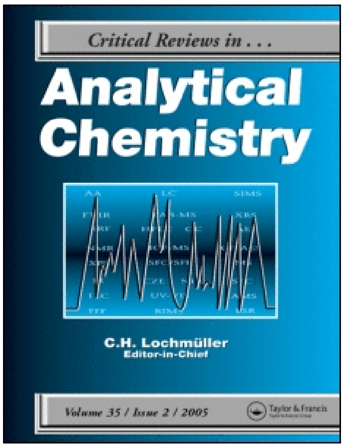

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Bridging the Gap Between Supercritical Fluid Extraction and Liquid Extraction Techniques: Alternative Approaches to the Extraction of Solid and Liquid Environmental Matrices

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Bridging the Gap Between Supercritical Fluid Extraction and Liquid Extraction Techniques: Alternative Approaches to the Extraction of Solid and Liquid Environmental Matrices

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ABSTRACT: Supercritical fluid extraction (SFE) sometimes yields poor recoveries as the result of inefficient analyte collection. Methods of collection and the important parameters for optimization are briefly discussed. The extraction of polar and/or high molecular weight analytes with (**common??**) supercritical fluids is frequently difficult and therefore, Conventional liquid extraction techniques are not obsolete. Many of the methods that are developing as alternatives to SFE for polar analytes are reviewed herein. The approaches are classified based on whether the analytes, extraction fluid, matrix, or experiment/instrumentation were varied. Advantages and disadvantages of each technique are described.

KEY WORDS: extraction, supercritical fluid, alternatives.

I. INTRODUCTION

Supercritical fluid extraction (SFE) was developed as a direct alternative to conventional liquid-solid extraction techniques such as Soxhlet and sonication. Frequently cited advantages of SFE include shorter extraction times, reduced solvent use, and the potential for analyte selective extractions by varying the pressure, temperature, or modifier used. Over the past 5 years, the shortcomings of SFE and modified SFE experiments with CO_2 and organic cosolvents were illuminated. Low extraction yields of many polar and/or high molecular weight analytes were encountered, particularly from wet and adsorptive matrices. Technical problems such as restrictor plugging and inefficient trapping of the analytes after extraction were also observed. A survey of analyte collection methods for SFE is included here. Finally, the large number of experimental variables, initially seen as an advantage, slowed the practical use of SFE because standard extraction conditions for a range of analytes and matrices were not established, thereby requiring a method development step for each sample.

Alternative approaches are being investigated. This review describes the alternatives that are currently in use as an attempt to "bridge the gap" between liquid extraction techniques and SFE for both solid and liquid matrices. Experimental approaches are categorized herein based on whether changes were made to the extraction fluid, the analytes, the matrix, or the experiment/in-

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strumentation. Positive and negative attributes of each technique are discussed.

A. Analyte Collection Methods in SFE

Restrictor plugging and poor analyte collection are two frequently cited difficulties that limit the reliability of SFE. Restrictor plugging results as the extraction fluid depressurizes across a length of tubing and the analytes are deposited on the restrictor walls, or when the restrictor tip freezes due to the Joule-Thomson cooling of the expanding CO_2.[1] Wet samples are particularly difficult because of the low solubility of H_2O in CO_2 and ice formation at temperatures $\leq 0°C$. Heating the restrictor or the collection vessel is sometimes used but at the risk of poorer analyte collection, as will be discussed below. Methods of analyte collection include cryogenic trapping in an empty container, deposition in a liquid solvent, or retention on a solid surface. Although cryogenic trapping is not very viable or frequently used, liquid solvents and solid surfaces are efficient and commonly employed.

1. Cryogenic Trapping in an Empty Container

One of the first collection methods used for SFE was cryogenic trapping. This method attempts to utilize the decreased volatility of analytes at very low temperatures. However, the results obtained are not especially promising. For example, polycyclic aromatic hydrocarbons (PAHs) were extracted from XAD-2 resins and collected in 100 ml volumetric flasks.[1] When the flask was cooled to 0°C but left open to the surroundings, recoveries were $\leq 8\%$. Upon cooling to liquid N_2 temperatures and sealing the flask (leaving only a small vent tube for exiting CO_2), yields improved to ~80%. The increased recoveries were attributed to the reduction of CO_2 aerosol formation that was purging the analytes from the flask and the elimination of analyte volatility concerns by freezing the analytes. In another study, PAHs were trapped in empty vials that were not cooled prior to use.[1] Although the vials were rapidly cooled by expanding CO_2, analyte recoveries did not exceed 40%. Overall, cryogenic trapping in an empty container is not feasible unless the analytes are extremely nonvolatile and the collection vessel is cooled with liquid N_2 before use, and therefore is not frequently used.

2. Deposition in a Liquid Solvent

One of the simplest collection methods found to produce high yields is to place the tip of the restrictor directly into a few milliliters of liquid solvent. Several problems arise, however. First, exiting CO_2 can cause aerosol formation and subsequent purging and evaporation of the solvent and analytes.[3] This can be reduced by keeping the flow rate low (generally ≤ 1 ml/min of liquid, equivalent to ~500 ml/min of gas) and by cooling the solvent. Small volumes of solvent must sometimes be added to keep an adequate volume in the collection vial. Replacement with solvent at room temperature can negate the cooling effect that CO_2 had on the original solvent, lowering the trapping and making evaporation even more likely.

Second, the analytes may not efficiently partition from the extraction fluid into the collection solvent. Solvent identity, volume or height, and temperature were found to be important parameters.[4] For example, phenol was much more efficiently trapped in methylene chloride or chloroform than in acetone, methanol, or hexane. A greater solvent height in the collection vial was shown to be more important than volume, because the solvent-analyte interaction time was longer before the gaseous CO_2 exited the vessel. Very volatile fragrance compounds were collected up to 11% more efficiently when the 2.5 ml solvent volume was 22 mm high compared with 8 mm high. Finally, yields were highest when the collection solvent was allowed to cool from the expanding CO_2 to temperatures as low as –40°C.[4] Cooling increases the solvent viscosity, decreasing the size of the CO_2 bubbles, and reducing aerosol formation. However, maintaining the solvent temperature at 5°C by placing the vial in a hollow aluminum block containing chilled H_2O eliminated the freezing of extracted H_2O and provided a steady flow rate.[4] Recoveries in that case were ≥92%. In another study, PAHs were more efficiently trapped in 15 ml acetone (86 to 100%) compared with 5 ml acetone (70 to 85%).[4] Analytes as volatile as benzene and n-nonane were collected in 15 ml methylene chloride (150 mm high) at ≥90% yields but somewhat less efficiently in 8 ml methylene chloride (85 mm high),[6] and isopropanol was trapped in 13 ml hexane (57 mm) at 72% but at only 61% in 4 ml hexane (18 mm).[7]

Third, heating the restrictor may be necessary to avert plugging.[1] The restrictor can be placed inside a stainless steel tube and wrapped with heating tape to a few centimeters short of the tip or placed inside an insulating aluminum block. Heating the restrictor inside an aluminum block is considerably less of a fire hazard and resulted in 90 to 95% recoveries of PAHs. This method was viable for the extraction of wet samples, including petroleum waste sludge containing 45% H_2O and pine needles consisting of 80% H_2O, and elemental sulfur. In another variation, the restrictor was heated and an organic solvent was introduced at the restrictor tip via a plastic tee to increase solvent contact with the depressurized analytes and maintain the solvent volume in the collection vial.[1] A stable flow rate and collection efficiencies of 81 to 95% for PAHs were achieved, but an extra pump was needed to deliver the organic solvent.

When the restrictor is heated to high temperatures (≥150°C) across its entire length, a glass transfer tube is used to avoid placing the hot restrictor directly into the solvent.[1,7,8] The transfer tube is positioned near the bottom of the vial, forcing the gaseous CO_2 (and analytes) to interact with the solvent before exiting the vessel. However, analytes are frequently deposited on the transfer tube and are hard to recover. For example, PAHs were recovered at only 30 to 65% using the transfer tube but at 90 to 95% with an unheated restrictor placed directly in the solvent.[1]

In summary, deposition in a liquid solvent is a viable collection method provided that the appropriate factors are considered. Flow rates of <1 ml/min liquid are essential for the collection of even nonvolatile analytes, and flow rates ≤0.5 ml/min may be necessary for the more volatile analytes. The efficient partitioning of analytes into the collection solvent was shown to vary with solvent identity and volume or height. The collection efficiency for a given set of analytes can be determined by spiking the analytes onto the outlet frit of the extraction vessel and immediately extracting at the conditions of interest. Because extracted analytes can also be purged from the collection solvent during the remainder of the extraction, spiking the solvent with analytes and purging with extraction fluid is also useful to quantitate losses. In general, the restrictor should be heated only when absolutely necessary. Because most plugging occurs at the restrictor tip, heating all but the last few centimeters is not useful, and the use of glass transfer tubes frequently yields poor results.

3. Retention on a Solid Surface

The first class of solid surfaces uses an inert material such as stainless steel beads or silanized glass beads.[3,9,10–12] Analytes are trapped by deposition on the cryogenically cooled surface. The material is then rinsed with a few milliliters of solvent to recover the analytes. Glass beads were capable of trapping hydrocarbons C_{10} to C_{40} with 75 to 95% yields at a temperature of –45°C.[3] Trapping was not affected by increasing the flow rate to 5 ml/min. However, volatile analytes such as acetophenone and N,N-dimethylaniline were recovered at only ~10% yield from stainless steel beads following an extraction with CO_2.[12]

The second class of solid surfaces utilizes a sorbent material such as silica, octadecyl siloxane (ODS), or other chromatographic stationary phase materials.[6,9,10–12] These traps utilize cryogenic cooling and analyte adsorption to specific sites on the surface. The traps can then be rinsed, potentially selectively for the analytes of interest, by appropriate choice of the elution solvent. A wide variety of sorbents are available for use. For example, Porapak Q was used to trap total petroleum hydrocarbons with ≥87% efficiency,[6] and a diol trap was most efficient for phenols.[11]

Both classes of traps work well for extractions with CO_2. However, when modifiers are used, the analytes may be rinsed from the trap by the modifier as the extraction proceeds. By elevating the trap temperature near the boiling point of the solvent, the solvent can be evaporated while the analytes are adsorbed. However, heating the sorbent eliminates the cryogenic cooling aspect, making it more difficult to efficiently trap volatile analytes. A five-component test mix with a range of polarities (acetophenone, N,N-dimethylaniline, n-decanoic acid, 2-naphthol, and n-tetracosane) was tested with ODS traps using CO_2 and 1, 2, 4, and 8% methanol in CO_2.[12] The volatile components, acetophenone and N,N-dimethylaniline, were trapped at ~100% with pure CO_2 and 1 to 2% methanol in CO_2 using trap temperatures of 5 to 20°C. With 4 and 8% methanol in CO_2 and trap temperatures of 30 and 50°C, respectively, recoveries of the volatiles were ~70 and ~20%. Analyte volatility considerations were more predominant with increasing temperatures. On the other hand, the nonvolatile analytes were most efficiently trapped with 1 to 2% methanol in CO_2 as the extraction fluid. A thin film of methanol on the surface of the ODS may increase interactions with the analytes. However, with 4 and 8% methanol in CO_2, recoveries of nonvolatiles were 60 to 100% at 40°C and 20 to 100% at 50°C, respectively. A thick film of methanol may be unstable during the extraction, may prevent dispersive interactions between the ODS and analytes, and may lead to reduced partitioning from the polar methanol layer to the nonpolar ODS.[12] Therefore, in most cases, solid surfaces are only useful when the extraction fluid contains ≤2% cosolvent in CO_2.

A final factor to consider is the choice of elution solvent. Polar sulfonyl urea herbicides were trapped on stainless steel beads and ODS.[9] Recoveries from ODS were as much as 45% lower because the ODS was poorly rinsed with acetonitrile. Therefore, analyte losses from sorbents may be attributed not only to inefficient trapping but to less than quantitative rinsing as well.

Overall, the collection method appropriate for a given application is dependent on the analyte volatility, the need for a heated restrictor, the presence of cosolvents in the extraction fluid, and the possibility of a selective extract by varying the solid surface. Because analytes as volatile as benzene can be quantitatively trapped in a small vial of solvent,[6] this simple and cost-effective method is a reasonable first choice. Regardless of the method chosen, prudent consideration of the parameters involved, such as solvent or solid surface identity and trapping temperature, is essential so that poor collection efficiency does not limit the extraction results.

II. "BRIDGING THE GAP"

A. Altering the Extraction Fluid

CO_2 is the most widely used extraction fluid because it is inert, nontoxic, available in high purity, and has a low critical temperature (31.1°C) and pressure (72.9 atm).[13] However, CO_2 lacks a permanent dipole moment and has a polarity comparable to liquid pentane or hexane.[14–16] Nitrous oxide, with a small permanent dipole moment of 0.17 D,[13] was one of the first tested alternatives.[17–25] However, N_2O is now avoided due to the risk of oxidation and explosion when the matrix organic content is high. Other alternatives are limited because of environmental hazards (freons), or critical temperatures and pressures too high for convenient use (H_2O and organic solvents such as methanol). Despite these apparent disadvantages, the search for a polar extraction fluid warranted their investigation, and several are discussed herein. Critical properties and dipole moments of these fluids are listed in Table 1.[13] The liquid mixtures of common associated solvents such as methanol with liquefied gases such as CO_2, are also alternative solvents. These "enhanced-fluidity liquids"offer the increased polarity and solvent strength of the organic liquid while maintaining the beneficial viscosity and diffusional properties of CO_2. Also included in this section is the use of temperatures ≥ 200°C for the extraction of solid matrices. Elevated temperatures are expected to provide the activation energy needed for analyte desorption from the matrix sites.

1. Freon-22

Chlorodifluoromethane or Freon-22 is nontoxic, nonflammable, and has a large dipole moment of 1.29 D. But, it is very expensive and has a relatively high critical temperature of 96°C.[13] Freon-22 is believed to be less ozone depleting than Freon-11 and Freon-12, but still damaging because it contains chlorine. Still, examples in which Freon-22 was shown to be advantageous as an extraction fluid include the recovery of seven steroids (including cortisone, shown in Figure 1),[26] PAHs and nitrated PAHs from diesel exhaust particulate matter,[27] polychlorinated biphenyls (PCBs) from river sediment, PAHs from petroleum waste sludge, elemental sulfur from bituminous coal, and α-pinene from pine needles.[19] The stronger interaction between the polar extraction fluid and matrix H_2O was credited with the increased recoveries.

2. Freon-23

Trifluoromethane, also known as fluoroform or Freon-23, has a large dipole moment of 1.65 D, a critical temperature and pressure lower than those of CO_2,[13] and is believed to be less hazardous to the atmosphere than Freon-22 because it does not contain chlorine. Freon-23 was tested for the extraction of sulfonyl urea herbicides and PAHs.[28] Despite the polar nature of the extraction fluid, sulfometuron methyl and chlorsulfuron (shown in Figure 1) were recovered from spiked Celite at only 43 and 52%, respectively. Recoveries with pure CO_2 were 30% lower, although >90% yields were obtained

TABLE 1
Critical Properties and Dipole Moments from Reference 13 of the Extraction Fluids Discussed

	T_c (°C)	P_c (atm)	Dipole moment (debye)
CO_2	31.1	72.9	0
N_2O	36.5	71.7	0.17
Freon-22 ($CHClF_2$)	96	48.5	1.29
Freon-23 (CHF_3)	25.9	46.9	1.65
Methanol	240	78.5	1.70
H_2O	374.1	218.3	1.85

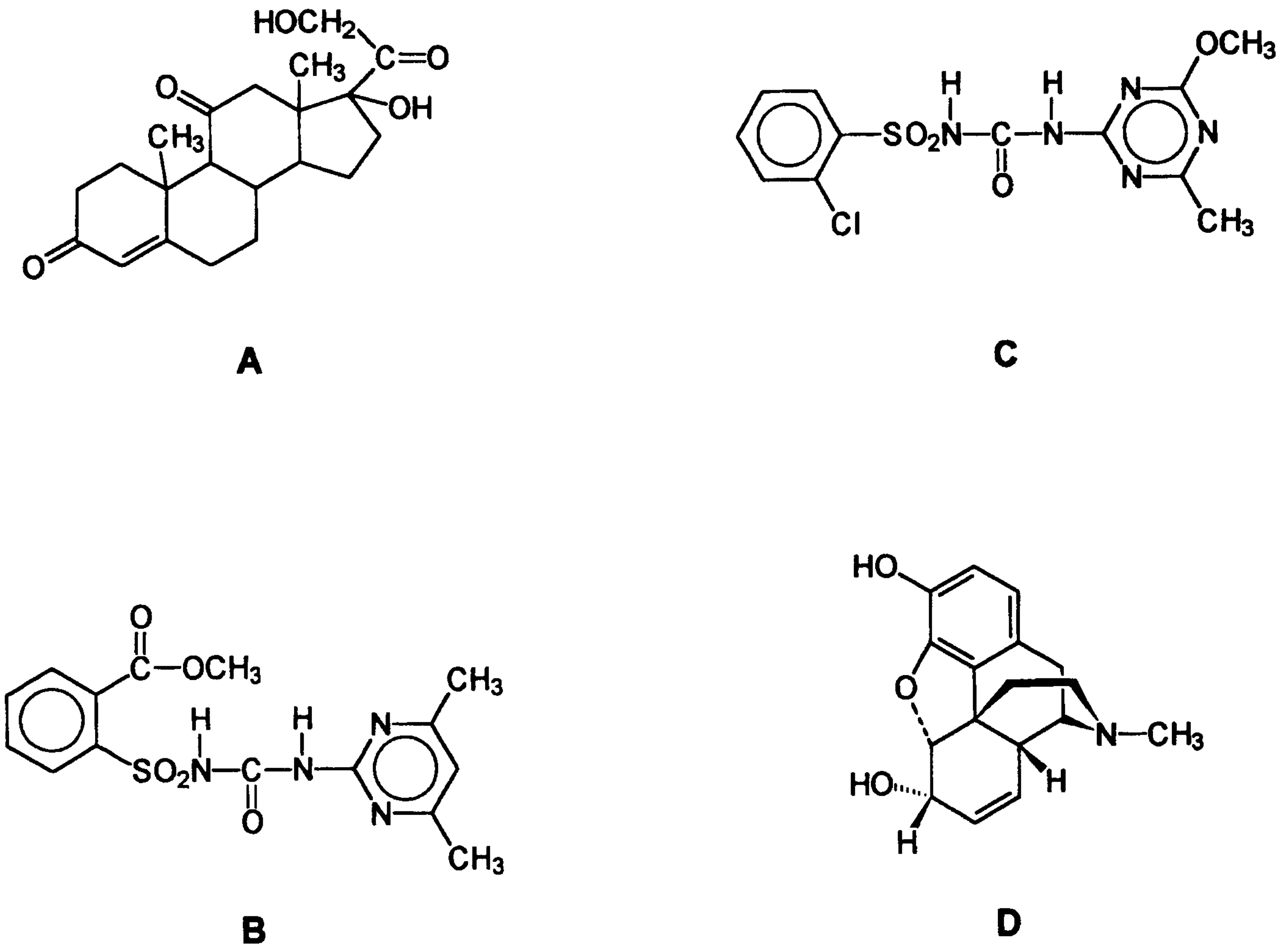

FIGURE 1. Structures of the analytes (A) cortisone, (B) sulfometuron methyl, (C) chlorsulfuron, and (D) morphine.

with a supercritical mixture of 2% methanol in CO_2. Freon-23 was 15 to 30% less efficient for the removal of PAHs from clay than either CO_2 or Freon-22. However, Freon-23 produced yields that were generally 15% higher than those with CO_2 for the extraction of triazines, organophosphorus pesticides (OPPs), carbamates, and anilides from glass beads, although overall recoveries did not exceed 80%.[29] Additionally, no selectivity was observed for analytes with differing polarities.

3. Methanol

Methanol is a common liquid solvent with a large dipole moment of 1.70 D.[13] The high critical temperature of 240°C generally deters the use of methanol as a supercritical fluid. However, supercritical methanol was investigated in one study for the recovery of bound (nonextractable) ^{14}C pesticide residues from soils and plants.[30] Recoveries were generally >60% and were comparable to or better than a high-temperature distillation technique. But, reactions between methanol and some of the pesticides or their metabolites occurred at the extraction temperature used, 250°C (for example, atrazine was converted to its methoxy analog).

4. Subcritical and Supercritical H_2O

Hawthorne and co-workers recently demonstrated the feasibility of using H_2O as an extraction fluid in the subcritical liquid or gaseous state.[31,32] The critical temperature and pressure of H_2O are 374.1°C and 218.3 atm, respectively.[13] Supercritical H_2O is corrosive in the presence of oxygen, leading to both analyte degradation and instrumental problems. For this reason, Hawthorne's work centered mostly around milder, subcritical conditions.

The high polarity of H_2O is reflected by the large dielectric constant, ϵ, of 79 at ambient conditions. At the critical point, however, ϵ is ~5 to 15 (see Figure 2), which is comparable to liquid methylene chloride, making supercritical H_2O an attractive solvent for nonpolar organic pollutants.[31,32] For example, the solubility of benzo[e]pyrene in H_2O at 99 atm increased ~25 million-fold by elevating the temperature from 25 to 350°C.[31] As shown in Figure 3, extracted analytes were collected in a few milliliters of chloroform, with the exiting H_2O extraction fluid forming a layer above the chloroform in the collection vial. After initially separating the liquid layers, the H_2O layer was extracted two more times with chloroform, and the combined chloroform extract was analyzed.

The variation in dielectric constant with temperature made class selective extractions based on polarity possible. Chlorinated phenols, PAHs, and alkanes were fractionated from an urban air particulate and a certified soil by raising the temperature from 50 to 250°C and then to 400°C.[31] The dielectric constant decreases from 71 to 29 to 8 at the three temperatures. Shown in Table 2, PCBs were recovered from an industrial soil and a river sediment at trace levels at 50 to 100°C but quantitatively at 250°C in 15 min at liquid conditions and in 5 min with steam at 300°C.[32]

This extraction fluid appears promising. H_2O is an inexpensive, environmentally sound solvent. Because the extraction fluid polarity is controllable with temperature, analytes of a given polarity can be recovered selectively from other analytes and extraneous matrix material. However, most standard SFE instruments currently available do not operate at temperatures >150°C and the possibility of corrosion due to oxidation is a considerable risk. The extra liquid-liquid extraction step to recover the analytes in the collection vial is also both time and solvent consuming.

5. Enhanced-Fluidity Liquids

As mentioned in the introduction, enhanced-fluidity liquids are mixtures of common associated liquids, such as methanol,

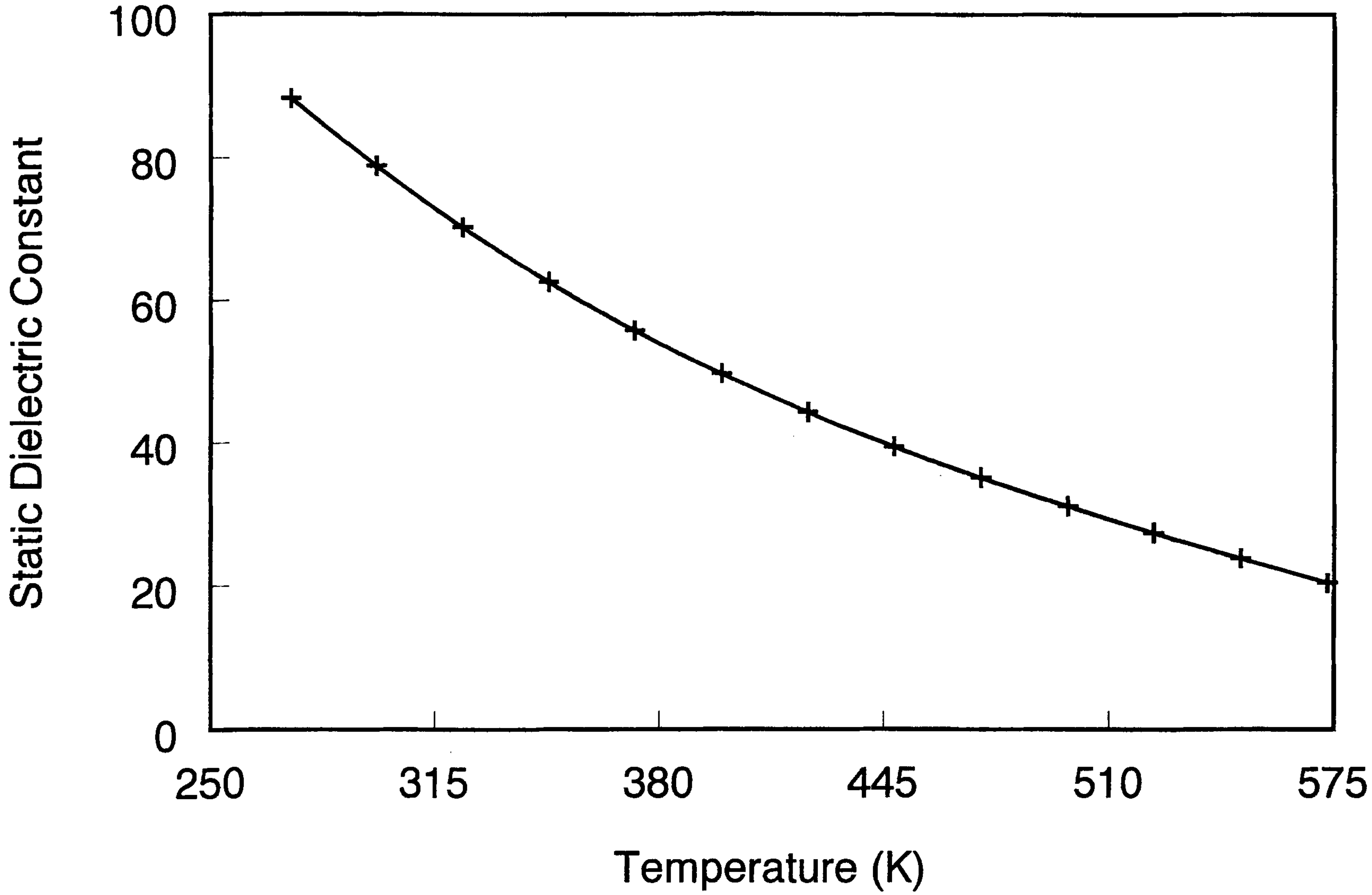

FIGURE 2. Variation of the dielectric constant (ϵ) of H_2O with temperature at constant pressure (99 atm). Data from Reference 33 were used to generate the curve.

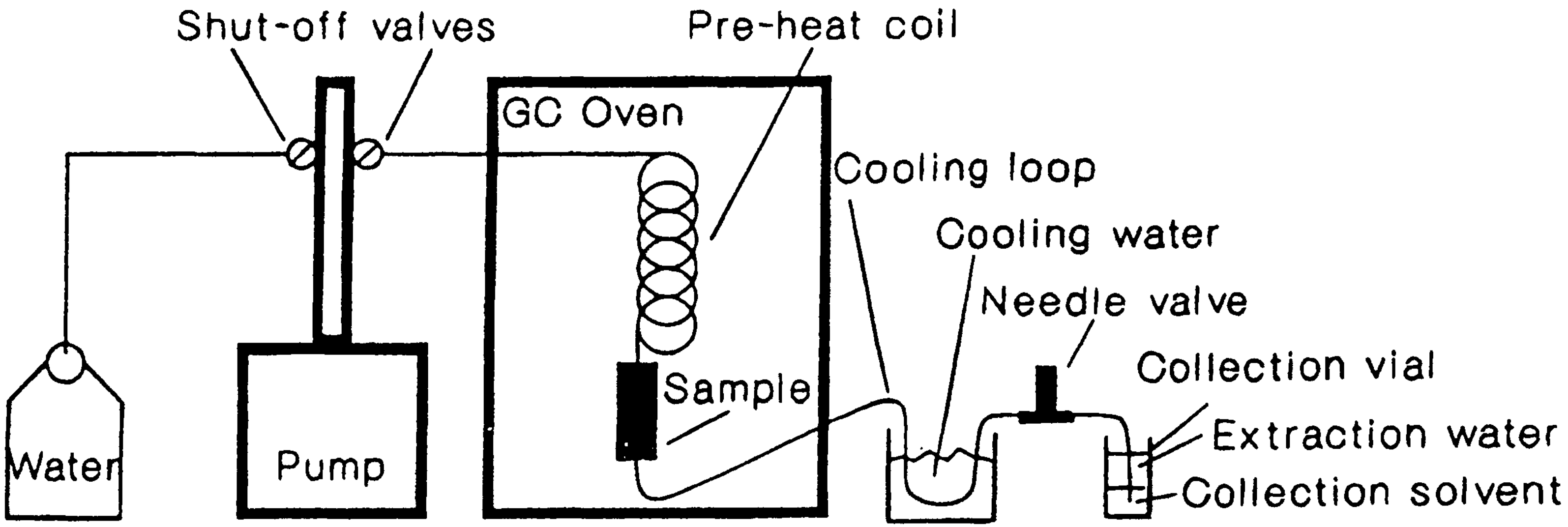

FIGURE 3. Schematic diagram of subcritical water extraction using cooling/collection system. From Reference 32. With permission.

TABLE 2
PCB Removal Efficiencies from Industrial Soil (CRM 481) Obtained by Sequential Extraction with Higher Temperatures

	Liquid water					Steam[a]
Temperature (°C)	50	100	150	200	250	300
H_2O dielectric constant	70	56	44	35	27	1
	Cumulative removal[b] (%)					
PCB-52	0	15	36	71	100	100
PCB-101	0	4	16	53	94	100
PCB-118	0	3	10	49	92	100
PCB-149	0	2	11	51	93	100
PCB-153	0	1	9	48	92	100
PCB-105	0	0	14	52	92	100
PCB-138	0	1	9	48	92	100
PCB-128	0	1	8	50	93	100
PCB-156	0	0	6	47	90	100
PCB-180	0	0	4	43	90	100
PCB-170	0	0	5	45	91	100

[a] At 50 atm, H_2O is liquid at 250°C and lower temperatures and steam at 300°C.

[b] One hundred percent removal is defined by the lack of any detectable species in the 16-h sonication extract (using methylene chloride and acetone) of the residues after a 60-min (10 min at each temperature) H_2O extraction.

Adapted from Reference 32. With permission.

with liquefied gases such as CO_2. Enhanced-fluidity liquid mixtures have low viscosities, approaching that of the liquefied gas, whereas the solvent strength is similar to that of the associated solvent. For example, the addition of as much as 50 mol % CO_2 is possible in these mixtures before the solvent strength decreases significantly from that of the original associated solvent. Enhanced-fluidity liquid mixtures are presently studied as possible alternative solvents for the extraction of polar analytes.[34,35]

Rosset and co-workers also studied the use of enhanced fluidity liquid mixtures for extraction of alkaloids. Thebaine, codeine, and morphine (shown in Figure 1) were extracted from poppy straw using subcritical mixtures.[36] No significant extraction was observed with CO_2. Quantitative yields were obtained with 50/50% (w/w) methanol/CO_2 after 3 h and with 24/6/70% (w/w) methanol/H_2O/CO_2 in just 20 min. Subcritical mixtures of 8.5/1.5/90% (v/v) methanol/triethylamine (TEA)/CO_2 and 6/6/3/85% (v/v) methanol/TEA/H_2O/CO_2 were used for the recovery of morphinic alkaloids in H_2O and urine after adsorption onto a C_{18} or silica sorbent.[37]

Reighard and Olesik used a C_{18} sorbent as a model system to optimize extraction conditions from an organic solid. Eleven phenolic and nitroaromatic pollutants were extracted from the C_{18} sorbent using supercritical CO_2 and enhanced-fluidity liquid methanol/CO_2 mixtures at 25 to 65°C.[38] An average recovery of 59% for 10 of the 11 analytes was found with CO_2 in 20 min, but 2,4-dinitrophenol was not recovered. Average yields for all 11 analytes improved to 75 and 71% with liquid 10/90 and 20/80 mol % methanol/CO_2 mixtures, respectively, in <8 min. Statistical analysis of the data illustrated that solvent composition was the most important variable, whereas the effect of temperature was minimal. For the same mixture composition, the extraction rate did not depend on the phase state of the solvent (liquid or supercritical).

Phenolics and nitroaromatics were also recovered from fortified house dust[39] and river sediment.[40] Zero to 40 mol % methanol/CO_2 and methanol/H_2O/CO_2 mixtures were evaluated as extraction solvents in both supercritical and liquid state conditions. Analytes with pK_as ≥ 10 were the most difficult to extract. Enhanced-fluidity liquid mixtures of methanol/H_2O/CO_2 and methanol/CO_2 provided higher extraction yields than supercritical CO_2, supercritical methanol/CO_2, or Soxhlet extractions. The average extraction yield was highest when using 32.1/7.9/60.0 mol % methanol/H_2O/CO_2 mixtures as the extraction solvent; however, the yields were also high when 20/80 mol % methanol/CO_2 was the extraction solvent. Table 3 shows a comparison of the extraction yields obtained using Soxhlet, 20/80 mol % methanol/CO_2, and 32.1/7.9/60.0 mol % methanol/H_2O/CO_2.[39] For the solvent mixtures studied, the extraction rate was again comparable for the same solvent composition under both supercritical and liquid conditions.

Comparable extraction solvent conditions were studied for the extraction of the phenolic and nitroaromatic compounds from sediment. 10/90 mol % methanol/CO_2 at 150°C or 20/80 mol % methanol/CO_2 at 100°C gave better recoveries for the analytes than the other methanol/CO_2 compositions studied. The best extraction condition for most of the analytes involved the addition of 10% H_2O (v/w H_2O/sediment) with the 10/90 mol % methanol/CO_2 mixture as the extraction solvent at 100°C. Ternary mixtures of methanol/H_2O/CO_2 were the best extraction solvent for the three analytes with pK_as ≤ 4.7.[40]

Four phenoxyacid herbicides were recovered from fortified house dust as well.[41] Phenoxyacids are relatively nonvolatile and require a derivatization step, most commonly to the methyl esters, prior to gas chromatographic analysis. Most of the recent studies of phenoxyacids focused on *in situ* derivatization or ion pair formation (see the next section, Altering the Analytes) to make the analytes less polar and more readily extractable. In this study, the phenoxyacids were recovered by increasing the polarity of the extraction fluid and derivatized after extraction, upon injection in the heated GC injector. Recoveries were <40% with CO_2 at temperatures of 25, 50, 100, and 150°C, but ranged from 83 to 95% using 10/90 and 20/80 mol % methanol/CO_2 at 100 and 150°C. However, recoveries of two analytes, dicamba and 2,4,5-TP, were comparable for all four temperatures tested, whereas 2,4-D and 2,4,5-T were more efficiently extracted at 100 and 150°C (see Figure 4A and 4B). Because the carboxylic acid side chains of 2,4-D and 2,4,5-T are less hindered by other substituents, these analytes were possibly more strongly adsorbed to the matrix relative to dicamba and 2,4,5-TP, and thus required both a high solvent strength extraction fluid (such as 20/80 mol % methanol/CO_2) and elevated temperatures (100 and 150°C) for efficient desorption and extraction.

Sixteen native PAHs were also identified in house dust extracts using supercritical and enhanced-fluidity liquid mixtures of methanol/CO_2.[42] Highest yields were obtained with 30/70 mol % methanol/CO_2 at

TABLE 3
Average % Recoveries ± One Standard Deviation, Relative to the Spiking Levels

	Percent recovery[a]				
Analyte	**Soxhlet**	**CO_2**	**10/90 mol % $MeOH/CO_2$**	**20/80 mol % $MeOH/CO_2$**	**32.1/7.9/60.0 mol % $MeOH/H_2O/CO_2$**
Phenol	74 ± 17	49 ± 23	72 ± 2	74 ± 9	85 ± 7
2-Chlorophenol	54 ± 13	63 ± 16	65 ± 2	76 ± 5	63 ± 6
o-Cresol	42 ± 12	49 ± 16	41 ± 2	50 ± 1	66 ± 8
m-Cresol	52 ± 13	53 ± 20	60 ± 3	63 ± 2	60 ± 6
Nitrobenzene	59 ± 4	87 ± 16	51 ± 3	75 ± 5	51 ± 4
2,4-Dimethylphenol	15 ± 7	24 ± 6	12 ± 1	22 ± 1	36 ± 6
2,4-Dichlorophenol	63 ± 14	61 ± 25	74 ± 3	73 ± 2	86 ± 7
2,4,6-Trichlorophenol	79 ± 24	92 ± 33	81 ± 7	105 ± 11	88 ± 5
2,4-Dinitrophenol	52 ± 34	35 ± 16	9 ± 2	0	23 ± 10
4,6-Dinitro-*o*-cresol	41 ± 14	24 ± 10	34 ± 10	35 ± 9	67 ± 21
Pentachlorophenol	81 ± 28	0	53 ± 14	94 ± 6	132 ± 26

[a] Extractions using CO_2, methanol/CO_2 and methanol/H_2O/CO_2 were done in at least triplicate at 238 atm and 50°C. The Soxhlet extraction used methylene chloride for 24 h and replicated 12 times.

liquid or supercritical conditions. With the exception of acenaphthalene and naphthalene, all analytes were recovered at >100% relative to 24 h Soxhlet extractions. Additionally, recoveries of 5 and 6 ring PAHs were ≤60 and 31% with CO_2 alone, but averaged 143 and 226% with 30/70 mol % methanol/CO_2.

The extraction of PAHs from spiked lignite and bituminous fly ashes using methanol/CO_2 mixtures under supercritical and liquid conditions was investigated.[43] Good recoveries from lignite fly ash were found with all conditions tested. None of the PAHs was recovered from bituminous fly ash until the temperature was raised to 150°C, regardless of extraction fluid composition. A 40/60 mol % methanol/CO_2 mixture at 150°C (supercritical) was found to be the best extraction condition; SFE experiments using 40 mol % modifier as in this study are generally not reported.

In all of these experiments, the extraction rates with enhanced-fluidity liquids were comparable to or better than those with supercritical fluids. Therefore, the favorable mass transport properties of supercritical fluids were preserved and the state of the fluid (liquid or supercritical) was generally inconsequential. The enhanced-fluidity liquid technique is quite appealing for the extraction of matrices with a high H_2O content where the solubility of H_2O in the extraction fluid is important and for thermally labile analytes where a high solvent strength fluid at low temperatures is needed. Enhanced-fluidity liquid extractions may be more exhaustive than SFE and may require an extract cleanup step.

6. High-Temperature SFE

The use of elevated extraction temperatures (≥200°C) was initially avoided because CO_2 density decreases with increasing temperature at constant pressure. However, analyte solubility in CO_2 is influenced by both CO_2 density and the analyte vapor pressure (which improves with increasing temperature).[44] Furthermore, the activation energy of desorption, needed to release the

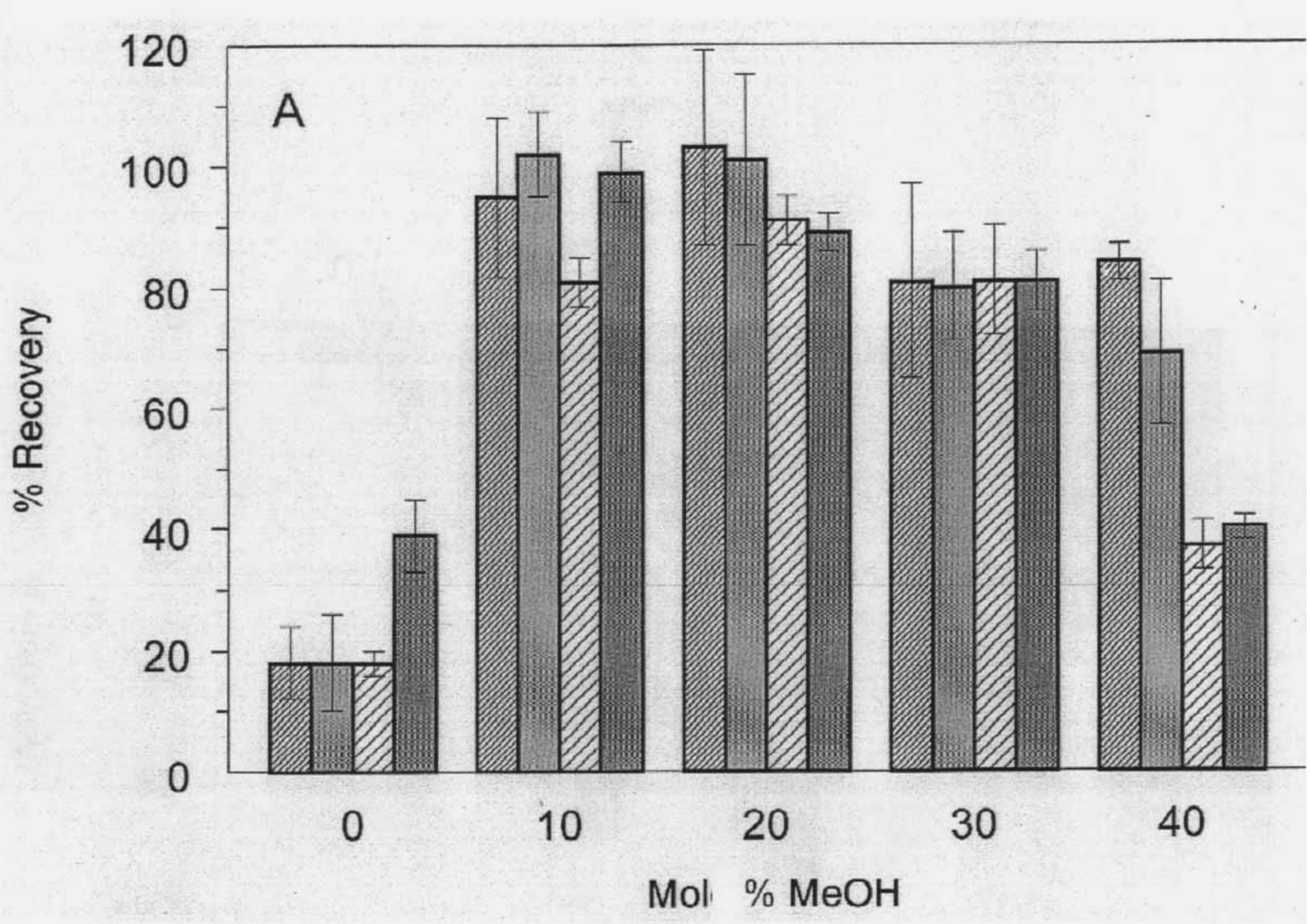

FIGURE 4. Overall % recoveries vs. mol % methanol in CO_2 at 442 atm and 25, 50, 100, and 150°C, shown from left to right for each composition, for phenoxyacid herbicides (A) 2,4,5-TP and (B) 2,4,5-T. Error bars signify one standard deviation. From Reference 39.

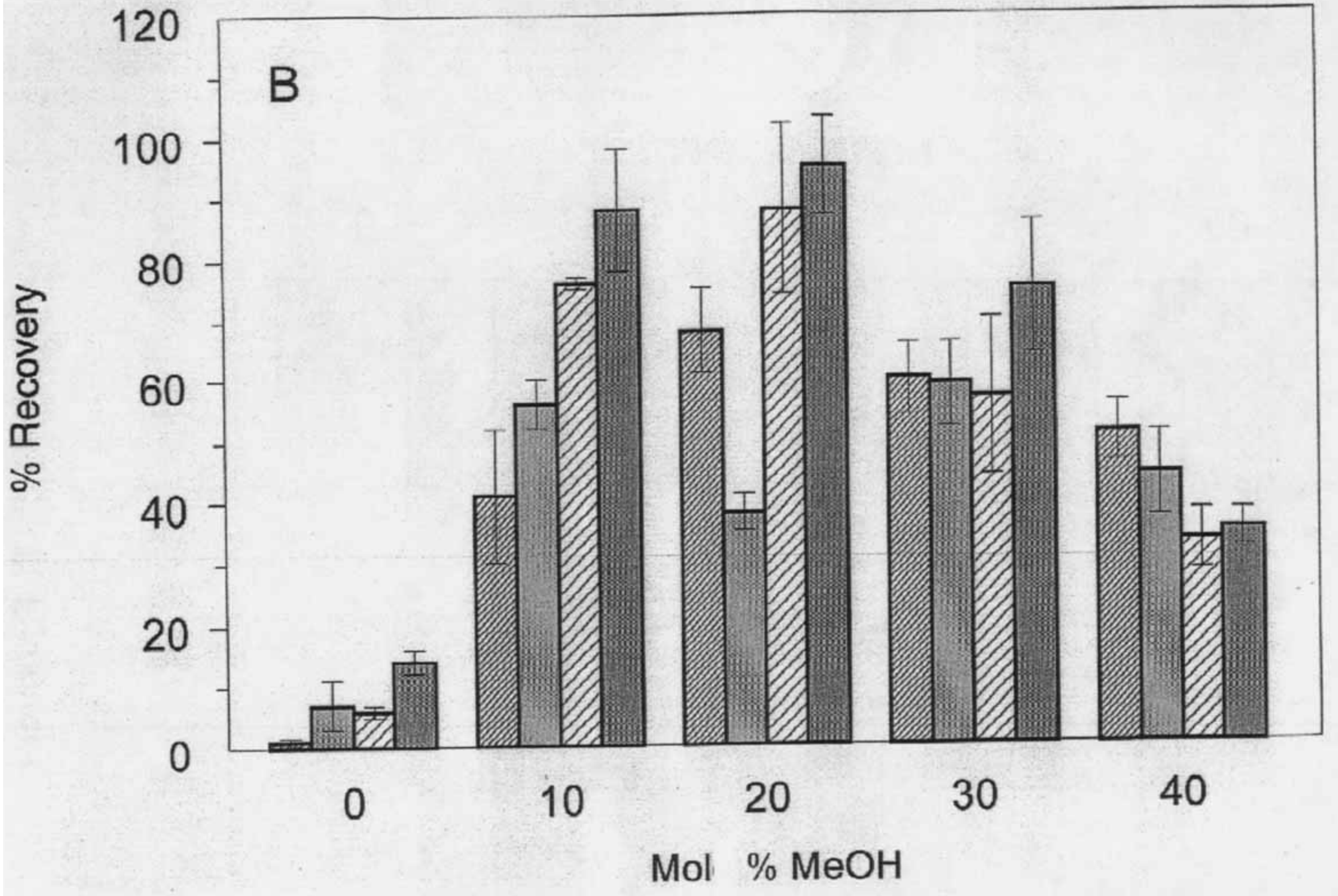

FIGURE 4B

analytes from the matrix, should be more easily overcome at higher temperatures.[44,45]

Analyte fractionation via temperature variation was recently investigated with good results.[45] A mixture of PCBs and PCDDs was spiked onto Florisil. Plots of % recovery vs. extraction time at various temperatures are shown in Figure 5. The PCBs were first extracted with CO_2 at 238 atm and 80°C for 15 min, conditions at which the PCDDs were not significantly extracted (see Figure 5A). The PCDDs were then removed with CO_2 at 612 atm and 200°C during an 80-min extraction (shown in Figure 5B). To extend the technique to native samples, fly ash was initially extracted with CO_2 at 640 atm and 250°C for 80 min to remove all of the analytes. The extract was then loaded onto Florisil with liquid elution of interfering components followed by CO_2 fractionation of the PCBs and PCDDs at the above listed conditions. The two steps were then performed simultaneously by connecting a Florisil column directly to the outlet of the extraction vessel containing the fly ash matrix.

Temperatures of 50, 200, and 350°C were tested for the extraction of PAHs, N- and S-heterocyclics, chlorinated phenols, and pesticides from soil and soot.[19] Increasing the temperature from 50 to 200°C with CO_2 had a positive effect on all analytes. A 350°C extraction temperature resulted in yields only comparable to the 50°C extractions although no thermal degradation was detected. Extractions at 350°C were more difficult to perform due to leaks around the vessel seals, and low molecular weight PAHs were formed from matrix organics. To determine the role of CO_2, thermal desorption with a nitrogen purge was tested. Recoveries of 0 to 50% with nitrogen were compared to 51 to 100% recoveries with CO_2 at 200°C from a railroad bed soil, indicating that the solvating power of CO_2 was important to the extraction.

In other studies, aliphatic and aromatic hydrocarbons were recovered from shale more efficiently at 350°C than at 50, 150, and 250°C,[47] PCDDs from fly ash and PAHs from marine sediment were detected at higher yields at 200 than at 120 or 40°C,[48] PCBs from river sediment and PAHs from urban air particulate were effectively recovered only when the temperature was raised from 50 to 200°C,[49] and PAHs were more efficiently extracted from marine sediment, diesel soot, and air particulate at 200 than at 80°C.[44] In this last study, the effect of temperature was found to be matrix independent.[44]

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

B. Altering the Analytes

When the analytes have low solubilities in CO_2 or the matrix-analyte interactions are strong, the addition of a reagent capable of chemical derivatization, complexation, or ion pair formation is a viable new approach. The reagent works either by modifying the analytes so that the resulting compounds are more soluble and/or less polar, or by interacting with adsorptive sites on the matrix thereby displacing the analytes and preventing readsorption. Thus, some of the examples cited in this section are also applicable to the next section where altering the matrix is considered. This technique was first applied to organic pollutants and was termed supercritical fluid derivatization and extraction (SFDE).[50] Recently, the methodology was extended to organometallic compounds

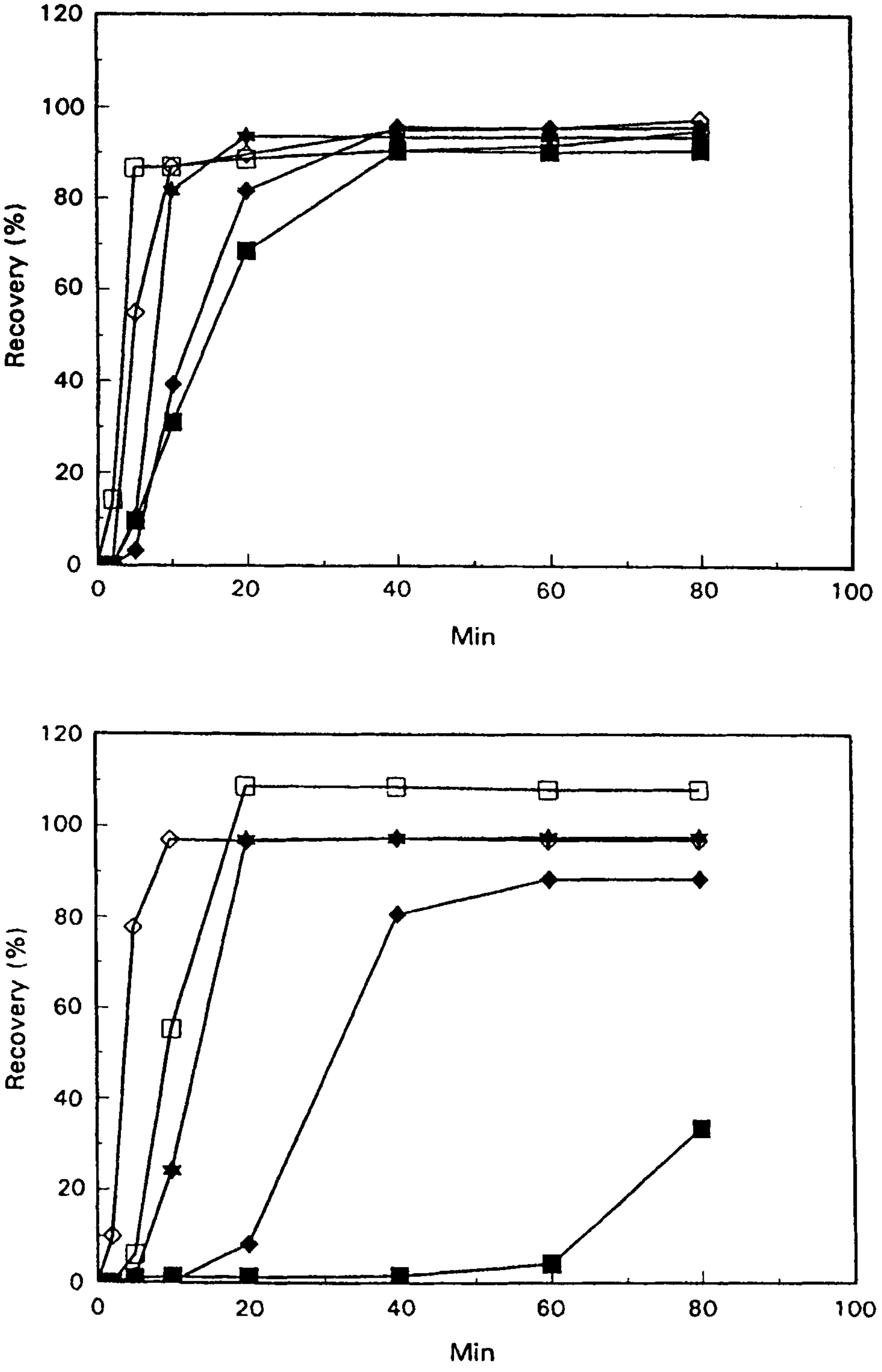

FIGURE 5. Effect of extraction temperature on the recovery of (A, top curve) 2,2′,3,4,5′-pentachlorobiphenyl and (B, bottom curve) 2,3,7,8- tetrachlorodibenzo-p-dioxin from Florisil using neat CO_2 at a density of 0.6 g/ml. (■) 50°C, (◆) 80°C, (★) 120°C, (□) 160°C, (◇) 200°C. From Reference 45. With permission.

and metal ions as well, making many of these analytes CO_2 extractable for the first time.

1. Organic Analytes

Tri-sil silylation reagent, a 2:1 (v/v) mixture of hexamethyldisilane and trimethylchlorosilane, was one of the first reagents tested. This reagent reacts with functional groups such as carboxylic acids, alcohols, phenols, and amines. For example, carboxylic acids are converted to trimethylsilyl esters. The extractions of caffeine from roasted coffee beans and Japanese tea,[50] lipophilic material (fatty acids and terpines) from marine sediment,[50] PAHs from harbor sediment and urban dust standard reference materials,[51] and 2,4-dichlorophenoxyacetic acid (2,4-D), a broadleaf weed control herbicide, from sand and soil[52] were studied. The PAH recoveries were six times higher with Tri-sil added than with CO_2 alone and two times higher than with 10% methanol added. However, low yields of 2,4-D (31%) were attributed to the interference of matrix moisture in the silylation reaction. Tri-sil is not a very good silyl donor and is rarely used today.

Neat N,O-bis-trimethylsilyltrifluoroacetamide (BSTFA, shown in Figure 6) is a more rapid and efficient silyl donor than Tri-sil.[53] In addition, the byproducts are more volatile and commonly elute well before the derivatized analytes during GC analysis. BSTFA was added to fly ash for the extraction of PAHs, halogenated phenols and aromatics, and dioxins.[54] Although some of the analytes do not form trimethylsilyl esters, all recoveries were enhanced in the presence of BSTFA relative to CO_2 alone or with methanol added to the sample.

Acetic anhydride, triethylamine, and H_2O were used to recover phenols as their acetyl derivatives. The extraction of chlorophenols from fortified and contaminated soils,[55] catechols, guaiacols, vanillins, and syringols from sediments,[56] and pentachlorophenol (PCP) from leather[57,58] were investigated. The derivatization was most effective for highly chlorinated phenols like PCP but was amenable to di- and trichlorophenols as well. Using acetic anhydride and pyridine, *o*-, *m*-, and *p*-cresol were recovered from spiked soil.[59,60]

A variety of reagents were tested for the SFDE of phenoxyacid herbicides. Phenoxyacids are prime candidates for this technique because derivatization to a more volatile and less polar form (most commonly the methyl ester) is required prior to GC analysis. Boron trifluoride,[52,61] pentafluorobenzylbromide (PFBBr),[62] trimethylphenylammonium hydroxide (TMPA),[61] tetrabutylammonium hydroxide (TBA) and methyl iodide,[63] tetrahexylammonium hydrogen sulfate and methyl iodide,[64] and methyl iodide alone[65] were all studied. Three sequential extractions with more TMPA added were necessary for quantitative recoveries of 2,4-D and dicamba (3,6-dichloro-2-methoxybenzoic acid) when the matrix organic content was high.[61] Yields of seven analytes from sand, clay soil, and topsoil ranged from 57 to 141% using TBA and methyl iodide, although excess methyl iodide prevented analysis by GC with electron capture detection.[63] 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were also extracted as methyl esters when methyl iodide and tetrahexylammonium hydrogen sulfate (a phase transfer reagent) were added to spiked diatomaceous earth, soil, urine, and rice.[64] The phase transfer reagent was later found to be unnecessary. Methyl iodide alone recovered organic acids such as 2,4-D, 2,4,5-T, and PCP from an anion exchange resin at 78 to 101% yields.[65]

Transesterification with methanol in CO_2 was demonstrated. Triglycerides in soybeans and soybean seeds, evening primrose seeds, and peanuts were converted to fatty acid methyl esters over methanol-treated alumina in the presence of CO_2.[66] Resin and fatty

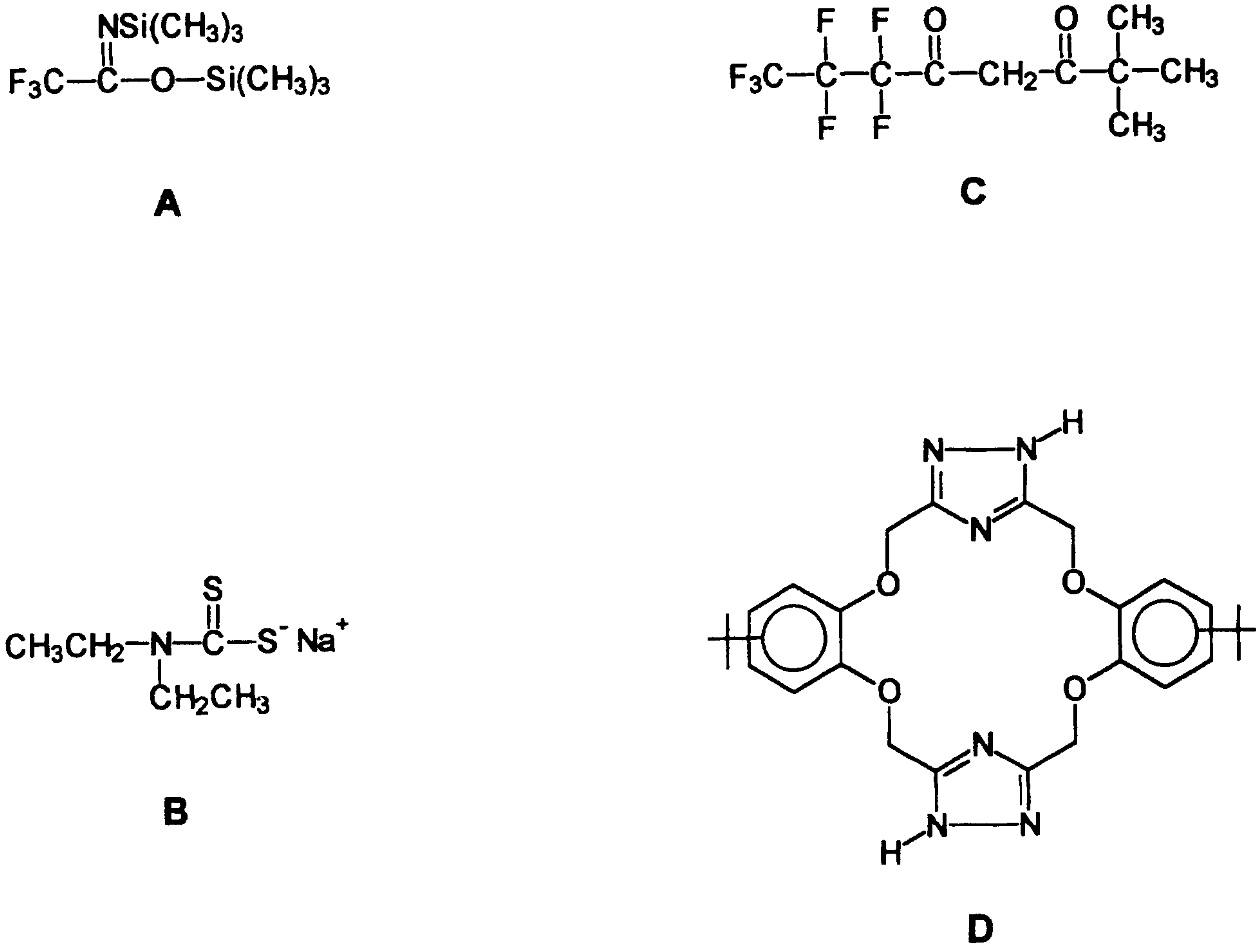

FIGURE 6. Structures of the reagents (A) BSTFA, (B) NaDDC, (C) FOD, and (D) t-butyl substituted dibenzobistriazolo-crown ether.

acids were extracted from sediment with the addition of methanol and formic acid. Recoveries improved from ≤3% with pure CO_2 to 88 to 267% relative to Soxhlet results.[67]

Ion pairing reagents were also tested. TMPA was utilized for the extraction of sulphonamides from diatomaceous earth with 82 to 108% yields.[68] Secondary alkanesulfonate (SAS) and linear alkylbenzenesulfonate (LAS) surfactants were recovered from sewage sludge as TBA ion pairs.[69] Three consecutive extractions with more TBA added lead to a 2.5-fold increase in yields over methanol modifier alone. Finally, the addition of a strong cation displacer, $CaCl_2 \cdot 2H_2O$ in methanol, yielded 87% of 2,4-D from soil.[52]

Strong advances were made in the extraction of some polar analytes by the use of SFDE. Additionally, even analytes that did not react with the reagents were more efficiently extracted because the reagents interacted with the adsorptive matrix sites. However, problems encountered include the need for multiple extractions with more reagent added when the matrix organic content is high, ineffective reagents in the presence of matrix H_2O or other modifiers, and excess reagent complicating analysis.

2. Organometallic Analytes

SFE was applied almost exclusively to the recovery of organic analytes until the last few years. However, organometallic compounds are frequently used in environmental applications. For example, uses of organotin compounds include agricultural and wood preservative fungicides, antifouling paint biocides, fire retardants, thermal stabilizers for poly(vinyl chloride), and catalysts in the production of silicones and polyurethane foams.[70,71] Most of the analytical methods developed focus on monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) due to toxicity and lack of a suitable method for MBT analysis. The use of SFE for the recovery of organometallics is still in its early stages with the majority of references reported in the last three years. Examples with and without the addition of a complexing reagent during the extraction step are included in this section to calibrate the reader to the present state of the technique.

Di- and trisubstituted tins were recovered from marine paint, potatoes, and almonds using on-line SFE/SFC with 0.3% (v/v) formic acid in CO_2.[72] TBT and triphenyltin (TPhT) were extracted from a fish tissue certified reference material using 10/90% methanol/CO_2; only 44% of TBT and 23% of TPhT were recovered.[71] The use of 20/80% (v/v) methanol/CO_2 lead to the 82% recovery of TBT from spiked soil and the 70 to 75% recovery of TBT from a certified reference sediment.[73] Aqueous ethylation with sodium tetraethylborate prior to solid phase extraction (SPE) of H_2O samples and SFE of the extraction disks with pure CO_2 resulted in 55 to 115% yields of butyl-, phenyl-, and cyclohexyltins.[21] The Sn-C_2H_5 bond was thermally more stable than the Sn-Cl bond and lowered the analyte polarity, increasing the solubility in CO_2.

Although the results described above without the use of a reagent during the extraction were promising, *in situ* complexation reactions were also investigated. Sodium diethyldithiocarbamate (NaDDC, shown in Figure 6) was one reagent tested for ionic organotins. Recoveries of trisubstituted species improved from 50 to 73% with CO_2 alone to 79 to 94% with NaDDC added prior to extraction (see Table 4).[21] Recoveries of dimethyltin, DBT, diphenyltin, and MBT increased from ≤21% to 11 to 93% with NaDDC added followed by extraction with CO_2 or 5/95% methanol/CO_2. In another study, the recovery of MBT was improved to 62% using NaDDC and 10/90% methanol/CO_2 while TBT and DBT were quantitatively recovered.[76] The authors attributed the low recovery of MBT to either

TABLE 4
Recoveries of Ionic Organotin Compounds by SFE[a]

		Percent recovery (percent RSD, n = 3)						
Matrix	Extraction fluid	Trimethyltin chloride	Trimethyltin bromide	Dimethyltin dichloride	Tributyltin iodide	Dibutyltin dichloride	Butyltin trichloride	Diphenyltin dichloride
Topsoil	Carbon dipoxide	50 (20)	73 (31)	7 (28)	71 (48)	21 (5)	5 (68)	5 (5)
Topsoil	Carbon dioxide with 5 percent methanol	61 (35)	66 (38)	6 (20)	72 (23)	7 (26)	17 (99)	12 (52)
Topsoil with NaCl	Carbon dioxide	70 (30)	78 (31)	6 (15)	52 (4)	4 (17)	4 (40)	5 (5)
Topsoil with NaCl	Carbon dioxide with 5 percent methanol	65 (16)	74 (14)	9 (38)	73 (10)	46 (39)	42 (61)	12 (11)
Topsoil with NaDDC	Carbon dioxide	79 (13)	86 (15)	78 (28)	94 (12)	93 (24)	11 (62)	32 (39)
Topsoil with NaDDC	Carbon dioxide with 5 percent methanol	76 (7)	87 (6)	72 (23)	81 (7)	92 (15)	48 (45)	41 (6)

[a] Extractions were carried out at 450 atm and 80°C, with 30 min static followed by 20 min dynamic. The compounds were derivatized prior to GC-AED analysis.

Adapted from Reference 75. With permission.

exhaustion of NaDDC during the extraction or loss of the now volatile analyte during collection in a small vial of solvent. The addition of diethylammonium diethyldithiocarbamate (DEA-DDC) followed by extraction with 5/95% methanol/CO_2 resulted in recoveries of <48% of monosubstituted, 7 to 106% of disubstituted, 75 to 119% of trisubstituted, and 76 to 123% of tetrasubstituted species from soils and sediments.[70]

In situ Grignard reactions were tested because organotin species are relatively involatile and derivatization is required prior to GC analysis. The sample must be very dry to prevent deactivation of the Grignard reagent. Treatment with C_2H_5MgCl and extraction with CO_2 lead to recoveries of 70% MBT, 92% DBT, and 102% TBT from an SPE disk.[21] *In situ* Grignard hexylation followed by CO_2 extraction yielded 76 to 114% recoveries for di- and trisubstituted species but only 15 and 40% for MBT and MPhT, respectively.[78]

The extraction of ionic alkylleads from sediment and urban dust was accomplished via methanol addition and CO_2 extraction with yields of 96% trimethyllead, 106% triethyllead, and 80% diethyllead.[79] Complexation with DDC was required after extraction and before analysis. Given the favorable results for *in situ* complexation of DDC with tin described above, the addition of DDC to the sample prior to extraction appears quite feasible for lead as well.

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

3. Metal Analytes

The extraction of metals by SFE was initially ignored due to the extremely low solubility of charged species in supercritical CO_2. Interactions between the ionic analytes and nonpolar solvent are very weak and charge neutralization is required.[80] Chelating or complexing reagents are commonly used to recover metals from H_2O. The reagents are generally soluble in organic solvents but not soluble in the aqueous phase.[81] For example, NaDDC is added to precipitate the M-DDC complex in aqueous solution then the M-DDC complex is extracted into an organic solvent. The potential for *in situ* chelation or complexation using CO_2 in place of an organic solvent, followed by SFE of the neutral species was successfully investigated over the past 5 years.

DDC forms stable complexes with over 40 metals and nonmetals. However, M-DDC solubility in CO_2 is quite low. The substitution of fluorine for hydrogen, producing a ligand such as bis(trifluoroethyl)dithiocarbamate (FDDC), was shown to improve metal-ligand (M-FDDC) solubilities by 2 to 3 orders of magnitude.[80,82]

FDDC was used for the extraction of Cu^{2+} from Celite and directly from H_2O,[80] Hg^{2+} from spiked filter paper,[83] and Cu^{2+}, Co^{2+}, Cd^{2+}, and Zn^{2+} from spiked filter paper, sand, and silica gel.[84] Extraction fluids were CO_2 or 5/95% methanol/CO_2. The addition of H_2O to the sample aided extraction by facilitating chelation and transport of the complex, and covering matrix sites reducing readsorption.[83] Recoveries were generally near 100%,[83,84] although percentages of RSDs were sometimes high and ranged from 2 to 70%.[84]

The ligand tetrabutylammonium dibutyldithiocarbamate (DBDTC) was found to be more soluble than DDC. DBDTC-saturated CO_2 was used for the recovery of Zn^{2+}, Cd^{2+}, and Pb^{2+} from H_2O,[85] divalent metals from freeze-dried and fresh bovine liver,[86] and

Cd^{2+} from metallothioneins dissolved in H_2O (although Cd^{2+} recovery from the solid protein was unsuccessful).[87]

Fluorinated β-diketones, such as 2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedione (FOD, shown in Figure 6), are commercially available and form stable complexes with trivalent species.[88] The β-diketones react with metals when the enol form deprotonates, coordinating the metal between the two oxygen atoms.[81] In the liquid phase, selective extractions are possible by varying the pH. For example, Tl (III) is extracted at pH 2, whereas Tl (I) is not recovered until pH 6. In addition, many of the metal chelates are sufficiently volatile for GC analysis.

The efficient extraction of lanthanides and actinides is of increasing importance for nuclear waste analysis and management. Uranyl ion (UO_2^{2+}), La^{3+}, Eu^{3+}, and Lu^{3+} were recovered from spiked filter paper with FOD and H_2O added followed by extraction with CO_2 or 5/95% methanol/CO_2.[88] Recoveries were <1% in the absence of the ligand with CO_2 extraction and 91 to 99% with FOD added and methanol modification. The metals were also extracted at very acidic pH, outside of the pH range where complexation was expected with liquid solvent extraction.

In an effort to eliminate the methanol modifier, tributyl phosphate (TBP) was added with various β-diketones to test the synergistic effect. With TBP alone in CO_2, <4% of the lanthanides were detected from spiked filter paper. Recoveries of 92 to 98% were found in the presence of various β-diketones and TBP.[89] Because the coordination number of trivalent species is generally 8 to 9, TBP probably fills vacancies not occupied by the β-diketone, preventing readsorption to the matrix.[89] La^{3+}, Eu^{3+}, and Lu^{3+} were recovered at 75 to 89% yields from H_2O with the β-diketone thienyltrifluoroacetylacetone (TTA) and TBP added.[89] Likewise, Th^{4+} and UO_2^{2+} were quantitatively recovered from filter paper, mine H_2O, and soils using TBP and FOD or other β-diketones without methanol modification.[90] Shown in Figure 7, the extraction of UO_2^{2+} from Kaolin ($Al_2SiO_5(OH)_4$), however, required the use of 5% ethanol in CO_2 with FOD and TBP added for a 93% yield.[91] In contrast, the effect of the β-diketone was minimal for the recovery of lanthanides from a 6 *M* HNO_3, 3 *M* $LiNO_3$ solution using a 30% (v/v) TBP/CO_2 mixture.[92] TBP reportedly acted as both a chelating agent and a solvent modifier. Recoveries of 85 to 92% for Sm^{3+}, Eu^{3+}, Gd^{3+}, and Dy^{3+} and 44 to 72% for La^{3+}, Ce^{3+}, Lu^{3+}, and Yb^{3+} were found.

Finally, a t-butyl substituted dibenzobistriazolo-crown ether (see Figure 6) was used to selectively extract Hg^{2+} in the presence of other divalent metals such as Cd^{2+}, Mn^{2+}, and Zn^{2+}.[93] The crown ether is selective based on ionic radius-cavity size compatibility. The extraction fluid was 5/95 mol % methanol/CO_2 and the presence of H_2O again aided extraction. Recoveries of 98, 95, and 94% from filter paper, sand, and H_2O, respectively, were reported while <4% of the other divalents were extracted. However, Au^{3+} was partially extracted in the presence of Hg^{2+}.

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

C. Altering the Matrix

The extraction analytes from liquids using supercritical CO_2 is difficult due to experimental problems of sample containment and restrictor plugging. Because H_2O and CO_2 have low solubilities in one another,[94]

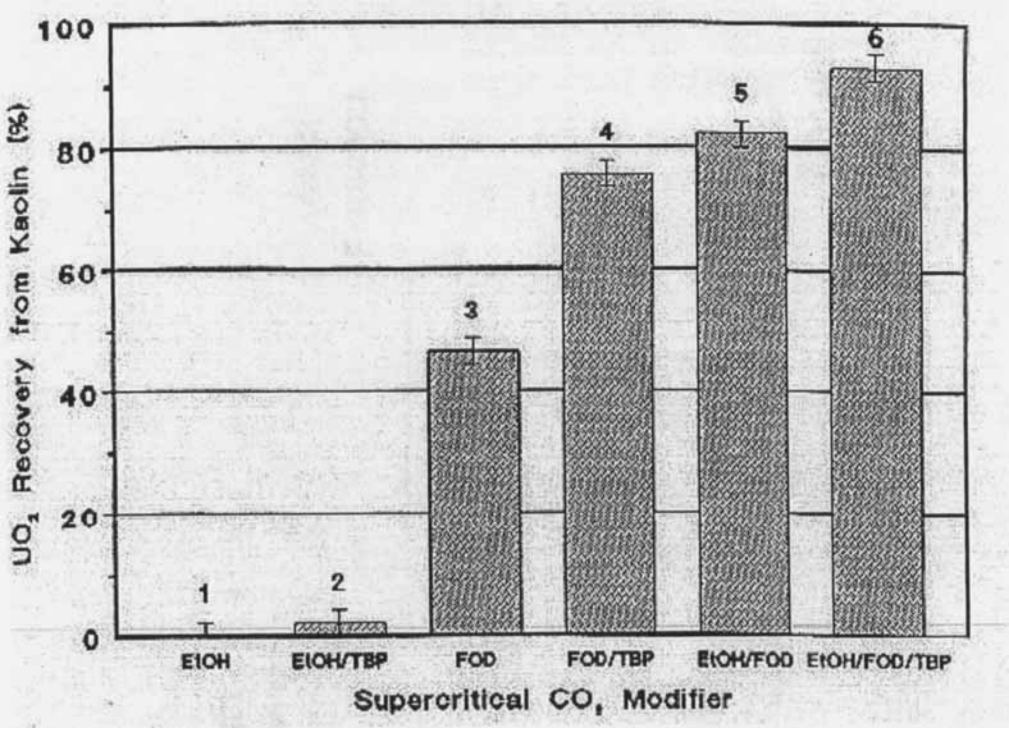

FIGURE 7. Recovery of uranium (VI) ions from Kaolin using different supercritical carbon dioxide modifiers as follows: 1 = 5% ethanol; 2 = 5% ethanol with 0.10 *M* TBP; 3 = 0.10 *M* FOD; 4 = 0.10 *M* FOD + 0.10 *M* TBP; 5 = 5% ethanol with 0.10 *M* FOD; 6 = 5% ethanol with 0.10 *M* FOD + 0.10 *M* TBP. From Reference 91. With permission.

significant time is required for analyte partitioning between the nearly immiscible phases. The direct SFE of analytes in H_2O was achieved with good results, however, and is briefly reviewed herein. Freeze drying a liquid sample and subsequently extracting the residue is a rather novel approach with some applications. A very promising alternative, SPE followed by supercritical fluid elution (SFE), is also discussed. SPE/SFE utilizes common sorbents to preconcentrate the analytes before elution using standard SFE instrumentation. Solvent use is greatly reduced compared to liquid-liquid extraction and the time for extraction is lessened relative to SFE of the original H_2O sample. As pointed out in the previous section, some of the derivatizing/ion pairing reagents used for the organic analytes also modify the matrix by interacting with adsorptive sites and preventing readsorption of the analytes.

1. Direct SFE of Liquid Matrices

The direct SFE of a liquid sample first employed a phase separator for the recovery of phenol and 4-chlorophenol from urine.[95] Consistent with SFE of solid matrices, Hedrick and Taylor then modified 10 cm × 1 cm i.d., 7 to 8 ml stainless steel vessels (Keystone Scientific, Bellefonte, PA and Suprex, Pittsburgh, PA).[96–99] The vessel (shown in Figure 8A) was filled half full and positioned vertically with flow of CO_2 down from the top through an inlet tube inserted below the H_2O surface. CO_2 flowed out of the vessel bottom via a tube inserted above the H_2O level in the cell. A series of 6-port

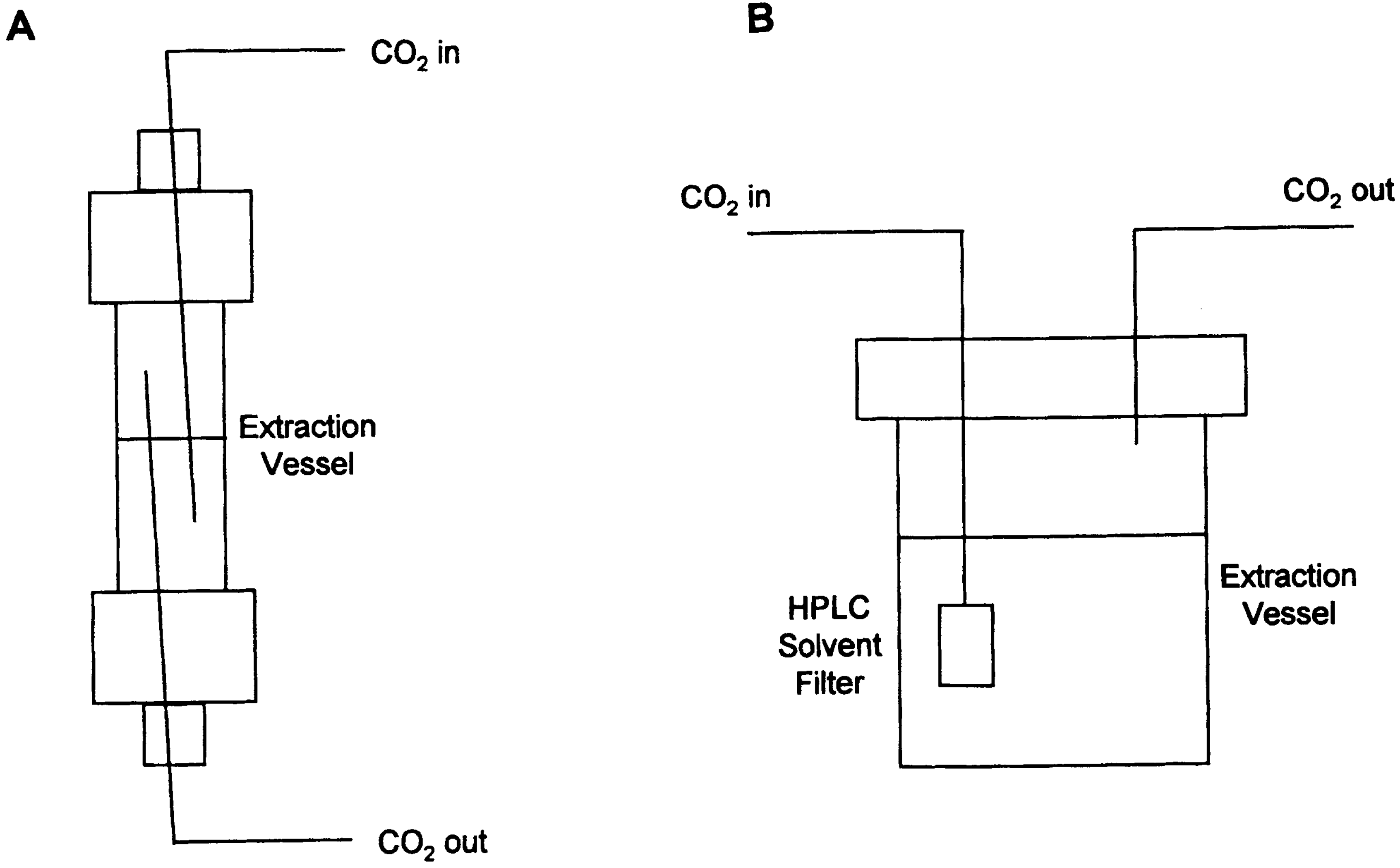

FIGURE 8. Liquid extraction vessels designed by (A) Hedrick and Taylor, and (B) Barnabas et al. Adapted from References 98 and 101, respectively. With permission.

switching valves and a recirculation pump were used to move supercritical CO_2 in a loop through the matrix. The CO_2 extract was then sampled and analyzed on-line or trapped in a liquid solvent or on a solid phase support for later analysis.

Analytes that are polar, of high molecular weight, and even very H_2O soluble may be efficiently extracted provided that the analytes are also soluble in CO_2 and sufficient time is allowed.[97] Examples of analytes recovered from aqueous matrices include diisopropyl methylphosphonate (DIMP),[96,97] triprolidine and pseudoephedrine,[96–98] six phenolics,[99] and phenoxyacid herbicides after the addition of 10% methanol or acetonitrile as modifiers.[100] Additionally, phenol was extracted with CO_2 from 6 *M* H_2SO_4 into H_2O for subsequent analysis.[97]

This vessel was also used extensively for the extraction of metals from H_2O. Due to the low solubility of metal ions in supercritical CO_2, a ligand was added either to the extraction fluid or directly to the sample to form a neutral complex that was then readily extractable. Dithiocarbamate-based ligands, particularly fluorinated species, were utilized for divalent metals.[80,85,86] Fluorinated β-diketones with or without TBP were used for lanthanides and actinides.[89,90,92] A crown ether was used for the selective extraction of Hg^{2+} in the presence of other divalent metals.[93] In all cases, <10% analyte was extracted without ligand and recoveries of 50 to 100% were reported when *in situ* chelation was possible. A more detailed discussion of the chelation procedure and cited references were given in the previous section.

Barnabas et al. later used a 50 ml, stainless steel vessel design shown in Figure 8B.[101] An HPLC solvent filter was placed on the end of the CO_2 inlet tube that was submerged in the solution. CO_2 exited the vessel via a tube positioned above the H_2O, effectively sampling the headspace. Although a considerably larger sample volume (~45 ml) could be used than in the Hedrick and Taylor vessel, the extraction time was greatly increased as well. Recoveries of the organochlorine pesticides (OCPs) lindane, aldrin, and dieldrin ranged from 40 to 75% after a 2 h extraction.[101] Alcohol phenol ethoxylate (APE, shown in Figure 9), a non-ionic surfactant, was removed from H_2O at 60% yield in 2 h.[102]

The direct SFE technique suffers from several limitations however. First, although the solubility of H_2O in CO_2 is quite low (~0.2 to 3 mol % at 25 to 100°C),[94] any H_2O that is transported from the vessel may contribute to restrictor plugging. This H_2O also causes problems in the analyte collection step. If the analytes are collected in a few milliliters of nonpolar organic solvent, a two-phase aqueous and organic mixture may result, and the matrix and analytes are simply transported from one vessel to another. H_2O can also activate solid phase traps, lowering the trapping efficiency.[96] Second, the Hedrick and Taylor extraction vessel holds ~3 to 5 ml H_2O and the Barnabas extraction vessel holds ~45 ml solution. When trace levels of pollutants are present, the extraction of a much larger sample volume (such as 1 L) may be necessary in order to concentrate the analytes sufficiently to exceed the limit of detection imposed by the analysis method. Third, the pH of H_2O in contact with CO_2 at pressures of 70 to 200 atm and temperatures of 25 to 70°C was recently measured at 2.80 to 2.95.[103] Therefore, bases are protonated and are much more soluble in H_2O than CO_2. If the analyte pK_a is >10, reactions with CO_2 are possible (for example, nitrogenous bases are converted to carbamates). Hedrick and Taylor found that a hydrocarbon, lipophilic moiety of considerable size (i.e., caffeine or larger) is necessary for a base to be extractable in CO_2.[98]

2. Freeze Drying the Sample

An interesting alternative to the direct SFE of a H_2O sample is to freeze dry the

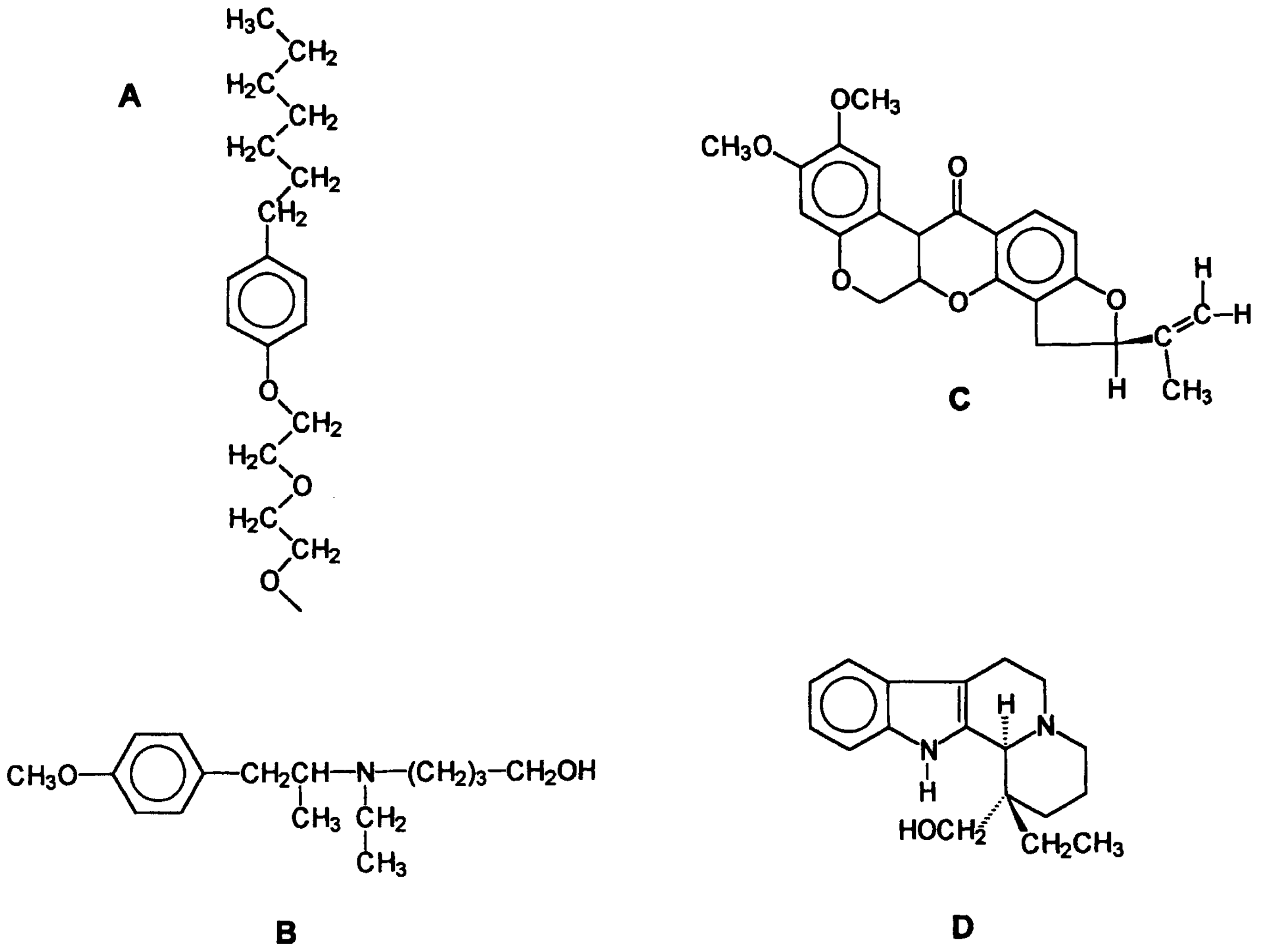

FIGURE 9. Structures of the analytes (A) alcohol phenol ethoxylate (APE), (B) mebeverine alcohol, (C) rotenone, and (D) RGH 2981 peripheral blood flow enhancer.

liquid and extract the residue.[104,105] This may be particularly attractive when analytes such as organophosphorus pesticides and carbamates decompose in H_2O upon prolonged storage. A H_2O sample spiked with atrazine and related herbicides was freeze dried with 0.6% glycine added as a stabilizer.[104] The residue was then extracted with CO_2. All of the analytes except fenitrothion were stable for at least 1 month, and recoveries exceeded those obtained by liquid-liquid extraction of the original sample. SFE was also applied to freeze-dried skim milk for the recovery of PCBs in Arochlor 1242. The residue was mixed with Florisil and extracted with CO_2.[105]

3. Solid Phase Extraction/ Supercritical Fluid Elution (SPE/ SFE)

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

SPE substantially reduces the volume of organic solvent required compared with liquid-liquid extraction methods, but elution with supercritical fluids offers a further solvent reduction. Moreover, SPE/SFE reduces or eliminates some of the difficulties encountered in the direct SFE of H_2O, including containing the sample in the extraction vessel and restrictor plugging associated with the minimal solubility of H_2O in CO_2. But, as shown in Figure 10, drying the disk prior to extraction can often lead to substantial losses of volatile components. SPE/SFE also provides a preconcentration step for the analysis of trace levels of pollutants. Whereas the concentration or removal of an aqueous/organic eluent after SPE is time consuming, the supercritical fluid eluent is easily removed from the extract because CO_2 goes off as a gas into the air.

Octadecyl (C_{18}) sorbents are by far the most widely used SPE materials. Examples of SPE/SFE using C_{18} are the isolation of a drug metabolite, mebeverine alcohol (shown in Figure 9), from dog plasma;[106] morphinic alkaloids from H_2O and urine;[37] PCB congeners from milk and blood serum;[107] and rotenone (see Figure 9), a natural product pesticide used for controlling fish populations, from H_2O.[108] In several cases, CO_2 modification with methanol or acetonitrile was necessary for rapid and efficient elution. Recoveries were generally quantitative.

Class selective extractions based on polarity were also possible. Three OCPs (lindane, aldrin, and dieldrin) and three OPPs (dichlorvos, diazinon, and malathion), shown in Figure 11, were selectively recovered from a C_{18} disk.[109] The disk was first eluted with pure CO_2 for the removal of OCPs then with CO_2 after the addition of methanol directly to the disk for elution of the OPPs. Recoveries exceeded 70% for all six analytes. The selective extraction aided in GC analysis because the nitrogen phosphorus detector used for the OPP analysis was less responsive in the presence of chlorinated species such as the OCPs, which were then quantitated by electron capture detection. In a similar study, three OCPs (heptachlor, isodrin, and dieldrin) were first extracted using CO_2 before six herbicides including simazine and diuron were then removed with 10/90% methanol/CO_2.[110] The OCPs were analyzed by GC and the herbicides were quantitated by HPLC with recoveries of 85 to 100% for all analytes.

C_{18} was also used for the SPE/SFE of PCBs and hexachlorocyclohexanes,[111] phenols,[61] PAHs,[112] OCPs,[101,112] nonionic surfactants,[102,113] and 43 semivolatiles includ-

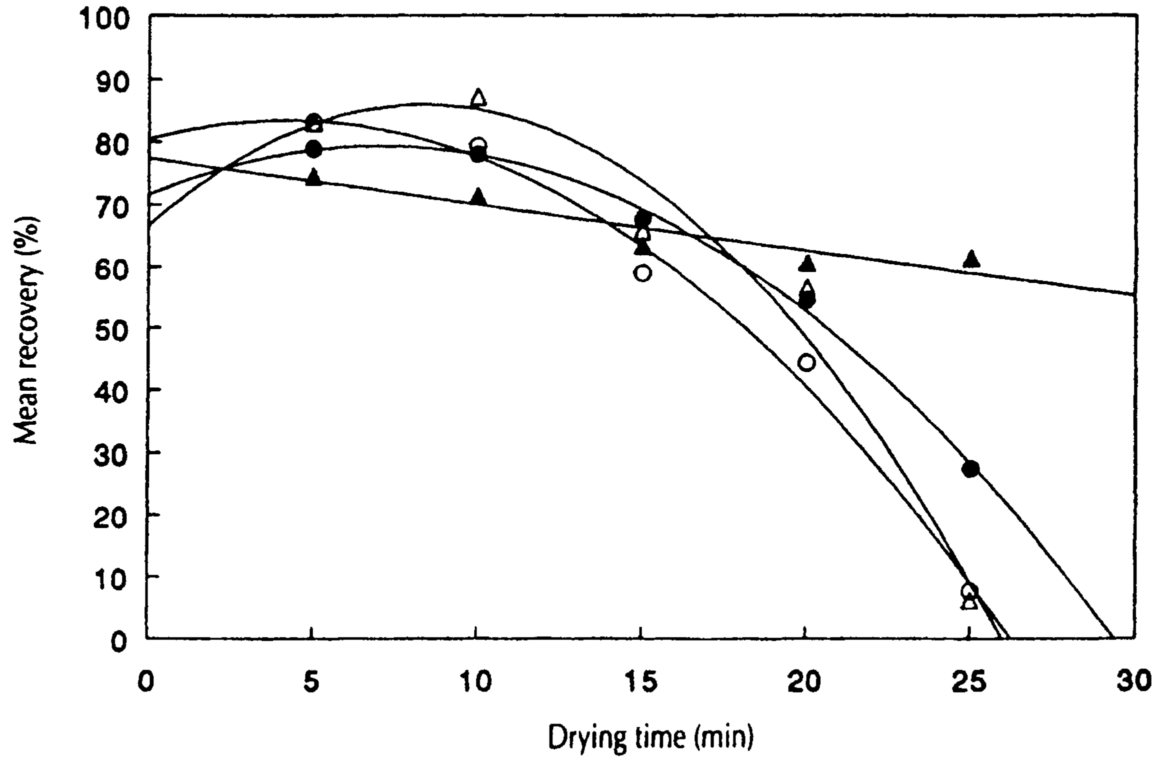

FIGURE 10. Effect of drying time on the loss of analytes (△ naphthalene, ○ acenaphthylene, ▲ anthracene, ● aldrin). From Reference 108. With permission.

ing PAHs, PCBs, phthalate esters, and OCPs.[114,115] Recoveries of three OCPs ranged from 77 to 99% and were on average ~20% higher than by direct SFE, described in the previous section.[101] Recoveries of the nonionic surfactant, APE, improved from 60% directly from H_2O to 100% after SPE/SFE.[102]

Organotins were recovered at ppb levels from synthetic seawater after retention on a C_{18} disk and an *in situ* Grignard reaction.[77] Limits of detection for the three analytes were 6 to 16 ng/L. Similarly, organotins were recovered from seawater, harbor H_2O, and river H_2O after derivatization with sodium tetraethylborate and enrichment on a C_{18} disk with high yields.[74]

Styrene-divinylbenzene (SDB) proved better than C_{18} for the extraction of phenols, averaging 83% recovery.[116] A C_8 disk followed by elution with 2% methanol in CO_2 was used for the recovery of sulfometuron methyl and chlorsulfuron (shown in Figure 1) at >90%.[117] Organic solvent use was reduced by 75% over liquid elution. Nitrotoluene explosives were concentrated on a phenyl sorbent and eluted with CO_2 or toluene/CO_2.[118] Finally, method development and evaluation of various SPE cartridges and disks for the recovery of PAHs and pesticides and phthalate esters[120] were previously reported.

Advantages of SPE/SFE are numerous. Although pretreatment of the disk or cartridge for sorbent activation cannot be avoided and generally consumes 10 to 20 ml organic/aqueous solvent, elution with CO_2 or modified CO_2 greatly reduces the subsequent solvent usage. By choosing a sorbent selective for the analytes of interest, a cleaner extract results. The need for a solvent concentration step is greatly reduced or eliminated as well. Finally, trace levels of pollutants can be concentrated from a large volume

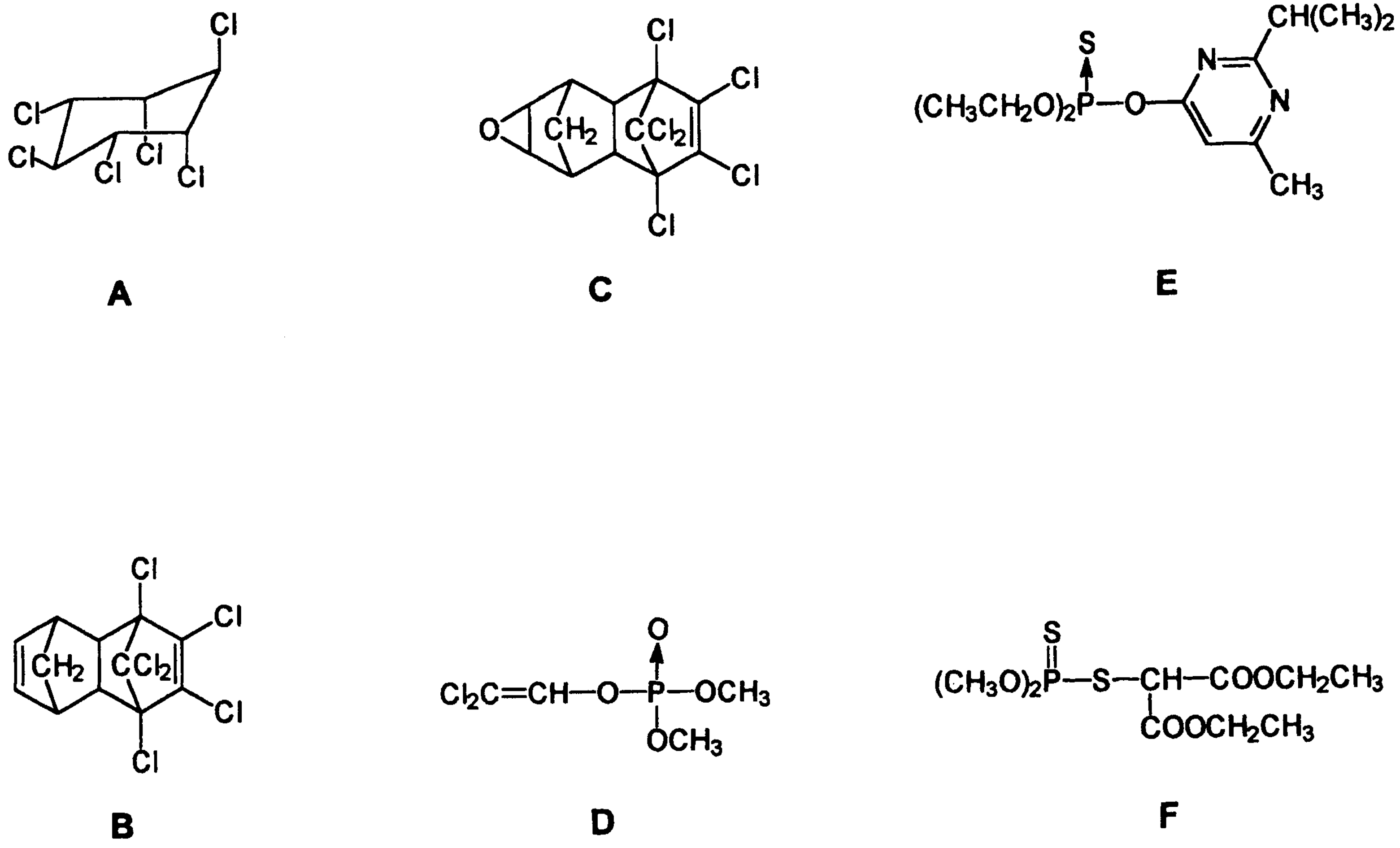

FIGURE 11. Structures of the OCPs (A) lindane, (B) aldrin, (C) dieldrin, and the OPPs (D) dichlorvos, (E) diazinon, and (F) malathion.

of H_2O to a small quantity of sorbent, reducing the volume of CO_2 needed and eliminating the problems associated with the direct SFE of H_2O. With the advent of more SPE phases, class-selective extractions from liquefied samples will become even more common.

D. Altering the Instrumentation/ Experiment

A high-pressure Soxhlet extractor typically utilizes liquid CO_2 as the extraction fluid instead of conventional liquid solvents. Conversely, microwave extraction and accelerated solvent extraction use smaller quantities (generally <30 ml) of traditional liquid-solid extraction solvents at elevated pressures and temperatures. In these techniques, reduced liquid solvent usage and shorter extraction times compared with conventional techniques are clearly the goals. Furthermore, standardized extraction conditions were developed, minimizing the need for a method development step for each sample that is currently required in SFE.

1. High Pressure Soxhlet Extraction

A high-pressure Soxhlet extractor was first described and utilized by Jennings[121] and later patented by J&W Scientific (Folsom, CA).[122] The extractor replaces conventional liquid solvents with substances such as CO_2, N_2O, and pentane that can be liquefied at temperatures of 0 to 20°C and pressures up to 102 atm. CO_2 is the most commonly used solvent. By inserting the vessel in a 40 to 45°C H_2O bath, CO_2 is vaporized until it hits an ice H_2O cold finger above the extraction thimble containing the sample, condenses, and drips into the thimble. The liquid CO_2 collects in the thimble and eventually spills over through the siphon tube to the boiling flask. At the conclusion of the extraction, the sample residue in the boiling flask is removed by syringe or by rinsing with a small quantity of solvent.

Examples shown include the extraction of volatile components from bananas and PAHs from coal fly ash.[121] Quantitative recoveries of morphine from blood, caffeine from kola nuts, and quinine from plants were obtained.[123] A variety of spices including clove, ginger, and sandalwood were extracted from plant materials in 2 to 2.5 h vs. 4 to 24 h by steam distillation with comparable yields.[124] More recently, OCPs and PCBs were recovered from certified potato, carrot, olive oil, butter, and lyophilized fish tissue samples,[125] but tributyltin was recovered from spiked sediment at only 21% yield.[73]

Although this technique was available in 1979, relatively few references are found in the literature. Organic solvent use is virtually eliminated compared with conventional Soxhlet extraction. However, the instrument cannot be operated at a variety of temperatures, thus excluding one of the most important variables in SFE.

2. Microwave Extraction

Microwave energy was explored as a means of rapidly extracting analytes without degrading or digesting the entire sample. The physics of microwave energy as well as general applications to analytical and environmental chemistry were recently reviewed.[126] In the microwave extraction of a solid matrix, the sample is saturated with a common organic or organic/aqueous solvent and irradiated for a short period of generally 0.5 to 10 min. After cooling, the supernatant is decanted and the matrix is rinsed several times. The combined extract is centrifuged, concentrated, or purified as necessary prior to analysis. Solvents lacking a dipole moment (i.e., hexane) do not efficiently absorb microwave energy. Therefore, at least 10% polar solvent is generally required. The absorption of energy by the solvent results in the disruption of weak hydrogen bonds, improved solvent penetration, and enhanced solvation.[127]

Environment Canada holds a patent on the microwave extraction technique and the trademark rights on a Microwave Assisted Process (MAP™).[128] This process uses a Kenmore microwave/convection oven and an energy magnetron generator. The patent describes the extraction of natural products such as garlic and mint using hexane, ethanol, or methylene chloride in several heating stages to avoid solvent boiling.

OCPs including endrin and dieldrin were extracted from spiked sediment using MAP™ with a 1:1 mixture of isooctane and acetonitrile.[129] Results were comparable to 6 h Soxhlet extractions. Other examples include the removal of crude fat from food products and OPPs (bromophos and parathion) from spiked soils using 30-s irradiation intervals.[130] Microwave extraction required 1/100 the time for Soxhlet extraction, and analyte degradation, sometimes a problem with Soxhlet, was avoided. The extraction of the lupin alkaloid sparteine and ^{14}C labeled RGH 2981 (see Figure 9), a peripheral blood flow enhancer, and its metabolite from seeds and rat feces was also studied.[127] Methanol/H_2O mixtures containing acetic acid or ammonia in varying proportions were tested. Results were comparable to or better than those by a shake flask (liquid-solid) technique.

A CEM Model MES 1000 microwave system (CEM, Matthews, NC), consisting of a magnetron tube and sample chamber or oven with a turntable holding up to 12 vessels, was also developed. Extraction vessels are polyetherimide, lined with perfluoroalkoxy or Teflon. The extraction solvent (~30 ml) was a 1:1 mixture of hexane and acetone. Extractions were typically performed on 5 to 10-g samples at 115°C for 10 min. PAHs, base/neutral compounds, phenols, and OCPs from marine sediments and soils,[131,132] PCBs from clay soil, topsoil, and sand,[133] and 187 compounds and 4 Arochlors from topsoil[134] were recovered at ≥70% yields. A comparison of the recoveries of phenols by microwave extraction, Soxhlet extraction, and sonication is given in Table 5.[132] Six phthalate esters were extracted from marine sediment and soil with recoveries of 70 to 91%,[135] OCPs were detected from marine sediment at yields greater than or equal to those by Soxhlet extraction,[136] and PAHs were recovered from certified marine sediments at >73% yields.[137] Imidazolinone herbicides were recovered from soil at 1 to 50 ppb levels using an ammonium acetate/ammonium hydroxide buffer at pH 10.[138] The average recovery was 92%.

In other studies, PAHs were extracted from highly contaminated soils using methylene chloride or hexane/acetone.[139,140] Yields were comparable to or better than 6-h Soxhlet extractions in methylene chloride and required 50% less solvent. Atrazines and principal degradation products were recovered from soils at >85% by first irradiating the soil sample in 25 ml H_2O followed by 0.35 *N* HCl in triplicate.[141] The fungal metabolite ergosterol and fatty acids were removed from a variety of agricultural products including mushrooms, corn, and grain dust using a methanol/NaOH mixture.[142] Fatty acids were simultaneously extracted and saponified by this process. PCBs and hexachlorocyclohexanes were efficiently removed from seal blubber and pork fat.[143] Because hexane was used as the solvent, a microwave transformer was utilized to transfer energy to the sample.

A Microdigest Model A301 microwave digester (Prolabo, France) was used for the leaching of organotin species from sediments using 0.5 *M* acetic acid in methanol.[144] Recoveries of MBT, DBT, and TBT from two reference materials were better than the certified values. When the reference materials were leached at a comparable temperature in the absence of a microwave field, ~50% of DBT and TBT and <10% of MBT were

TABLE 5
Performance of MAE, Soxhlet, and Sonication Extraction Procedures for Selected Phenols (ERA Soil Lot 330)[a]

		MAE		Soxhlet		Sonication	
	Certified level[b] (mg/kg)	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)	Average recovery (%)	RSD (%)
4-Chloro-3-methylphenol	5.14	90	1	77	5	74	13
2,4-Dichlorophenol	9.05	70	1	52	15	64	26
2-Methylphenol	12	50	1	33	15	49	18
3-Methylphenol	8.7	54	1	43	5	56	21
Pentachlorophenol	14.6	72	3	66	3	34	72
2,4,6-Trichlorophenol	6.49	90	2	41	47	67	27
2-Fluorophenol (surrogate)	5.0	60	4	50	23	62	15
Phenol-d_5 (surrogate)	5.0	69	1	66	21	73	13
2,4,6-Tribromophenol(surrogate)	5	102	1	92	20	151[c]	36

[a] These analyses were performed by GC-MS following the procedures given in Method 8250. The Soxhlet extraction was performed according to EPA Method 3540 and the sonication extraction was performed according to EPA Method 3550. The number of determinations was three.

[b] Reported by ERA.

[c] High recovery due to matrix interferant.

Adapted from reference 132. With permission found, validating the need for the microwave energy. Butyl- and phenyltins were also leached from certified sediments using 0.5 *M* ethanoic acid in methanol with recoveries exceeding the certified values.[145]

Recently, microwave energy was used for the direct extraction of H_2O samples. MAP™ was applied to the headspace analysis of volatiles such as benzene, toluene, ethylbenzene, chlorobenzene, xylenes, and dichlorobenzenes at ppb to ppm levels.[146] The vapor above the H_2O was sampled using a standard headspace sampler. PCBs were extracted from 500 ml H_2O containing 10 g NaCl and 50 ml isooctane at ~70°C.[147] Isooctane was chosen as a cosolvent due to its near immiscibility with H_2O and because it readily dissolves PCBs. The addition of NaCl caused "salting out" but also aided in uniform heat transfer within the vessel. The organic phase was then analyzed with recoveries of 68 to 85%. Finally, chlorinated benzenes at ppt levels were removed from 1 L H_2O with NaCl added.[148] After heating, helium was used to purge the analytes from the vessel into a small vial of hexane.

Advantages noted in the above references include shorter extraction times and minimal solvent usage relative to Soxhlet or sonication, the ability to extract multiple samples at once, portability and potential for use in the field, and trace analysis of even somewhat polar analytes in H_2O. A comparison of techniques is further summarized in Table 6.[140] Disadvantages include degradation of some thermally labile components and lack of selectivity in the extraction such that a clean-up step is frequently necessary prior to analysis.

3. Accelerated Solvent Extraction

Accelerated solvent extraction (ASE™) was introduced by Dionex Corporation (Sunnyvale, CA) in 1994. ASE™, shown schematically in Figure 12, uses the solvents traditionally used for liquid-solid extraction techniques at elevated pressures (102 to 136 atm) and temperatures (50 to 200°C) for solid and semisolid samples.[149–151] The technique capitalizes on improved analyte solubilities and kinetics of analyte desorption from the matrix at temperatures above the boiling points of the solvents. The sample in a stainless steel vessel is saturated with solvent and heated. The most frequently utilized conditions are a 1:1 mixture of hexane and acetone at 136 atm and 100°C. During a 5 to 10 min static extraction period, the heated, expanding solvent is vented to a collection vial. Fresh solvent is introduced at the end of the static step prior to a compressed nitrogen purge to flush the extraction cell. A 10-g sample requires ~12 min and ~15 ml solvent for extraction.

Extracted analytes include OPPs and phenoxyacids from clay, loam, and sand,[149] PAHs from marine sediment[151] and contaminated soil,[152] 56 basic, neutral, and acidic compounds from soils,[150] and PCBs from sewage sludge and oyster tissue.[151] Recoveries in all cases were comparable to or better than the certified values of those obtained by conventional methods, and no matrix dependence in the overall extraction yields was observed.

The same methodology was applied to the extraction of 16 PAHs from lignite and bituminous coal fly ashes using standard SFE equipment.[43] Extractions with methylene chloride at 136 atm and 150°C yielded >70% recoveries of midmolecular weight PAHs. High molecular weight PAHs were recovered at >62% and low molecular weight PAHs were recovered at yields less than or equal to Soxhlet extraction.

Advantages of ASE™ over conventional techniques include avoidance of localized heating and numerous washes with sonication, and greatly decreased time and solvent consumption over Soxhlet and wrist shake. A potential disadvantage is that the extractions tend to be exhaustive, leading to non-

TABLE 6
Additional Factors for Consideration for the Extraction of PAHs from Contaminated Soils

	Soxhlet extraction	Microwave-assisted extraction	Supercritical fluid extraction
Sample mass	10.0 g	2.0 g	1.0 g
Extraction time	6 h	20 min (plus 30 min for cooling and pressure reduction)	1 h (plus 10-15 min for temperature-pressure equilibration)
Sequential or simultaneous operation	Five separate assemblies used	Up to 12 vessels can be used simultaneously	Sequential in the system used
Equipment cost	Low (<$1000)	High (~$15,000-20,000)	Highest ($25,000- 40,000)
Operator skill	Low	Medium	High
Organic solvent volume used for extraction	100 ml of CH_2Cl_2	40 ml (acetone-CH_2Cl_2)	CO_2, 12 ml MeOH and 5 ml CH_3Cl for collection

Adapted from Reference 140. With permission.

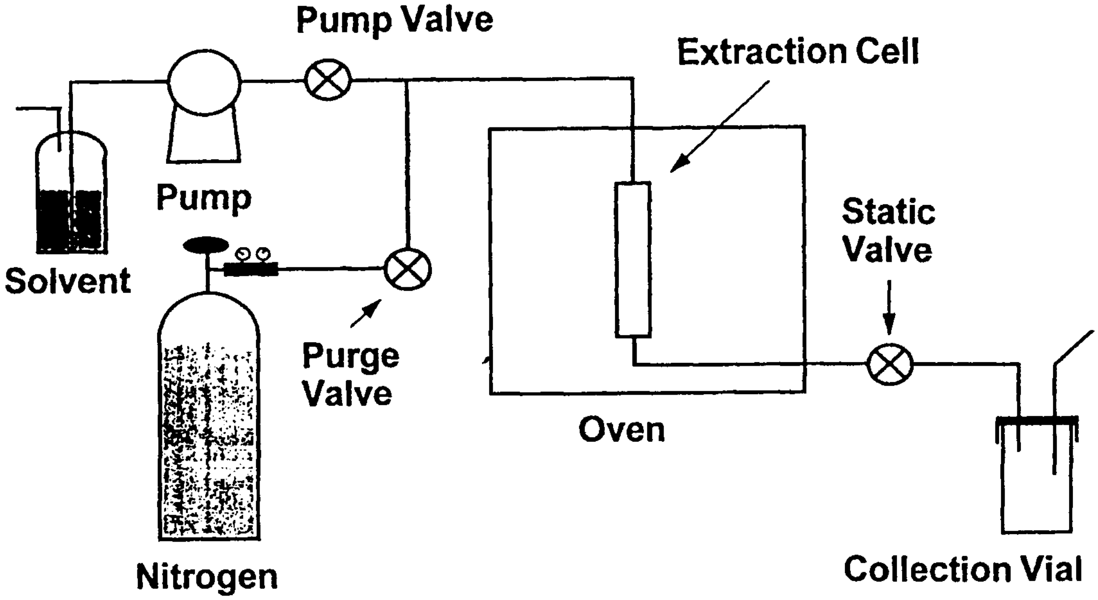

FIGURE 12. Schematic of accelerated solvent extraction (ASE) system. From Reference 151. With permission.

selective extracts requiring additional cleanup.

III. CONCLUSIONS

Supercritical fluid extraction and traditional liquid extraction methods represent opposite ends of the spectrum, with advantages and shortcomings recognized for both techniques. Neither is ideal for all analytes from all matrices. The alternative approaches that developed predominantly over the past 5 years to bridge the gap between SFE and liquid extraction were discussed in this review. Some of the most promising alternatives appear to be the use of subcritical H_2O at a variety of temperatures for polarity-selective extractions, enhanced-fluidity liquids for intermediate strength extraction of polar analytes, microwave extraction, and accelerated solvent extraction for aggressive extraction of polar analytes. In addition, *in situ* complexation or chelation is useful for the extraction of organometallics and metal ions that are not ordinarily soluble in CO_2, and SPE followed by SFE is viable for aqueous samples. All of the methods described focus on reduced solvent usage and shorter extraction times. Some methods, such as *in situ* chelation and high-temperature SFE, are potentially analyte selective, whereas others, including enhanced-fluidity liquid extraction, microwave extraction, and ASE may require further cleanup prior to analysis. Because a single technique that is universal for all samples is still not available, alternatives will continue to develop to meet the needs for sensitive, precise, and environmentally sound extraction methods.

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