Modifier Effects in the Supercritical Fluid Extraction of Solutes from Clay, Soil, and Plant Materials

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The effect of polar modifiers on matrix swelling has been examined under supercritical conditions. Plant materials and clays which would typically be used in supercritical fluid extraction have been examined. The extent of swelling of modifier-saturated plant and clay matrices was observed via a high-pressure sapphire view cell. Extractions of radiolabeled solutes from these matrices were conducted separately. The relationship between the extractability and the swelling in these materials was determined. A correlation based on these results suggested a mechanism to explain the interaction between the supercritical fluid, the modifier, and the matrix.

INTRODUCTION

The determination of residual amounts of agricultural products in soils and plants is of regulatory and environmental significance. Soils are complex systems composed of many ingredients including silt, clay, and organic matter; plants consist mainly of organic matter. The use of pure supercritical fluids for the extraction of solutes from various environmental media has been conducted successfully in the past.[1,2] In the extraction of solutes using supercritical carbon dioxide, the carbon dioxide is considered a nonpolar solvent, with a liquid solubility equivalent to hexane at moderate densities. Solute molecules exhibiting significant nonpolar character are readily solubilized in unmodified supercritical CO_2 and, therefore, are extractable. As the polarity of analytes of interest is changed via the presence of polar functional groups, the use of pure carbon dioxide to extract, i.e., solubilize, the solute becomes more difficult. Previous experimental evidence has demonstrated the need for modifiers or entrainers to extract polar or moderately polar analytes and achieve acceptable recoveries.[6]

Matrices are known to influence extractability in supercritical fluid extraction (SFE) as well as in classical extraction methods.[6] In an effort to determine the influence of the matrix, the effect of polar modifiers on the swelling of the matrix has been examined. The swelling of modifier-saturated plant and clay matrices subjected to modified supercritical CO_2 in a high-pressure sapphire view cell has been observed. In conjunction, extraction of radiolabeled organic compounds from these matrices was conducted using a supercritical fluid extractor followed by liquid scintillation counting of the extract to determine recovery. Thus, the relationship between the extractability and swelling in these materials was determined. A correlation based on the results obtained suggests a mechanism to explain the interaction between the supercritical fluid, the modifier, and the matrix.

Several phenomena may affect supercritical fluid extractions. These include solute and modifier polarity, physical and chemical state of the solute and the matrix, solubility of the solute in the modifier, miscibility of the modifier–supercritical fluid mixture under a wide variety of temperature and pressure conditions. Chromatographic theory explains several of these interactions as taken from the evaluation of a packed-bed system.[8] Correlations between chromatographic packed beds and supercritical fluid extraction in a packed cell have been drawn.[3,22,23] These relationships cannot explain all of the potential interactions in the extraction cell, but they do provide a starting point for potential phenomena that may exist.

Some workers have reported that solubility does not completely explain recoveries of some solutes from complex matrices.[5,6] An examination of the interaction of the modifier and the supercritical fluid with the matrix is important to understand potential phenomena affecting extractions. The experiments and results outlined herein describe a proposed mechanism during the supercritical fluid extraction process.

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Solute–Modifier Interactions. Many mechanisms have been proposed to explain the effect of modifiers on the extraction of solutes from various substrates. The first of these is the consideration of the solubility of the solute in modified supercritical fluids.[4] Enhanced extractability is achieved when solute–modifier interactions are examined. Molecular group interactions between modifiers and solutes that give rise to hydrogen bonding have been suggested as a potential influence of modified supercritical fluids in SFE.[5] Although other interactions are proposed and studied, solubility is a principle consideration in extractability.

Modifier–Matrix Interactions. It is not expected that the recovery of solutes from complex matrices should depend on the solubility of the solute in the modifier–supercritical fluid mixture alone. Matrix swelling resulting from the interaction of the modifier and the matrix has also been proposed as a predominate interaction in SFE.[6] A relationship between the interaction of the modifier with the matrix and the rate of mass transfer of the solute from the matrix can be developed. The rate of solute mass transfer is known to change with varying matrix compositions, for example, soil versus plant materials. In fact, mass-transfer differences have been exhibited in the supercritical fluid extraction of the same solute molecule from soils of varying compositions.[24] Additionally, solute extraction from matrices can vary with the amount of contact time that has transpired. In laboratory studies of field samples, agricultural products and byproducts are well-known to become bound to the matrix with time and field weathering. These aged samples have been difficult to extract using supercritical fluid conditions that yielded acceptable recoveries for freshly fortified and short-term field samples. However, experimental results where the sample matrix was saturated with water prior to supercritical fluid extraction yielded acceptable recoveries for these bound residues. The use of water as a modifier was prompted by the use of water in the reaction of caffeine from coffee.[7]

With the classical extraction of caffeine from coffee by either SFE or liquid–liquid extraction with methylene chloride, a modifier–matrix effect is influential in overall extractability. In both types of extraction, water is added as a swelling agent to obtain acceptable results. Liquid modifiers added to the point of matrix saturation cause more matrix swelling than supercritical fluids or gases. With swelling, the interior volumes of the matrices experience increased exposure to the total mobile-phase mixture, consisting of the modifier and the supercritical fluid. Because of this increased exposure, sample matrix swelling enhances the rate of mass transfer of the desired analyte into the extraction fluid.[6] Then, provided the modifier or the supercritical fluid–modifier mixture is a good solvent for the solute, higher extraction efficiencies result.[9,10]

The objective of this work was to observe the matrix swelling, which occurs in an extraction vessel, when swelling agent was introduced and determine whether the observations could be correlated with matrix composition and analyte extractability. Swelling experiments were conducted to mimic extraction conditions in the SFE cell. Typically in analytical SFE, modifier has been introduced into the cell by two separate methods. Both means of modifier introduction were examined in the swelling experiments.

There are three basic factors or dimensions of significance in assessing the swelling of a material. The first factor is the nature of the swelling agent, which could be liquid, gas, supercritical fluid, or modified supercritical fluid. The swelling agents we used were pure liquids and modified supercritical fluids. Generally, it is found that solids will exhibit a greater degree of swelling when saturated with a liquid (as opposed to other swelling agents, i.e., gases or supercritical fluids).[11–13] The second factor or dimension is the nature of the matrix. The third dimension is the type of solid swelling, intracrystalline or osmotic, that occurs. In intracrystalline swelling, the liquid penetrates the interior of the solid. The crystals imbibe the liquid and as a result swell. This is also termed "interlayer swelling". The interaction force at this stage is on the order of thousands of atmospheres.[14] In the second swelling form, after the intracrystalline layers are swollen, double-layer repulsion or electrostatic forces become predominant. This is termed "osmotic" or "intercrystalline" swelling. Osmotic swelling is usually measured by the amount of pressure that must be applied to prevent further swelling. The net interaction force at this stage is on the order of a few atmospheres.[14]

Soil and Clay Swelling. For most soils, obvious swelling of the entire matrix in the presence of organic swelling agents is uncommon and generally not visibly noticeable due to the heterogeneity of the soil. Swelling in soils is governed by the distribution of cations, anions, and repulsive electric forces between the charged particles of the soil. However, a pronounced, composition-dependent swelling can occur for highly mineralized soils, i.e., those with high clay percentages.

Clays which are predominantly made up of montmorillonite clay will swell measurably, those which are composed of kaolinitic clay will not.[15] Kaolinite, $(Al_2Si_2O_5(OH)_4)_2$ occurs commonly in soils as hexagonal crystals. Most kaolin structural units are held together in the basal plane by H-bonding between the oxygen and hydroxyl. Such bonding prevents swelling of the mineral beyond its basal spacing or *c*-spacing of 0.72 nm. Montmorillonite, $[Al_{1.67}Na_{0.33}]Si_4O_{10}(OH)_2$, has a basal spacing of 0.98–1.8 nm and a layer charge which is lower than kaolinite. Montmorillonite swells more easily than kaolinite clay. The range of spacing on the basal planes varies with exchangeable cations and the degree of interlayer solvation; this explains the larger *c*-spacing than seen with the nonswelling kaolinite. Montmorillonite has a high colloidal activity, including a high plasticity and cohesion, swelling, and shrinkage. It shows base exchange not only for positive inorganic ions but also for large cations. The presence of these large organic ions alters the clay dispersibility and allows for swelling of the lattice structure.[20]

EXPERIMENTAL SECTION

Materials. Two pure clays, sodium, magnesium montmorillonite and kaolinite, were utilized. These were obtained from the American Colloid Co., Arlington Heights, IL 60004-1434. The clays were dried in a 55 °C oven to a constant weight. The pea leaves were provided by DuPont Agricultural Products, Experimental Station, Wilmington, DE 19880-0402. Pea leaves were dried under a nitrogen atmosphere to constant weight.

The solutes utilized were commercial DuPont herbicides. These are Diuron [3-(3,4-dichlorphenyl)-1,1-dimethylurea] and the active ingredient in Express, tribenuron methyl [2-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)(methylcarbamoyl)]sulfamoyl]-benzoic acid methyl ester], a sulfonylurea herbicide. The solute chemical structures are shown in Figure 1 with the radiolabeled carbons indicated.

Procedures. *Swelling.* A schematic of the high-pressure gas sorption apparatus is shown in Figure 2. The apparatus consists of two units: a control unit for maintaining the system at a constant pressure and a thermostated bath unit to provide a constant temperature. Components used in this apparatus are listed in Appendix I. The bath unit is equipped with a steel reinforced double-layer polycarbonate Lexan view window con-

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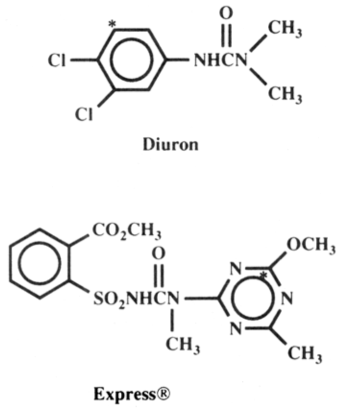

Figure 1. Structures of solute molecules using in extraction and swelling experiments illustrating position of ^{14}C label with an asterisk.

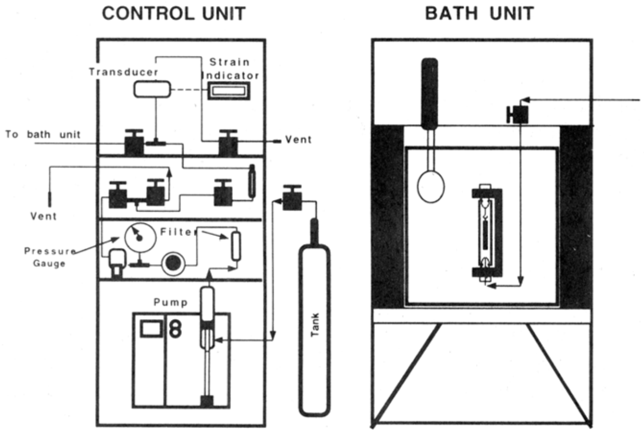

Figure 2. High-pressure gas sorption apparatus used in swelling experiments (a) Control unit contains syringe pump and supply tank. (b) Bath and viewing unit contains constant-temperature controller and Lexan view window. The bath unit shows the positioning of the sapphire view cells.

tainer. This window is supported on the inside by $^1/_8$-in. 304 stainless steel.

The main component of the apparatus is a high-pressure sapphire view cell assembly detailed in Figure 3.[15] The sapphire cell was purchased from Saphikon Inc. (Milford, NH) and is rated to a maximum pressure of 8900 psi. This cell provided a safety factor of 2 above the maximum pressure used in the experiments, 4450 psi. The cell dimensions to obtain this pressure rating were length 20.32 cm (8 in.), o.d. 1.577 cm (0.621 in.), and i.d. 1.12 cm (0.441 in.). The sapphire tube wall thickness was ~0.23 cm (0.09 in.).

For swelling observations involving soils, clays, and plant materials, a Hastalloy B holder accommodates a Pyrex sample tube, actually a standard 5-mm NMR tube, inside the sapphire view cell. This sample tube packed with the soil of interest receives the flow of supercritical fluid from the pump. There is not a gas-tight seal between inner sample tube and the outer sapphire glass cell; therefore, the pressure inside and outside the Pyrex sample holder (contained inside the high-pressure sapphire vessel) is the same during the swelling experiments. This prevents the Pyrex tube from being subjected to the high pressures used for the extractions and rupturing. The only tube that needed to be rated to handle the high pressures was the exterior sapphire view cell.

In a typical experiment, the soil or clay matrix is dried to a constant weight and then sieved through a 35-mesh tray. To increase overall volume of the samples and provide even distribution of the matrix in the cell, an equal weight of 80-mesh porous glass beads with a pore volume of 0.85 mL/g is mixed with the sample and loaded into the inner sample tube. Finally, an ASTM standard weight of 2.5 kg (5.5 lb) is dropped onto the matrix to ensure consistent compaction from a distance of ~3 ft.[16] The force exerted by this compaction is calculated to be less than 50 psi pressure and does not represent any significant pressure influence on the matrix used in these experiments. The filled glass tube is then inserted into the Hastalloy B holder. The holder provides the seal for the sapphire view cell and minimizes scratching of the sapphire tube with handling, providing safer operation under high pressure. The sapphire vessel and seals are positioned into an aluminum C-clamp; see Figure 3 (Colburn Lab, University of Delaware, Newark, DE 19711). The entire assembly is then placed into the custom-built constant-temperature bath (Figure 2b). With this design, accidental breakage of the sapphire tube under high pressure will be contained within the bath, providing for safe operation.

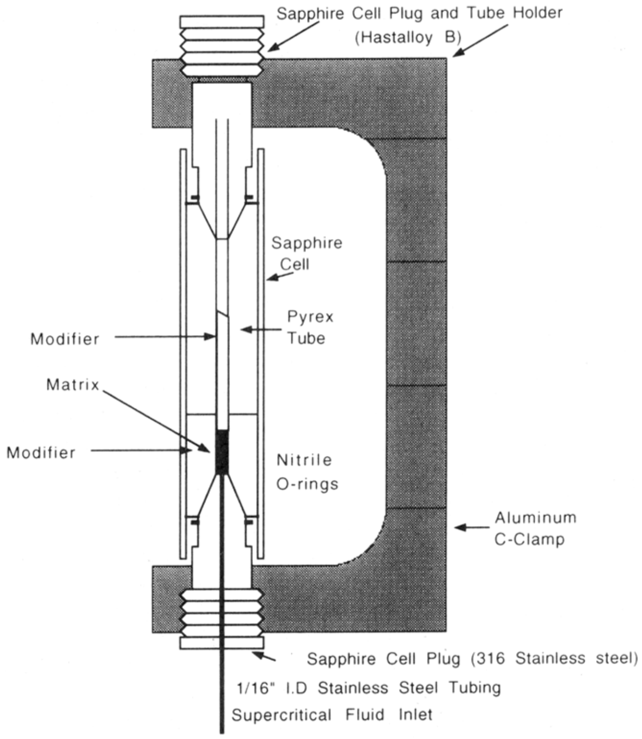

Figure 3. Sapphire vessel assembly for unsaturated fluid experiments. The modifier and the sample holder introduced into the sapphire cell are followed by the supercritical fluid. This experimental setup mimics the extraction conditions exactly.

Two methods of introducing modifier during analytical-scale supercritical fluid extraction have been routinely used. One method is to saturate the matrix in the extraction cell with liquid modifier and then conduct the extraction with the supercritical fluid. The second method is to use modified supercritical fluids as the extractant phase (and the swelling agent) at or above the modified phase's critical pressure and temperature. These two separate techniques of introducing the modifier into the system were evaluated, requiring two swelling cell designs. The first design allowed for the observation of the matrix in the presence of a gas or supercritical phase saturated with modifier. This is illustrated in Figure 3. Specifically, a quantity of modifier was introduced on the sample and into the annulus formed by the inner tube and the sapphire cell. After temperature equilibration, the assembly was pressurized to the pressure of interest by introducing CO_2 or N_2 into the view cell. At the temperature and pressure of the system, carbon dioxide or any other supercritical fluid was saturated with the modifier on the basis of the modifier solubility in the fluid. The matrix was also conditioned or soaked during the initial introduction of the liquid modifier alone. The level of the matrix was then monitored with a cathetometer (Fischer Scientific Co., Pittsburgh, PA 15219). The error in this level measurement was ±0.25 mm. The swelling of the matrix was defined as the percent change in the height of the matrix as measured by the cathetometer. Generally, the matrix height was on the order of 2.5 cm (25 mm). Thus, typical swelling measurements were expected to have a precision of ±1%. Swelling values in this report had an associated precision value of ±1%, which is not always visible on the figures since the symbols used represent a value larger than this error.

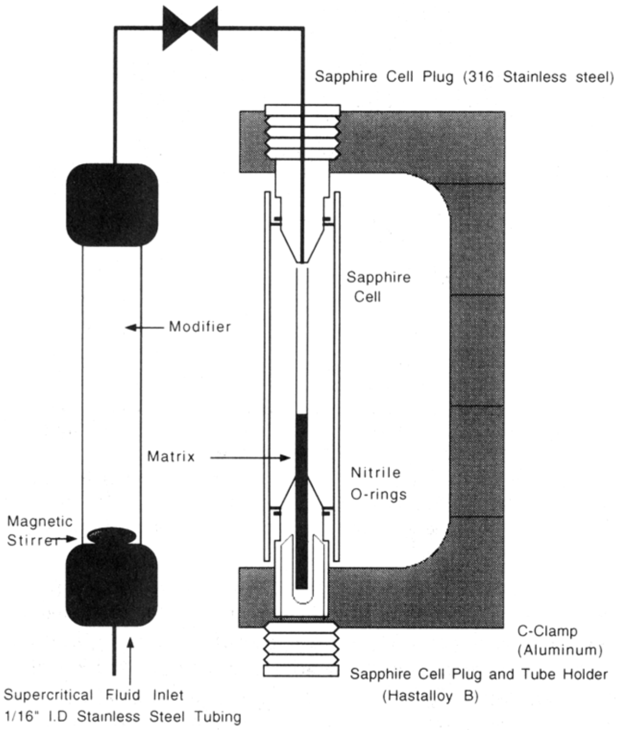

Figure 4. Sapphire vessel assembly for saturated fluid experiments. Supercritical fluid is bubbled through the modifier container under elevated temperatures and pressures. Based on the temperature and pressure chosen, the modifier concentration varies and saturates the supercritical fluid.

The second introduction technique utilized a saturator column containing the modifier prior to the apparatus, as shown in Figure 4. As designed, this method mixes the modifier and the supercritical fluid prior to introduction into the sapphire view cell. The stainless steel mixing chamber is filled with modifier and then the fluid is loaded into the chamber. At this point, the contents of the chamber are mixed with a submersible magnetic stirrer. After equilibration, the chamber contents, modifier-saturated supercritical CO_2, are transferred into the sapphire vessel assembly containing the matrix. After the matrix becomes saturated with the modifier–supercritical fluid mixture, the visual change of matrix height due to swelling is monitored with the cathetometer.

The difference between these two introduction systems accounts for some variables that may exist during typical supercritical fluid extraction experiments. Variability of recovery due to these differences could be caused by the swelling of the matrix due to contact with the modifier prior to the introduction of the supercritical fluid (technique 1, Figure 3) or the effect of a two-phase, supercritical fluid and liquid, extractant phase (technique 2, Figure 4).

Extraction. The instrument utilized for extraction was a Suprex 50A (Suprex Corp., Pittsburgh, PA) supercritical fluid extractor that was modified to handle the extraction of 12 separate samples. The construction and design of this instrument has been described in detail.[18] Briefly, this multivessel extractor can analyze 6 or 12 samples in a segmented parallel fashion in 3 h or less. Timing has been set up so that periods of static and dynamic extraction overlap within the individual vessels. Time overlap eliminates dead time, which would occur if the samples were extracted serially. In addition, the design eliminates the use of a restrictor. With no restriction, flow is dead-ended (i.e., restricted) via a switching valve. Supercritical fluid extractions are then conducted in a static mode (no flow). Once the extraction is complete, the dead-ended valve is repositioned to allow flow. Subsequently, pressure and density are rapidly reduced to prevent significant losses of the supercritical fluid and the extraction effluent is transferred for collection. In this instrument design, the modifier if used to conduct the extraction is introduced via a liquid-pumping system separate from the supercritical fluid pump. Our system used an LKB Model 2150 (LKB-Produkter AB, Bromma, Sweden) dual-piston pump for pumping modifier and entrainer fluids. Any suitable liquid pump could be substituted. With the addition of this pump to deliver liquids, modifier is introduced directly into the extraction vessel allowing for saturation of the matrix with the liquid modifier prior to the introduction of the supercritical fluid. Swelling of the matrix is thus possible.

Table I. Extraction and Swelling Conditions

	extraction	swelling
sample size, g	1.0–2.0	0.2–0.5
modifier vol, mL	20–25	20–25
temp, °C	45	45
pressure	variable	variable
time	variable	variable

The radiolabeled solutes, diuron and tribenuron methyl (Figure 1), were dissolved in methylene chloride to yield a solution with a concentration of ~100 ppb (10 000 dpm/mL). One milliliter of this solution was then deposited onto 1–2 g of the matrix under consideration and dried under a nitrogen stream for 3–4 h. The dried mixture was then loaded into 10-mL extraction vessels (Keystone Scientific, State College, PA). The extraction method involved initial addition of modifier to the extraction vessel followed by static extraction with supercritical CO_2, after which the cell was rapidly depressurized and the extract collected (dynamic flushing). Liquid scintillation counting of the extracts was conducted. The precision associated with the liquid scintillation counting is less than ±10%.

Conditions for extraction and swelling are outlined in Table I. Although the results of these experiments are correlated in this report, the individual samples used for the extraction and swelling experiments were not the same. Matrices examined were from the same characterized lots of soil and clays and the same field for the plant materials. Pea plants were grown at DuPont's Stine farm site (Newark, DE).

RESULTS AND DISCUSSION

This work used montmorillonite and kaolinite as control clays to examine swelling caused by supercritical fluids. Montmorillonite is a swellable clay; kaolinite clay is not. These two clays were chosen to provide extreme values in terms of matrix swelling. Real soils, which would be mixtures of these clays and other nonswelling materials, i.e., organic matter, silt, and sand, were initially considered to have swelling properties intermediate to these two extreme clay measurements. If a major fraction of the soil clay belongs to the montmorillonite group, then the examined soil was expected to swell considerably. If a solute was present in the intracrystalline regions of this swellable clay, then extraction yields would be enhanced with osmotic swelling of the clay matrix. If the clay fraction was predominantly kaolinite or a nonswelling clay, then swelling was minimal and solutes trapped in the intracrystalline regions would not realize enhanced extraction.

Saturation Effects. In the presence of unmodified supercritical CO_2, both the montmorillonite and kaolinite clays did not swell. Figure 5 illustrates the swelling of montmorillonite clay in the presence of supercritical CO_2 with water as the modifier. The swelling percentages shown in this graph are cumulative at each point. These results were obtained using the method of modifier introduction shown in Figure 3, i.e., where the modifier is introduced into the annulus and the Pyrex tube and then the vessel is pressurized with supercritical CO_2, at a constant temperature and variable pressure. For each point in Figure 5, the matrix swelling was monitored while coming to equilibrium, at least 5–6 h. At all the pressures examined, swelling increased with time; the most dramatic increases were apparent in the first 100-min time period. As for all modifiers in a supercritical fluid, solubility changes with temperature and pressure conditions.

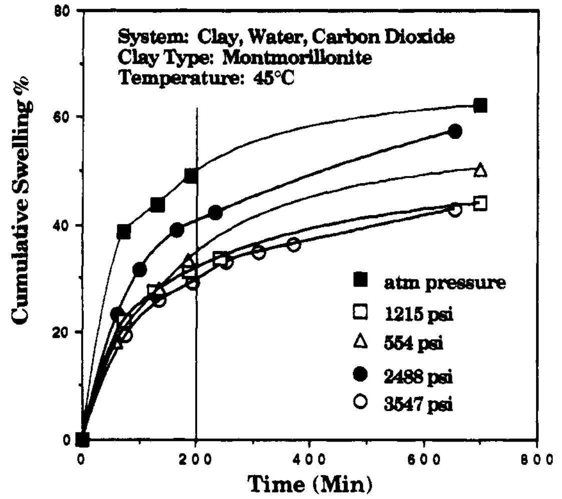

Figure 5. Rate of swelling for the montmorillonite clay, water, and carbon dioxide system at 45 °C at varying pressures. Optimum pressure for swelling under supercritical fluid conditions is ~2500 psi.

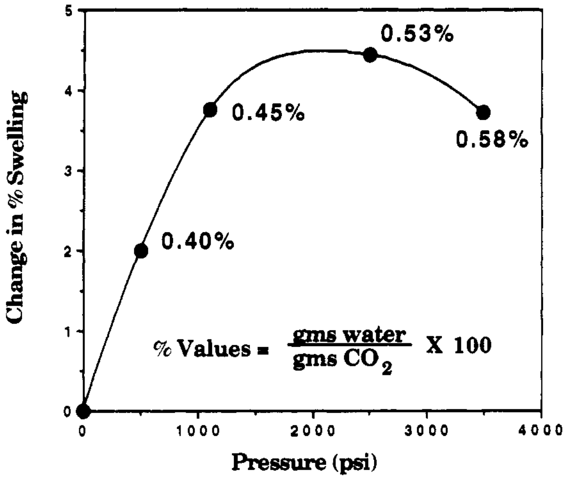

Figure 6. Normalized percent swelling for the montmorillonite clay, water, and carbon dioxide system at 45 °C at varying pressures. The amount of water saturated in the carbon dioxide varies with pressure; these water saturation values are calculated and illustrated for the individual pressures.

The trace amounts of water solubilized in the CO_2 for these experiments are calculated on the basis of temperature and pressure and shown for one equilibration time period (a cross section of Figure 5 from the x-axis) and all pressures in Figure 6.

As pressure was adjusted, the percent swelling varied. To explain the swelling results illustrated in Figure 5, it is necessary to visualize what is taking place inside the sapphire swelling tube. At 0 psi (on the graph, but actually at atmospheric pressure), normal swelling behavior is achieved; i.e., the introduction of the liquid to the solid matrix has caused it to swell. As the matrix–liquid mixture is allowed to equilibrate, this swelling increases but eventually levels off after ~3 h (~200 min on the graph). Intracrystalline swelling is the predominant swelling mechanism at this pressure. As additional pressure is introduced into the cell, e.g., at pressures of approximately 550 and 1200 psi on the graph, the matrix is first compacted below the level that would have been achieved with the initial sample packing into the tube. Therefore, these pressures applied to the matrix cause compaction which the swelling must overcome.

The greatest swelling change occurred at a pressure of ~2500 psi. At 2500 psi and 45 °C, 5.25 mg of water was calculated to be dissolved in the carbon dioxide, as is illustrated in Figure 6. This is not the maximum amount of water introduced over the course of the experiment, suggesting that there is no linear correlation between matrix swelling and increased amount of water present in the supercritical phase. The maximum in swelling that occurs at 2500 psi can be explained as follows: the matrix is unsaturated at lower pressures; around 2500 psi the water solubilized in the CO_2-rich phase is finally able to penetrate the crystalline layers of the montmorillonite matrix and swelling results. The swelling is less than the swelling that occurs with a liquid, since the matrix swelling never reaches the level of the initial unpressurized matrix. However, significant water is present to cause observable separation of the crystalline layers of the montmorillonite clay and overcome the compression of the system.

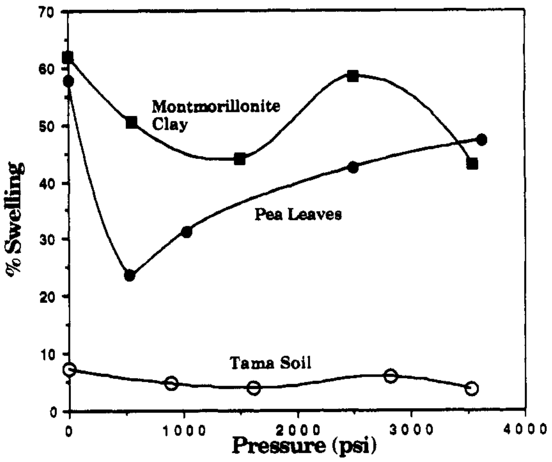

Figure 7. Saturated swelling of montmorillonite clay, pea leaves, and Tama soil (OM 3.1%, clay 14%, silt 68%, pH 6.3) with water and carbon dioxide in the system at 45 °C and varying pressures.

At pressures greater than 2500 psi, swelling starts to diminish and contraction occurs. This is most likely due to compression, caused by the hydrostatic pressure effect on the crystalline layers.

It should be noted that under the same conditions that were used for montmorillonite, kaolinite clay demonstrated no appreciable swelling with a water-modified carbon dioxide system.

Figure 7 shows swelling of montmorillonite clay, Tama soil, and pea leaves in the presence of a water-saturated supercritical fluid at a constant equilibration time. In these experiments, the matrices were saturated with CO_2–water premixed phase prepared as illustrated in Figure 4. Below 1000 psi, the clay (and pea leaf) matrices showed an appreciable compression due to the pressure applied. For the saturated montmorillonite system at low pressures, i.e., below 1500 psi, there is enough water to penetrate the crystalline layers and cause swelling; however, at the same time, osmotic repulsion between adjacent crystalline regions occurs. At these low pressures, osmotic swelling is greater than swelling of the crystalline regions. But as pressures gradually increase, the electrostatic forces giving rise to osmotic repulsion are suppressed and intracrystalline separations increase. The resulting compression and swelling profile is a sum of the two types of swelling: osmotic and intracrystalline. The point at which compression and swelling are equal corresponds to the minimum observed in Figure 7. For this clay system, the minimum occurs at ~1500 psi. This is within the same order of magnitude observed for Na-montmorillonite osmotic swelling in aqueous systems.[19]

At pressures above 1500 psi, the montmorillonite clay behaved similarly to the first modifier introduction technique experiment described above and in Figures 5 and 6, with the occurrence of a maximum at ~2500 psi.

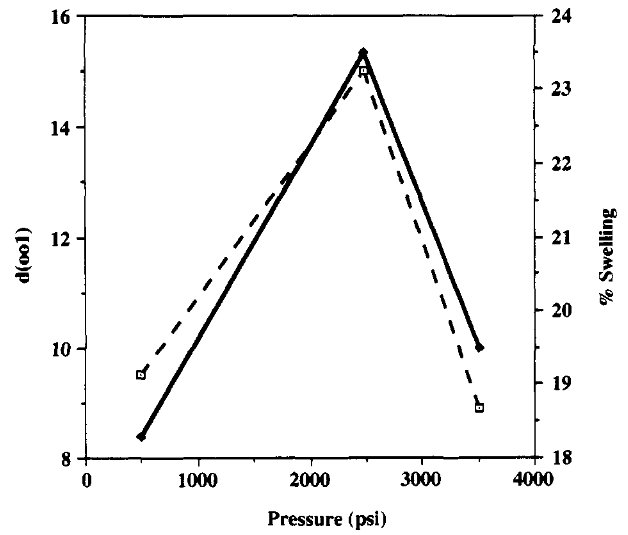

Figure 8. Correlation between percent swelling and change in *d*-spacing based on X-ray diffraction results.

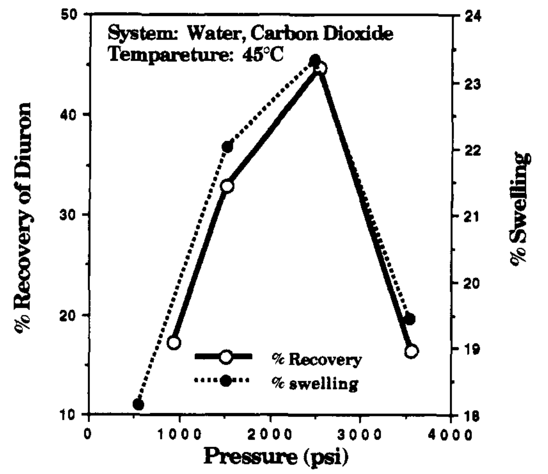

Figure 9. Correlation between percent recovery of diuron from montmorillonite clay with percent swelling of the same clay material at constant temperature and varying pressure. The precision of the percent recovery values is ±5% RSD and the precision of the percent swelling values is ±1%.

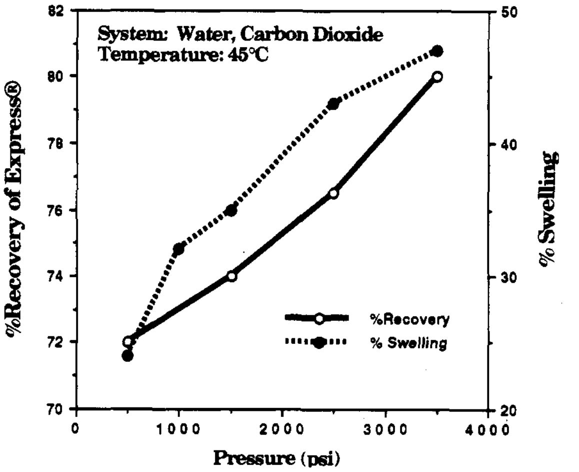

Figure 10. Correlation between percent recovery of Express herbicide from pea leaves with percent swelling of pea leaves at constant temperature and varying pressure. As in Figure 9, the precision of the percent recovery values is ±5% RSD and the precision of the percent swelling values is ±1%.

At pressures greater than 2500 psi, hydrostatic pressure again is expected to begin to affect the intercrystalline region, causing a decrease in the matrix swelling. These results illustrated this matrix depression.

As a confirmatory method beyond visual swelling measurements, X-ray diffraction analysis was used to measure the separation distance of the crystalline layers. Specifically, the *d*(001) spacing between the montmorillonite clay layers was measured as a function of system pressure. These experiments were conducted to assess the degree to which the swelling depends on interlayer or osmotic swelling, that is, to be able to know whether the liquid penetrated the interior layers or is only adsorbed at the surface. When an X-ray diffraction pattern is found to be different before and after swelling, then liquid has been taken up into the interior of the solid. If the diffraction pattern is unchanged, it is reasonable to assume that the liquid is only absorbed at the surface of the solid.[21] Our experimental outcome, illustrated in Figure 8, shows that the amount of montmorillonite clay swelling was directly proportional to the separation distance, *d*(001) spacing, between the crystalline layers. Therefore, liquid has been taken up into the interior of the solid. As can be seen in Figure 8, the X-ray diffraction analysis of swollen montmorillonite clay samples indicates a parallel behavior of change in *d*-spacing with percent swelling. These samples were exposed to ambient conditions for ~15 min prior to X-ray analysis.

The saturated swelling of pea leaves, used here as representative of a total organic matrix (Figure 7), shows an initial drop in swelling with pressure, as did the montmorillonite clay. This drop is most likely due to the impact of pressure, i.e., compression upon the system causing an initial decrease in the matrix level. Over the course of the experiment, this is gradually overcome by swelling of the matrix. This result suggests that the same type of mechanism responsible for clay swelling could account for swelling in the pea leaves. At higher pressures, there is a gradual approach to a maximum in swelling. It appears that, at the maximum pressure investigated, maximum swelling has not been reached. However, because of instrument limitations and a self-imposed safety factor on our swelling apparatus, our experiments were not conducted at levels higher than 4000 psi. Therefore, any later depression at higher pressures of the pea leaves was not investigated.

Tama soil was included in these swelling experiments to expose a system containing organic and inorganic components to the same conditions to induce swelling. This soil, which had 14% uncharacterized clay, showed the same trends at a much smaller magnitude. Again, under these same conditions, kaolinite clay did not exhibit appreciable swelling.

Correlations between Swelling and Extraction. The percent swelling of the matrix versus the extraction efficiency of analytes of interest from montmorillonite clay and ground pea leaves are illustrated in Figures 9 and 10. In these experiments, water-modified carbon dioxide was the extraction fluid as well as the swelling agent. Excellent correlation between recovery and swelling was achieved. Over the pressure range investigated, montmorillonite clay swells. This swelling is attributed to the expansion of the clay crystalline structure. With the expansion of the inner crystalline structure, solutes such as the radiolabeled diuron and tribenuron methyl herbicides entrained in the crystalline layers become more accessible to the extractant solvent, i.e., the modified supercritical CO_2. Thus, the solute extractability is seen to be dependent on the intracrystalline swelling of the clay, and the modifier chosen for the extraction should possess characteristics that will not only solubilize the analyte of interest but will also swell the matrix particles. Mixtures of modifiers, might therefore, be more successful in increasing extractability. For example, water could be used as the swelling agent and methanol or acetonitrile could be used as

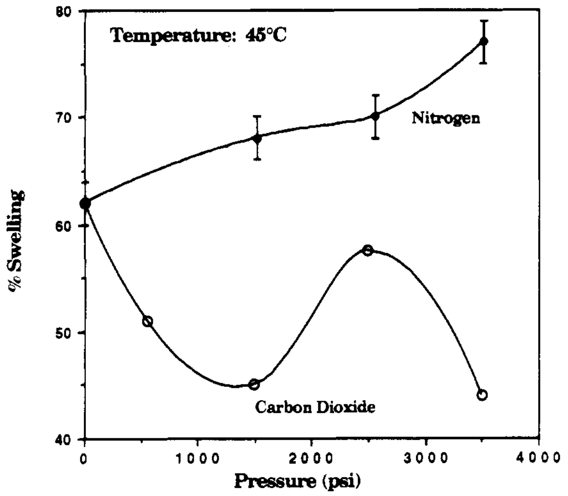

Figure 11. Comparison of percent swelling in montmorillonite clay with water as the modifier in supercritical carbon dioxide and nitrogen gas at 45 °C and varying pressures.

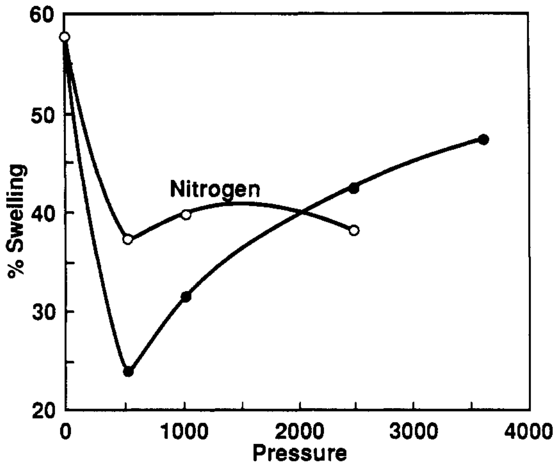

Figure 12. Comparison of percent swelling in pea leaves with water as the modifier in supercritical carbon dioxide and nitrogen gas at 45 °C and varying pressures.

the solubilizing agent in a mixed-modifier system. It should be noted here, that percent extraction efficiency numbers were based on one static extraction step of the individual matrices and recoveries are less than accepted values of 100 ± 25%. With bound residues, we have found that repeated static extraction steps with fresh fluids, similar to classical liquid–liquid extractions with fresh solvent, yield higher recoveries. These one step extractions were conducted to conform to swelling experiments where each individual sample was allowed to swell once. The effect of multiple introductions of fluid for swelling purposes is the subject of future research. Extraction results were replicated three times; precision associated with these results was ±5% RSD.

Kinetic Effects. Knowledge about the rate of mass transfer is needed to assess extraction from complex matrices, such as plant or soil materials. The effect of increasing the equilibration time for the montmorillonite clay system at constant pressure can be found in Figure 5. It is apparent that most of the swelling, approximately 30–50%, takes place within the first few minutes. This observation supports our experimental extraction results. Generally, 60–75% of the extraction yield is obtained within the first few minutes of the extraction process. These results also suggest that if extractions were conducted for longer periods of time, swelling would be maximized and extraction efficiencies for bound or entrained solutes increased. The results do support the use of static instead of dynamic extraction for those problems where the analyte of interest is thought to be contained in the intracrystalline regions of the matrix.

On a practical note, the time periods examined for the swelling experiments are much larger than those typically used for analytical-scale SFE. If the swelling of the matrix aids in the extractability of a solute, then most extractions are conducted in too short a time frame. However, these results illustrate that contact time with a modifier can significantly enhance extractability, i.e., recovery.

Effect of Supercritical Fluid Type. A comparison of the percent swelling in supercritical nitrogen (P_c = 33.5 atm) and supercritical carbon dioxide was made to determine if there were unique characteristics of supercritical CO_2 in the swelling process. Again, pea leaves and montmorillonite clay were investigated. Results of these experiments can be found in Figures 11 and 12. As illustrated in these constant-temperature experiments, the trends of the percent swelling in supercritical carbon dioxide were not reproduced by supercritical nitrogen. Unique properties of the supercritical carbon dioxide can account for the physical swelling observations seen with montmorillonite clay. However, the mechanism that produces these different results is not understood and needs further investigation.

The swelling exhibited in pea leaves, chosen as representing pure organic matter, can be simply explained. With the first introduction of pressure on the system, a physical collapse or compression is demonstrated. This compression is greater with carbon dioxide then with nitrogen (see Figure 12), but occurs with both fluids. As the matrix swells, this initial compression is slightly overcome. With continued pressure increases, the swelling appears to level off with nitrogen gas but continues to rise with the supercritical carbon dioxide fluid. Currently, this is attributed to the unique properties of supercritical carbon dioxide and its permeability to the plant material.

Ultimately, as observed, supercritical carbon dioxide does not behave in the same manner as supercritical nitrogen for these matrices.

Effect of Modifier Type. Pea leaves were chosen as the representative matrix. With these leaves, little or no mineral or clay constituents are present to influence extractability. For pea leaves, swelling can be correlated to polarity in the modifier series found in Table II, where the dielectric constants of the modifiers used are listed. When water was used as the modifier, the swelling was maximized. Of this list of modifiers, the polarity of water is highest, based on its high dielectric constant. Likewise, as the dielectric constants of the different modifiers decrease, the swelling of the pea leaves matrix also decreases. This phenomenon holds except for acetone and tetrahydrofuran (THF) in this series. THF has previously been observed to yield distinct results when used as a modifier in carbon dioxide, which is attributed to the shape of the THF moiety.[22]

Percent swelling versus percent recovery from ground pea leaves in the presence of different types of modifiers is illustrated in Figure 13. These results demonstrate that linear correlations between extractability and matrix swelling are perhaps oversimplifications of all of the complex interactions which may be occurring in the supercritical fluid extraction system.

The swelling exhibited in Figure 13 is attributed to the interactions between the polar plant material and the polar modifiers. The polar pea leaf matrix adsorbs modifiers of similar polarity; however, extraction yields are not controlled by the swelling of the matrix only. In addition to the matrix polarity, the modifier or the supercritical fluid must have the ability to solubilize the solute and obtain acceptable extraction efficiencies. These swelling results suggest that not only does

Table II. Modifier Dielectric Constants[7]

water	80	ethanol	24.6
acetonitrile	37.5	acetone	20.7
methanol	32.7	tetrahydrofuran	7.6

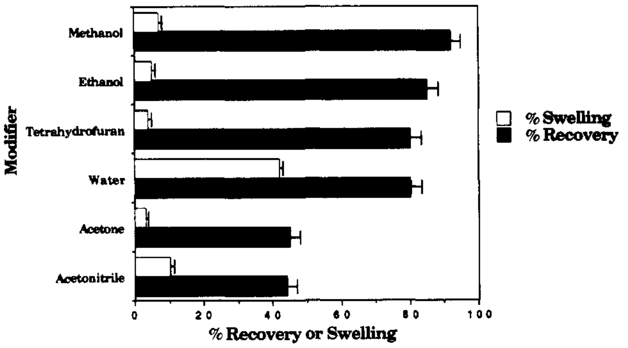

Figure 13. Correlation of percent extraction efficiency and percent swelling for pea leaves with different modifiers at constant temperature (45 °C) and constant pressure (172 atm, 2530 psi).

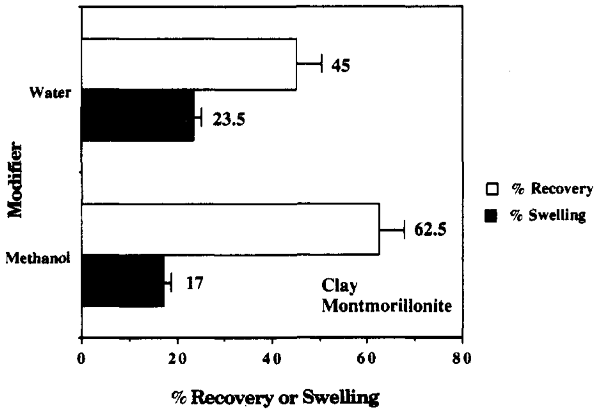

Figure 14. Correlation of percent extraction efficiency and percent swelling for montmorillonite clay with methanol and water modifiers at constant temperature (45 °C) and constant pressure (172 atm, 2530 psi).

accessibility to adsorption sites or areas of entrapment need to be considered but that solubility of solute molecules is also important.

The interactions that control the extractability from matrices as a function of modifier type could best be accounted for in terms of solute–modifier interactions in addition to modifier–matrix interactions. For example, in the extraction of tribenuron methyl from aged pea leaves, the best recoveries were achieved with a 50:50 water–methanol mixture as the modifier. It is hypothesized that the water acts as the matrix swelling agent and methanol acts as the tribenuron methyl solubilizing agent. Therefore, the combination of both modifiers affect better extraction yields of this sulfonylurea compound from pea leaves than does either modifier alone.

The rate of swelling of montmorillonite clay in the presence of water and methanol was examined in Figure 14. The greatest amount of swelling was observed during the first few minutes after exposure to the modifier. Again, water was found to be the better swelling agent. But methanol showed the better extraction efficiency when used alone. As samples were aged, a mixture of water and methanol proved to be the best overall modifier for the highest extraction efficiencies.

Other matrices need to be investigated with a wide variety of modifiers to make accurate predictions of polarity and matrix interactions.

CONCLUSIONS

Matrix swelling in the presence of supercritical CO_2 with water as a modifier has been examined for the clays montmorillonite and kaolinite and for plant tissue. Swelling due to modifiers has been observed with saturated and unsaturated supercritical fluids. Greater swelling occurred in the saturated fluid systems; pure supercritical CO_2 did not cause matrix swelling in any of the systems examined. At low pressures, the montmorillonite clay shows intercrystalline swelling. At higher pressures, intracrystalline hydration of the montmorillonite clay dominates the swelling behavior as was evidenced from X-ray diffraction analysis.

A direct correlation between the extraction efficiency and swelling from saturated matrices was obtained for the clay and pea leaves as a function of pressure. For the montmorillonite clay, the correlation was achieved over the pressure range where the intracrystalline swelling of the clay should predominate. Kaolinite clay did not swell, and recovery did not change with pressure.

Modifier polarity seems to predict high extractability and swelling in organic matrices such as pea leaves. When inorganic components are present in the matrix, other interactions of modifier are witnessed and mechanisms are not as yet understood.

Kinetic effects of swelling suggest that longer contact times should produce greater swelling and higher recoveries, but the majority of swelling takes place very rapidly. The swelling process of supercritical carbon dioxide is unique when compared to supercritical nitrogen.

In summation, matrix swelling was found to be an important factor in modifier-enhanced supercritical fluid extraction; maximum swelling was not found at maximum pressure. Different modifiers were seen to impart different degrees of swelling on the matrices. Greater recoveries from clay and pea leaves saturated with these different types of modifiers were enhanced by matrix swelling.

APPENDIX

Components of Swelling Apparatus

1. Varian 8500 HPLC pump	Varian Sunnyvale, CA 94089
2. high-pressure filter	High Pressure Instruments Erie, PA 16505
3. high-pressure gauge	High Pressure Instruments
4. rupture disk	High Pressure Instruments
5. check valve	High Pressure Instruments
6. two-way straight valve	High Pressure Instruments
7. strain indicator	Daytronics Miamisburg, OH 45342
8. pressure transducer	Daytronics
9. constant-temperature circulator	American Scientific Products McGaw Park, IL 60085-6787
10. sapphire cell	Saphikon, Inc. Milford, NH 03055
11. Pyrex tube	Hamilton Company Reno, NV 89520
12. gas tanks	Scott Specialty Gases Plumsteadville, PA 18949-0310
13. water tank	DuPont Agricultural Products Experimental Station Wilmington, DE 19880-8402
14. sapphire seal	Saphikon, Inc.
15. sapphire clamp	University of Delaware Colburn Lab Newark, DE 19711

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