection as well as extraction of aqueous samples will be part of the consensus on how to perform SFE.

References

1 Lee, M.L.; Markides, K.E. (Eds) Analytical Supercritical Fluid Chromatography and Extraction,Chromatography Conferences, Inc. Provo, Utah, (1990).

2 Czybryt, J.J.; Meyers, M.N.; Giddings, J.C., *Phys.Chem.* **1970,** 74 4260.

3 Schultz, W.G.; Randall, J.M., *Food Tech.* **1970,** 24, 94.

4 Gars, H.J., Ber. Bunsenges. *Phys. Chem.* **1984,**88, 894.

5 King, J.W., *J. Chromatogr. Sci.* **1989,** 27, 355.

6 Van Leer, R.A.; Paulitis, M.E., *J. Chem. Eng. Data* **1980,** 25, 257.

7 Debendetti, P.G.; Kumar, S.K., *AIChE J.* **1988,** 34, 645.

8 Ong, C.P.; Ong, H.M.; Li, S.F.Y.; Lee, H.K., *Microcol. Sep.* **1990,** 2, 69.

9 Friedrich, P.G.;Prude, E.H., *J. Am. Chem. Soc.* **1984,** 61, 223.

10 Harvala, T.; Alkio, M.; Komppa, V., *Chem. Eng. Res. Dev.* **1987,** 65, 388.

11 Fagernas, L., *Acta Chem. Scand.* **1986,** 40, 543.

12 Tanaguchi, M.; Nomura, R.; Kijma, I.; Kobayashi, T., *Agric. Biol. Chem.* **1987,** 51, 413.

13 Hedrick, J.L.; Taylor, L.T., Unpublished Results

14 Hawthorne, S.B.; Krieger, M.S.; Miller, D.J., *Anal. Chem.* **1989,** 61, 736.

15 Hawthorne, S.B.; Miller, D.J., *J. Chromatogr. Sci.* **1986,** 24, 258.

16 Janda, V.; Steenbeke, G.; Sandra, P., *J. Chromatogr.* **1989,** 479, 200.

17 Onuska, F.I.; Terry, K.A., *J. High Res. Chromatogr. Chromatogr. Commun.* **1989**, 12, 357.

18 Ikushima, Y.; Saito, N.; Hatakeda, K.; Ito, S.; Asano, T.; Goto, T., Bull. *Chem. Soc. Jpn.* **1986**, 59, 3709.

19 Liebman, S.A.; Sarver, E.W.; Snyder, A.P.; Fifer, R.A.; Shaw, F.; Harper, A.M., Presented at "Joint International Symposium on the Compatibility of Plastics and other Materials with Explosives, Propellants, and Pyrotechnics and Processing of Explosives, Propellants, and Ingredients" 1988.

20 Squires, T.G.; Paulitis, M.E., (Eds) Supercritical Fluids: Chemical and Engineering Principles and Applications *ACS Symp. Ser.* No 329, Washington D.C.

21 Xie, Q.L.; Markides, K.E.; Lee, M.L., *J. Chromatogr. Sci.* **1989**, 27, 365.

22 Wright, B.W.; Frye, S.R.; McMinn, D.G.; Smith, R.D., *Anal. Chem.* **1987**, 59, 640.

23 Hawthorne, S.B.; Miller, D.J., *J. Chromatogr.* **1987**, 403, 63.

24 Hawthorne, S.B.; Miller, D.J., *J. Chromatogr. Sci.* **1989**, 27, 347.

25 Hawthorne, S.B.; Miller, D.J., *J. Chromatogr. Sci.* **1990**, 28, 2.

26 Wright, B.W.; Wright, C.W.; Gale, R.W.; Smith, R.D., *Anal Chem.* **1987**, 59, 38.

27 Hawthorne, S.B.; Miller, D.J., *Anal. Chem.* **1987**, 59, 1705.

28 Lopez-Avilia, V.; Dodhiwaia, N.S.; Beckert, W.F., *J. Chromatogr. Sci.* **1990**, 28, 468.

29 Schneiderman, M.A.S; Sharma, A.K.; Locke, D.C., *J. Chromatogr.* **1987**, 409, 343.

30 Ebeling, H.; Franck, E.V., *Ber. Busenges. Phys. Chem.* **1984,** 88, 862.

31 de la Tour, C., *Ann. Chim. Phy.* **1822,** 21, 127.

32 Sugiyama, K.; Saito, M.; Hondo, T.; Senda, M.,
J. *Chromatogr.* **1985,** 332, 107.

33 Skelton, R.J.; Johnson, C.C.; Taylor, L.T.,
Chromatographia **1986,** 21, 3.

34 Engelhardt, H.; Gross, A., *J. High Res. Chromatogr. Chromatogr. Commun.* **1988,** 11, 38.

35 Blumberg, R., Liquid-Liquid Extraction Harcourt, Brace, Jovanovich (1988) : New York.

36 Levy, J.M.; Ritchey, W.M., *J. High Res. Chromatogr.Chromatogr. Commun.* **1985,** 8, 503.

37 Levy, J.M.; Ritchey, W.M., *J. High Res. Chromatogr. Chromatogr. Commun.* **1987,** 10, 443.

38 Lee, M.L.; Xu, B.; Huang, E.C.; Djordjevic, N.M.; Chang, H.-C. K.;Markides, K., *J.Microcol. Sep.* **1989,** 1, 7.

39 Poole, C.F.; Dean, T.A., J. High Res. Chromatogr. Chromatogr. Commun. **1986,** 9, 626.

40 Poole, C.F., Personal Communication

41 Chester, T., Personal Communication

42 Miller, J., Chromatography: Concepts and Contrasts John Wiley and Sons, New York , 1988.

43 Chester, T., Personal Communication

44 Henry, R. Personal Communication

45 Rastogi, S.C., *Chromatographia* **1990,** 29, 441.

46 Robinson, A.B.; Partridge, D.; Turner, M.; Teranishi, R.; Pauling, L.J., *Chromatogr.* **1973,**85, 19.

47 Nunez, A.J.; Gonzales, L.F.; Janak, J.J., *J. Chromatogr.* **1984,** 300, 127.

48 Drozd, J.; Novak, J., *J. Chromatogr.* **1979,** 165, 141.

49 Wiebe, R.; Gaddy, V.L., *J. Am. Chem. Soc.* **1934,** 56, 76.

50 Wiebe, R.; Gaddy, V.L., *J. Am. Chem. Soc.* **1941,** 63, 475.

51 Elgin, J.C.; Weinstock, J.H., *J. Chem. Eng. Data* **1959,** 4, 3.

52 McHugh, M.A.; Krukonis, V.J. Supercritical Fluid Extraction: Principles and Practice Butterworths, Boston ,1986.

53 Ehntholt, D.J.; Thrun, K.; Eppig, C., *Intern. J. Environ. Anal. Chem.* **1983,** 13, 219.

54 Roop, R.K.; Akgerman, A.; Dexter, B.J.; Irvin, T.R., *J. Supercrit. Fluids* **1989,** 2, 51.

55 Roop, R.K.; Akgerman, A.; Irvin, T.R.; Stevens, E.K., *J. Supercrit. Fluids* **1988,** 1, 31.

56 Thiebaut, D.; Chervet, J.P.; Vannort, R.W.; Dejong, G.J.; Brinkman, U.A. Th.; Frei, R.W., *J. Chromatogr.* **1989,** 477, 51.

57 Merk Index, 11th Edition , S. Budavari Ed, Merkand Co., Rahway, NJ 1989.

58 Langes Handbook of Chem, 12th Edition McGraw Hill Book Co. NY 1972.

59 Berry,A.J.,Games, D.E., Perkins,J.R. *J. Chromatogr.* **1986,** 363, 147.

60 King, J.W., Johnson, J.H., Friedrich, J.P. *J. Agric. Food Chem.* **1989,** 37, 951.

61 Mulcahey, L.J., Effect of Modifiers in Supercritical Fluid Chromatography and Extraction, Doctoral Thesis, Virginia Polytechnic and State University 1991.

Vita

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