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Review

Supercritical fluid extraction in environmental analysis

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Abstract

This review focuses on recent attempts to understand the SFE process and on applications of that understanding to increase SFE recoveries of pollutants from environmental solids. Methods to decrease restrictor plugging and to increase the collection efficiencies of extracted analytes are evaluated. Approaches to obtain quantitative extractions include fluid choice, extraction flow-rate, modifiers, pressure and temperature are presented as is the potential for SFE to extract ionic organics and metals. Finally, the need for a better definition of "quantitative" extraction and the use of SFE in certification exercises for complex environmental samples is discussed.

Contents

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1. Introduction

Through the last twenty years detection methods in analytical chemistry have developed into powerful techniques that can separate, identify and quantify a large number of sample compounds in a time span of hours or even minutes. It is, therefore, striking that analysts often rely on sample preparation methods that have changed very little since the turn of the century, as is the case with Soxhlet extraction. These techniques often require several hours or even days to perform and use large amounts of pesticide-grade organic solvents that result in dilute extracts which not only have to be concentrated, but also often need a thorough clean-up before analysis. In contrast, an ideal sample preparation method should be rapid, simple, and inexpensive to perform yet yield quantitative recovery of target analytes without loss or degradation. It should also generate an extract ready for analysis without additional concentration or class fractionation while producing little or no laboratory waste.

In the last five years, it has become increasingly clear that supercritical fluid extraction (SFE) has the potential to meet some or most of the above-mentioned requirements [1–6]. The time requirements and the use of large amounts of very pure, expensive, and often toxic solvents in connection with the conventional methods have led to the increased interest in developing SFE. Since 1982, more than 700 reports have been produced on this method. Of these ~170 were published before 1990 and more than 500 between 1990 and 1994. Publications before 1990 primarily reported processing (rather than analytical) applications, mainly in the food industry or samples with high amounts of target compounds [2,7,8]. Analytical applications of SFE began in the late 1980s, and from 1990 to 1992 most reports were on spiked samples (i.e., a sample fortified with the analytes of interest) and/or highly contaminated samples [9–14]. Only in the last two to three years have reports on samples containing low (trace) amounts of native contaminants started to emerge [15–19]. Since 1990, the analytical uses of SFE for environmental analysis has matured into a competitive technique with the main focus on durable methods for fast routine extraction from complicated real matrices. This development has resolved some of the discrepancies between SFE and quantitative determinations using conventional liquid solvent extraction methods. These techniques now show a much higher consensus than before and SFE has even been found to successfully participate in certification procedures on environmental matrices on equal terms with conventional extraction methods. In fact, with appropriate conditions (e.g., the use of modifiers and elevated temperatures) SFE sometimes leads to higher recoveries than obtained using traditional extraction procedures. This development has prompted the need for a definition of quantitative recovery.

Further evidence of the growth of analytical SFE is the rapid advances in instrumentation. Even as late as the end of the 1980s, nearly all analytical SFE reports utilized "home-built" equipment since virtually no suitable instrumentation was available. In contrast, at least three manufacturers now offer fully automated SFE systems. Few, if any, analytical instruments have seen such rapid development.

This paper will not try to review all publications on environmental SFE in the last few years, as this has already been done by others [4,20–22]. Instead, it will focus on the important phenomena involved in SFE and try to illustrate these phenomena using representative contributions from the most current literature.

2. Short introduction to the SFE experiment

The SFE experiment can be divided into three sequential steps, i.e., initial partitioning of the analyte from the matrix active sites into the supercritical fluid, elution of the analyte from the extraction cell, and collection of the analyte in the SFE trapping system [23]. Each of these steps must be quantitatively efficient for SFE to yield quantitative recoveries. For example, new workers in SFE frequently misinterpret low recoveries as poor extraction (steps 1 and 2),

when the true cause of poor recoveries could be poor collection efficiencies.

The first step in the SFE process, the initial partitioning of the analytes from the matrix active sites into the supercritical fluid, is frequently the step that limits the rate (and ultimate recovery) of SFE for heterogeneous environmental matrices. While this initial desorption process is poorly understood, it has been shown to depend on factors such as diffusion of the analyte in or between matrix active sites and the ability of the supercritical fluid to initially displace the analyte from active sites [24–26]. For many environmental samples, the rate of this initial desorption process can control the overall SFE rate. The second step, chromatographic elution, depends on the amount of fluid flow versus sample size and conventional chromatographic partitioning between the extraction fluid "mobile" phase and the matrix (which, of course, depends on analyte solubility) [27]. Finally, the third step, collection, is the most instrument-specific step, and depends heavily on the restrictor system and trapping systems used.

While it is not the purpose of this report to present a theoretical framework for SFE, proper development of quantitative SFE methods is greatly helped by an understanding of these three steps, and comments related to each step will be made throughout the manuscript. It should also be noted that the theoretical framework for SFE of environmental samples is presently being developed to include both kinetic and chromatographic processes, and the interested reader is referred to Refs. [2,24–28] for related theoretical discussions.

3. Extraction

3.1. Fluid choices in SFE

In practice, more than 90% of all analytical SFE is performed with carbon dioxide (CO_2) for several practical reasons. Apart from having relatively low critical pressure (74 bar) and temperature (32°C), CO_2 is non-poisonous, not flammable or explosive, chemically relatively inactive, available in high purity at relatively low cost, is easily removed from the extract, and creates no environmental problems when used for analytical purposes. In the supercritical state, CO_2 has a polarity comparable to liquid pentane and is, therefore, best suited for lipophilic compounds. The main drawback of CO_2 is its lack of polarity for the extraction of polar analytes.

The second most common choice of extraction fluid for analytical SFE has been N_2O. This fluid was considered better suited for polar compounds because of its permanent dipole moment [3,29]. One of the applications where N_2O has shown significant improvements when compared to CO_2 is for the extraction of polychlorinated dibenzodioxins from fly ash [30,31]. Unfortunately, this fluid has been shown to cause violent explosions when used for samples having high organic content and should, therefore, be used only when absolutely necessary [32].

Other more exotic supercritical fluids which have been used for environmental SFE are SF_6 and Freons. SF_6 is a very apolar molecule (although easy polarizable) and as a supercritical fluid, it has been shown to selectively extract aliphatic hydrocarbons up to around C-24 from a mixture containing both aliphatic and aromatic hydrocarbons [33]. Freons, especially $CHClF_2$ (Freon-22), has on several occasions been shown to increase the extraction efficiency compared to extraction with CO_2. Hawthorne et al. [29] showed that $CHClF_2$ extracted PCBs from a river sediment with higher efficiency than pure CO_2 or CO_2 modified with methanol (MeOH). For PAHs from a petroleum waste sludge and a railroad bed soil, $CHClF_2$ gave dramatically improved recoveries when compared to CO_2 and N_2O. In contrast, Klink et al. [34] did not experience any significant difference in the extraction efficiency between CO_2 and $CHClF_2$ for the extraction of fatty acids and sterols from plant tissues and sediment. This may however, be because $CHClF_2$ was used at subcritical conditions in these experiments.

Although supercritical H_2O has often been used for the destruction of hazardous organics [35], the high temperature and pressure needed ($T > 374$°C and $P > 221$ bar) together with the

corrosive nature of H_2O at these conditions, has limited the possible practical applications in environmental analysis. H_2O at subcritical conditions has, however, been shown to be an effective fluid for the extraction of several classes of environmental pollutants [36]. Hawthorne et al. [36] has shown subcritical H_2O (250°C and 350 bar) capable of effectively extracting PAHs from two certified matrices (a soil and urban air particulates) in 15 min. They also showed that more polar compounds (e.g., chlorinated phenols) can be quantitatively extracted from spiked samples at lower temperatures. Since H_2O is an environmentally acceptable solvent that possesses a large polarity range (mainly dependent on the temperature rather than pressure), this fluid may have a bright future in environmental sample preparation of thermally stable compounds.

3.2. Selection and use of modifiers

Since CO_2 is relatively non polar, a few percent of a polar modifier is often added to enhance the solubility of more polar compounds or to increase the ability of the supercritical fluid to better displace analytes from matrix active sites. These modifiers are usually organic solvents that are added to the extraction fluid. They can be added using three different procedures [37,38]:

1) Direct addition of modifier to the sample. This approach is economical and easy to perform, but has the drawback in that a static (non-flowing) extraction step must be performed to allow the modifier to contact the sample on pressurization, since in the following dynamic (flowing) step the amount of modifier in the cell will decrease with the sweeping of the extraction cell. However, this approach is by far the simplest way to evaluate several modifiers in the least time, and has been demonstrated to yield quantitative and reproducible recoveries [38–40].

2) Premixed fluids prepared by addition of the modifier to the liquid CO_2 in the storage cylinder. In this technique the percentage of modifier has been shown to increase as much as a factor of two during the use of the cylinder [41,42]. While changes in modifier concentration could lead to possible changes in extraction efficiencies, this does not appear to be a significant problem since investigators that have used premixed modifiers report reproducibilities normally expected for analytical SFE [11,18,19,39,43].

3) The most accurate and reproducible method of modifier addition is using a separate modifier pump, provided that the pumping system compensates for the compressability (density) of the CO_2 so that a constant concentration of modifier results [37,44]. A modifier pump also gives the potential to change modifier concentrations during an extraction (which cannot be done with the above techniques).

As of yet, there is no clear understanding of the specific mechanism that a modifier acts through, apart from the enhancement in solubility when a higher amount of modifier is used [45]. For example, the use of MeOH (3.5%) in CO_2 has been reported to enhance the solubility of 2-aminobenzoic acid by up to 620% [46]. In the extraction of linear alkylbenzene sulfonate (LAS), very low recoveries were achieved using pure CO_2 and N_2O, but on adding MeOH to the CO_2 the recoveries became quantitative [21] because of the increased solubility of the LAS. However, for many environmental analytes, a low concentration of modifier (e.g., 1 vol%) is as effective as a higher concentration (e.g., 10 vol%), indicating that the modifier acts by competing with the target analytes for the active sites on the sample matrix rather than increasing analyte solubility [38]. In the work of Langenfeld et al. [38], the effects of eight different modifiers and one modifier mixture were tested at two different concentrations (1 and 10 vol%) on two certified reference materials. The results obtained showed that the modifier identity generally had a larger impact on the extraction efficiency than did the concentration of the modifier.

A variety of different modifiers has been tried over the years, but MeOH remains the most popular of those tried, despite the fact that several recent studies have shown that other organic modifiers are more effective for samples, including PAHs from sediment, soot, and air

particulate matter [38,47], nitro-PAHs from diesel soot [48], and chlorinated dioxins from fly ash [31]. In a similar study, SFE of primary aromatic amines from soil using different aliphatic amines as modifiers in N_2O was studied by Oostdyk et al. [49]. They came to the conclusion that the extraction efficiency was independent of the modifier concentration but strongly dependent on the size of the individual aliphatic amines used as modifiers. This supports the theory of modifier interaction with active sites on the matrix. The results of these modifier survey studies clearly demonstrate that modifier selection should be based on a survey of modifiers having different types of polarities.

It should be kept in mind that modifiers have the potential to reduce the trapping capacities of the extraction system (especially sorbent traps) in comparison with pure extraction fluids [11,43,50]. Therefore, a trapping efficiency study should be performed whenever a modifier is added [see Section 4.2]. Also, modifiers generally tend to produce extracts with a higher content of matrix compounds and interferences [11,43,44,50], which may require additional extract clean-up prior to analysis. It is also necessary to avoid using modifiers that interfere with the analysis step when solvent trapping is used, e.g., modifiers that cause interference with detectors, such as chlorinated solvents for GC–ECD and aromatic solvents for HPLC-UV. If these precautions are followed, modifiers provide a powerful tool in SFE.

3.3. Effect of water on SFE

Most environmental samples contain at least some water, which may either help or hinder the extraction process. Water can affect the mechanical performance of SFE by causing restrictor plugging (especially when water content exceeds a few percent), and also can affect the extraction process itself. The most obvious approach to avoiding restrictor plugging is to dry the sample, but both oven and room temperature drying can lead to substantial losses of even relatively non-volatile analytes as has been demonstrated by Burford et al. for a petroleum waste sludge [51]. Oven drying at 105°C caused losses of species as non-volatile as *n*-octadecane (b.p. = 316°C), and even drying at room temperature caused substantial losses of *n*-tetradecane and more volatile species (Table 1). An alternative to drying samples is to mix the sample with a drying agent,

Table 1
Losses of volatiles and semi-volatile organics from a wet petroleum waste sludge using different drying methods

Analyte	Analyte concentration (μg/g)	% Lost (RSD, %)[a]		
		Sample oven dried for 1 h at 105°C	Sample air dried for 18 h	Mixed with drying agent[b]
n-Octane (C_8)	1499 ± 275	>99	>99	97 (49)
n-Decane (C_{10})	1096 ± 231	>99	>99	51 (11)
n-Dodecane (C_{12})	1880 ± 257	>99	83 (3)	25 (11)
n-Tetradecane (C_{14})	1618 ± 132	>99	49 (9)	30 (7)
n-Hexadecane (C_{16})	536 ± 35	>99	8 (10)	17 (7)
n-Octadecane (C_{18})	138 ± 6	63 (2)	10 (9)	15 (4)
n-Pentacosane (C_{25})	31 ± 2	0 (6)	7 (2)	10 (2)
Phenol	3066 ± 573	72 (12)	66 (1)	19 (6)
m-,*p*-Cresol	5242 ± 743	61 (11)	37 (1)	19 (4)
C_2-alkylphenol	1321 ± 183	35 (11)	0 (9)	11 (2)

[a] Adapted from Ref. [51].
[b] The sample which contained 30% water was mixed 1:1 with anhydrous magnesium sulfate.

and a recent survey on the characteristics of 21 different drying agents has been reported [51]. However, this procedure can also lead to substantial losses of volatile and semi-volatile analytes since mixing a wet sample with drying agents can cause substantial heating of the sample. For example, mixing the petroleum waste sludge with magnesium sulfate caused significant losses of tetradecane and more volatile species (Table 1). Burford et al. concluded from these results that drying agents should be placed at the outlet end of the cell (rather than mixed with the sample) to obtain the highest recoveries of volatile species while avoiding restrictor plugging. An additional problem with drying agents is the potential to selectively retain some analytes. For example, Burford et al. reported that several different drying agents retained phenols and anilines if the sample was dry, however none of the drying agents retained these species if the sample was wet [51].

The effect of water on the actual extraction step (i.e., the partitioning of the analytes from the matrix to the supercritical fluid) is not well understood. The presence of water has been reported to both increase and decrease recoveries, depending on the system studied. For example, Hawthorne et al. reported substantial increases in SFE recoveries for PAHs from a waste sludge when the sample was dried from 45 to 2 wt% water [29]. Similar results were found by Snyder et al., who reported lower recoveries of organophosphate and organochlorine pesticides from soil when the water content was increased [52]. In addition, lower recoveries were obtained when completely dry samples were used compared to samples containing 5% water. Lee and Peart reported lower recoveries of PCBs and chlorinated benzenes (ca. 75%) from completely dry sediments, but saw no reduction in recoveries and good agreement with Soxhlet extraction when the water content was increased from 11 to 50 wt% [53]. Although the role of water in SFE is unclear, it appears that having a small amount of water present (e.g., 1 or 2%) is advantageous, possibly by keeping clay plates hydrated [54] or by covering adsorption sites. However, additional amounts of water may either increase or decrease extraction efficiencies by unknown mechanisms. Clearly, the role of water in SFE needs further study.

3.4. Temperature and pressure effects in SFE

Pressure and temperature are the two most important physical parameters in SFE and they have both theoretical and practical implications for the extraction process. Together they define the density of the supercritical fluid. The maximum extraction fluid density is achieved with high pressures at temperatures close to the critical temperature of the fluid. The common belief among analytical chemists is that the maximum solubility of a solute in the supercritical fluid is achieved at the maximum fluid density. This statement however, is not necessarily true. It is more correct to say that the maximum solubility is achieved at the highest density at a given temperature [24]. It is also important to point out that the solubility of a substance in a supercritical fluid is affected by two factors, the volatility of the substance and the solvating effect (related to density) of the supercritical fluid [24]. This means that raising the fluid temperature can greatly increase the solubility for compounds with significant vapor pressures. For example, the solubility of anthracene increases by only a factor of 5 when increasing the CO_2 pressure from 150 to 400 bar (at a constant 50°C) as shown in Fig. 1. In contrast, raising the temperature from 50 to 200°C at 150 bar increases anthracene solubility by a factor of 23, and at 400 bar by a factor of 48 [55]. Note that the large increase in solubility with higher temperature occurs despite a ca. 50% drop in CO_2 density when the temperature is raised from 50 to 200°C. Although little solubility data in CO_2 is available for elevated temperatures [4], it appears that increasing temperature (at constant pressure) should generally increase solubility for analytes with sufficient vapor pressure to be analyzed by GC. However, the solubility of organics not having significant vapor pressure is likely to drop with elevated temperature because of the drop in CO_2 density [4].

Recent studies have demonstrated dramatic

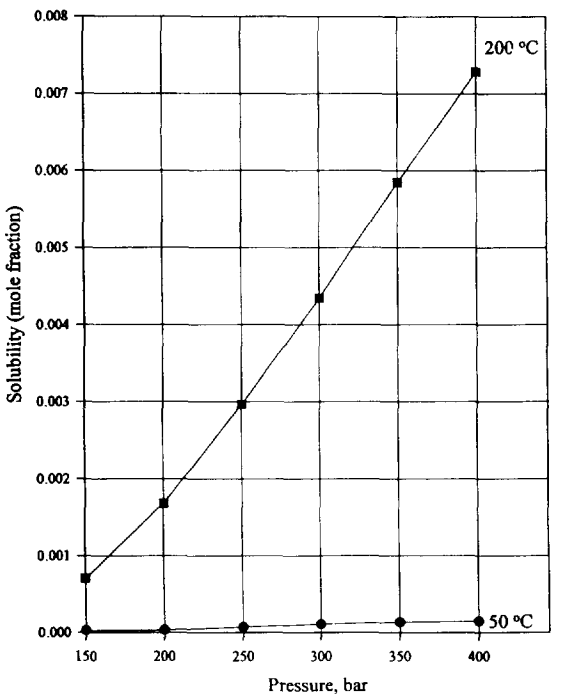

Fig. 1. Effect of temperature and pressure on the solubility of anthracene in supercritical CO_2. Note that increasing temperature yields much higher solubility than increasing pressure. Adapted from Ref. [55].

increases in SFE recoveries with pure CO_2 by raising extraction temperature. Langenfeld et al. [6] studied the temperature and pressure effects using pure CO_2 on PCBs and PAHs from three certified reference materials. They found that at 50°C, raising the extraction pressure (355–659 bar) had no effect on extraction efficiencies from any of the samples. While high recoveries were obtained in 40 min from a highly contaminated soil regardless of temperature, PCBs from a sediment and PAHs from air particulates were efficiently extracted only if the temperature was raised to 200°C. At 200°C, PCBs were effectively extracted at any pressure (152–659 bar), while both higher temperature and pressure increased the recovery of PAHs from air particulates. A later study on the extraction of chlorophenols, S- and N-heterocycles, and pesticides (atrazine and prometon) from soil, and PAHs from soil and soot demonstrated that 405 bar of pure CO_2 at 50°C usually yielded much lower recoveries than Soxhlet extraction (as low as a few percent), while 200°C SFE at 405 bar generally gave good agreement after 30 minutes with 18-hour Soxhlet extractions [56]. However, further increases in temperature to 350°C did not generally increase recoveries and was suspected of causing degradation of some of the analytes [56]. An example of the effect of temperature on the extraction of PCBs is illustrated in Fig. 2 [57]. Note that while the extraction appears to be nearly complete after 40 minutes at 50°C, increasing the SFE temperature to 200°C significantly increases the amount of the PCB extracted from the same sample. The authors [56,57] concluded from these results that temperature is more important than pressure for achieving high extraction efficiencies when the interactions between pollutant molecules and sample matrices are strong. It was argued that increasing SFE temperature could be a useful alternative to adding organic modifiers for achieving high extraction efficiencies from environmental samples.

The use of high temperature SFE can be limited by available instrumentation since the majority of commercial SFE instruments can only operate at ≤ 150°C. However, such temperatures have also been shown to enhance recoveries. For example, Robertson et al. [58] reported ca. 2-fold increases in the recovery of triazine pesticides by raising temperatures from

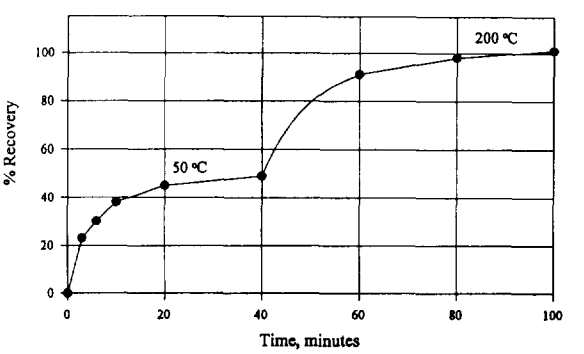

Fig. 2. SFE extraction rate of PCB 52 from a certified river sediment (SRM 1939) using pure CO_2 at 641 bar. Sequential extractions of the same sample were performed first for 40 min at 50°C, and then at 200°C for an additional 60 min. 100% recovery is based on Soxhlet extraction values certified by NIST.

Table 2
Temperature effect on the extraction of PCBs from a certified reference material (SRM 1939)

PCB No.	Certified value (ng/g)	% Recovery (%RSD)[a]			
		40°C (30 min)	60°C (30 min)	80°C (30 min)	97°C (30 min)
28	2210	42 (1.3)	54 (4.4)	65 (4.1)	72 (5.9)
52	4480[b]	49 (2.9)	58 (3.7)	65 (4.9)	67 (4.6)
128	100[b]	51 (3.3)	57 (1.5)	62 (7.3)	69 (3.6)
180	160[b]	69 (2.4)	78 (4.9)	85 (3.5)	87 (4.6)

[a] Triplicate extractions at constant density (0.75 g/ml) with pure CO_2.
[b] Indicative values

80 to 140°C. As shown in Table 2, a more moderate increase from 40 to 97°C yielded ca. 30% higher recoveries of PCBs from a certified river sediment. Similarly, increasing temperature from 65 to 150°C (at 400 bar) increased the recovery of heavy hydrocarbons (No. 5 fuel oil) by ca. 50% [59].

Although increasing SFE temperature and adding modifiers have both been shown to increase SFE recoveries from difficult samples, the combined effect of these two approaches has received little attention. However, Yang et al. [47] recently studied the combined effect of temperature and modifier on SFE of PAHs from different matrices. Raising the extraction temperature from 80 to 200°C increased SFE recoveries of PAHs from marine sediment, air particulates, and diesel soot using either pure CO_2 or CO_2 modified with either MeOH, toluene, or diethylamine for all of the samples, demonstrating that the effect of temperature is independent of the sample matrix. In contrast, the effect of the modifiers was highly dependent on the matrix at both temperatures. MeOH modifier yielded little, if any, increase in recoveries over pure CO_2. Toluene was effective on only two samples (sediment and air particulates), while diethylamine was effective on all three samples. For the effective modifiers, the combination of modifier and 200°C yielded the highest recoveries, demonstrating that the modifier and temperature effects are additive. In general, diethylamine as modifier at 200°C gave the highest PAH recoveries, and yielded good agreement with Soxhlet extractions. The results suggest that extraction using CO_2 with modifiers at high temperature could be the most exhaustive SFE method for the extraction of particularly difficult samples.

At present, it is not clear why increasing extraction temperatures often yield such dramatic increases in extraction efficiencies, although at least two major effects may be responsible. First, increasing temperature would be expected to increase the kinetics of the desorption process [6,27,56,60], and, therefore, increase the overall extraction rate of analytes that are limited by their slow rate of initial desorption (e.g., the PCB molecules that do not extract until the temperature is raised as shown in Fig. 2). Second, as discussed above, increasing temperature can greatly increase the solubility of analytes that have significant vapor pressures (e.g., "GC-able" compounds), despite the decrease in density that occurs with increasing temperature at constant pressure. While further investigations are needed to determine the relative importance of these mechanisms, it is clear that increasing SFE temperature is a powerful approach to increasing SFE efficiencies for many environmental analytes.

3.5. Flow-rate and sample size considerations in SFE

Having selected the supercritical fluid, the pressure and temperature conditions, and (if needed) a suitable modifier, the focus can turn to considerations about the flow-rate, sample size, and whether to use a pure dynamic (flowing)

extraction or a combination of static (stopped flow) and dynamic extraction.

The amount of sample needed for SFE is mainly determined by the detection limit of the analytical method, the size of available extraction cells, and the size of a statistically representative sample. If the sample amount chosen does not fill the extraction cell size, the remaining volume should be filled with an inert material (e.g., rinsed sand or Na_2SO_4), especially if the CO_2 flow is from bottom to top, or if the cell is horizontal [18,23]. Since smaller cells are less expensive, simpler to use, and are more reliable, SFE samples are typically ≤ 10 grams, although larger cells are available from some suppliers.

Flow parameters often determine the success or failure of SFE, and can also be varied to provide information on the dynamics of the extraction process. It is clear that if the flow of supercritical fluid is insufficient to sweep the cell void volume, the effectiveness of the extraction is reduced. A minimum of 4–5 cell volumes should be used in order to ensure that the cell void volume is sufficiently swept. However, sweeping the sample volume several times does not guarantee good extraction efficiencies because the kinetics of the extraction process (rather than the elution of the analytes) often limits the overall extraction rate [23,24,27,60]. In fact, changing the extraction flow-rate is a simple way to determine whether the extraction is limited by chromatographic retention (e.g., the equilibrium process controlled by the adsorption of analytes to accessible active matrix sites and by analyte solubility) or limited by the kinetics of the initial desorption process [23]. If changing the extraction flow-rate changes the extraction rate proportionally, then the extraction is primarily limited by chromatographic retention (including solubility). In such cases, increasing extraction flow-rate and/or changing extraction conditions to increase analyte solubility will increase the extraction rate and efficiencies. This is demonstrated in Fig. 3 (top) for the extraction of the PAH benzo[a]pyrene from a highly contaminated soil. Note that the extraction rate is proportional to the SFE flow-rate. For such samples, a faster extraction rate will be achieved by using smaller samples, and static extraction will not be as effective as dynamic extraction, since the sample will be exposed to a smaller volume of extraction fluid.

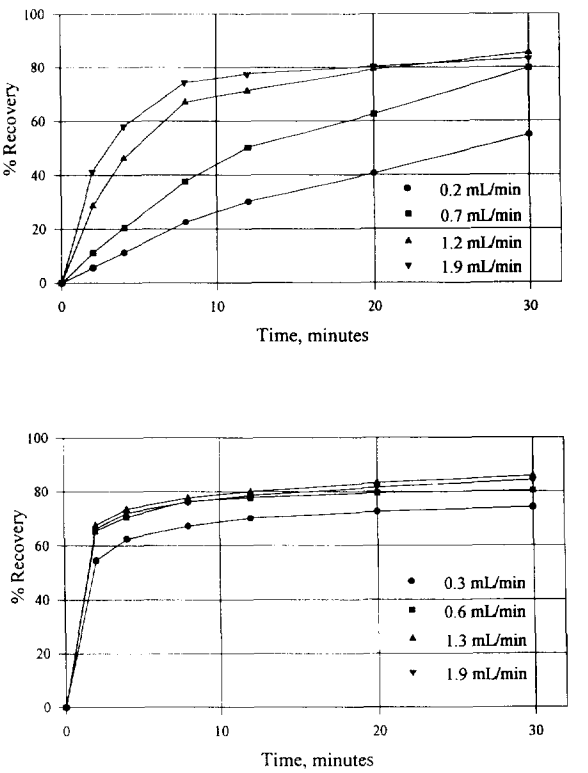

Fig. 3. Effect of SFE flow-rate on PAH extraction rates from two soils (400 Bar, 60°C). Top: Extraction of benzo[*a*]pyrene from a highly contaminated soil (ca. 100 to 1500 μg/g of individual PAHs, benzo[*a*]pyrene concentration of ca. 100 μg/g). Note that the extraction rate is proportional to the SFE flow-rate and, therefore, mainly limited by the chromatographic retention (including solubility). Bottom: Extraction of benzo[*a*]pyrene from a soil contaminated at lower levels (ca. 5 to 50 μg/g, benzo[*a*]pyrene concentration of ca. 45 μg/g). In this sample, the extraction rate is not affected by the SFE flow-rate, but is primarily dependent on the kinetics of the desorption process.

Very different behavior is seen when the same PAH is extracted from a soil contaminated at much lower levels (allowing better interaction of the PAHs with matrix active sites). For this sample, the extraction rate is not dependent on the SFE flow-rate (Fig. 3, bottom), demonstrating that the kinetics of the desorption process controls the SFE rate for benzo[a]pyrene from

this sample, and the extraction rate is not controlled by chromatographic retention (including solubility). For such a sample, a large sample will be extracted at a similar rate as a smaller sample (assuming that the void volume is swept sufficiently), and a static extraction step will be nearly as effective as a dynamic extraction step performed for the same time (assuming that sufficient dynamic extraction is performed after the static step to elute extracted analytes)[23,61].

3.6. Derivatization and extraction of polar compounds

Derivatization of organic and inorganic compounds has frequently been used in order to make polar, non-volatile or thermally labile compounds amenable to GC analysis. Since derivatization schemes generally make analytes less polar, the same concept has been used to derivatize analytes in situ in order to make them easier to extract by SFE. As discussed previously, most fluids used for SFE lack the ability to extract polar compounds. The fluids employed are, however, relatively inert and the rapid diffusion characteristics in SFE facilitate in situ derivatization. This procedure increases the number of polar analytes that can be extracted using SFE, as well as providing extracts that can be analyzed by GC. Derivatizations have been performed in two basic modes. Most simply, the reagent is added directly to the sample, a static extraction is performed (to allow the derivatization to occur under SFE conditions), and then a dynamic SFE step is used to elute the derivatized analytes. Alternatively, reagents have been added to the CO_2 by flowing the CO_2 through a cell containing the reagent prior to the sample cell. In this second mode, a constant supply of reagent can be supplied during dynamic SFE.

Hawthorne et al. [62] and later Lopez-Avila et al. [63] have shown the potential for the use of in situ chemical derivatization in SFE of chlorinated phenoxy acid herbicides from soils and sediments. Hawthorne et al. used trimethylphenylammonium hydroxide (TMPA) to achieve a $>90\%$ recovery from sediments and soil in 90 min at 80°C. They also used TMPA for the derivatization of phenols from water and fatty acids from E. Coli yielding good recoveries in 15 to 30 min. Lopez-Avila et al. applied pentafluorobenzyl bromide (PFBBr) and triethylamine (TEA) for this derivatization using a 60 min. static extraction followed by 30 min. dynamic extraction (400 atm and 100°C) to achieve varied recoveries of 13 chlorophenoxy acid herbicides. Lee et al. [64] have shown the in situ derivatization of eight chlorinated phenols from sediments using acetic anhydride in the presence of triethylamine. From sediments spiked at 50–500 ng/g, the authors obtained 84–100% recovery of the different chlorinated phenols when extracted with CO_2 at 80°C and 370 bar using 10 min static and 5 min dynamic extraction with a flow-rate of 2 ml/min. The effect of temperature was also studied and it was concluded that 110°C gave the best overall recovery. Using sequential extractions it was discovered that these conditions only yielded $\sim 80\%$ recovery when applied to real sediments with native analytes. Nevertheless, SFE achieved as high (or higher) recovery as Soxhlet extraction and steam distillation with relative standard deviation of 5–10%. Field et al. [65] used tetraalkyl ammonium salts as ion-pairing reagents to extract linear alkylbenzene sulfonates (LAS) and linear alkylsulfonates (SAS) from sewage sludge. The extracts were then analyzed without further treatment by injecting the extracts via a heated split/splitless injection port (where the ion pairs are converted to the alkylated LAS or SAS). With GC–MS analysis of the extracts, good quantitative agreement with conventional liquid solvent extractions was achieved using a 15-minute static/15-minute dynamic SFE procedure.

While derivatization has primarily been used to increase the solubility of polar analytes, derivatization can also have a positive influence on the extraction of non-polar compounds by reducing the polarity of matrix active sites and thereby facilitating the release of bound compounds. Hills and Hill [66] used a mixture of silanes as a modifier/derivatizing agent to improve the recovery of PAHs from two certified materials. They found that the mixture of silanes was about two times more efficient than CO_2 modified with

10% MeOH and in some cases gave values higher than the certified concentrations.

3.7. Extraction of metals

One of the most recent and interesting applications of SFE is concerned with metal-containing compounds. This area has previously been dominated by ion chromatography and HPLC and has focused on total metal determination. A good example of this is the determination of mercury that primarily has been quantified as total mercury, disregarding possible speciation. Using SFE, however, it should be possible to achieve speciation for the organometallic compounds as well as to determine the total content. This has been studied recently by Liu et al. [67]. In preliminary experiments (spiking level 2.5 μg/g) the organomercury compounds were extracted by SFE using CO_2 with 5 and 10% MeOH. The extracts were analyzed by GC–AED after derivatization with pentylmagnesium bromide giving recoveries of 64–89%. This was followed by determination of the remaining inorganic mercury using an enzyme-linked immunosorbent assay (ELISA) specific for Hg^{2+}. This group has also evaluated the extraction of organotin compounds using MeOH modified CO_2 (5%) and diethylammonium diethyldithiocarbamate as a complexing agent added directly to the soil or sediment sample [68,69]. The extracts were then derivatized with pentylmagnesium bromide prior to GC–AED analysis. On spiked samples, recoveries were quantitative for most di- and trialkyl organotin compounds. The mono substituted species, however, could only be extracted in yields of less than 20%. Using this method on a certified reference sediment, they achieved recoveries of 108% for tributyltin and 95% for dibutyltin.

Cai et al. [70] studied the SFE of organotin compounds using in situ derivatization with hexylmagnesium bromide. They achieved good recoveries (74–114%) from spiked samples and only the mono-alkyl species gave poor recoveries. From real samples the dibutyltin recoveries were also low, but tributyltin recoveries were near-quantitative from two certified reference materials.

Wai and co-workers and Liu et al. have demonstrated that metal ions can be extracted by SFE using CO_2 containing a suitable chelating agent [71–73]. They have reported the application of SFE for the extraction of Cu^{2+}, CO^{2+}, Cd^{2+}, Zu^{2+}, and Hg^{2+} by CO_2 containing the fluorinated ligand lithium bis(trifluoroethyl)dithiocarbamate (LiFDDC). The negatively charged ligand ($FDDC^-$) reacts with the metal ions to form neutral metal chelates that become soluble in supercritical CO_2. This in situ chelation-SFE technique could become important for the pre-concentration of trace metals from environmental samples and metal-containing wastes. The success of this technique for environmental applications depends largely on the effectiveness of the ligand binding, and the solubility of the ligand–metal complex.

The recovery of trace heavy metals (Cd^{2+}, Zn^{2+} and Pb^{2+}) from aqueous media using different alkyldithiocarbamates as complexing agents was investigated by Wang and Marshall [74]. They found increasing solubilities of the complexes upon increasing the chain length of the alkyldithiocarbamates. Using CO_2 saturated with tetrabutylammonium dibutyldithiocarbamate they achieved 73–94% removal in 5 to 15 min of the inorganic ions. Higher than 95% removal was achieved in 10 min by acidifying the aqueous matrix prior to extraction.

Lin et al. have reported on the SFE of lanthanides and actinides by CO_2 containing different fluorinated β-diketones as chelating agents [75,76]. Fluorinated β-diketones are important for this purpose because some of them are commercially available and are known to form stable complexes with lanthanides and actinides. Extraction and separation of actinides by SFE is of special interest because of the potential application to nuclear waste analysis. Extractions involving mixed ligands like β-diketones and tributylphosphate [76] as well as thenoyltrifluoroacetone and tributylphosphate [77] has also been reported. Most of these applications give fairly good recoveries from water, sand and filter paper depending on which ligands are used and

the mixing. However, further studies are still needed to evaluate the use of these methods to real samples.

3.8. Fractionation and selectivity in SFE

Most reports on SFE refer to the speed of extraction (typically 30–60 minutes) as one of the main advantages of this technique over other conventional methods. However, in terms of analyst time required, SFE's time advantage is less important since conventional techniques (e.g., Soxhlet and sonication extraction) do not require much supervision during a typical 12–24 hour extraction. On the basis of reducing analyst time, one of the best advantages that SFE can provide is the cleanness of extracts. This relies on the higher selectivity of SFE when compared to liquid solvent extraction. With conventional solvent extraction, extracts of environmental solids frequently contain large amounts of matrix organics which must be removed using sample clean-up or fractionation steps prior to extraction analysis. Since clean-up steps are time consuming and an important source of analytical error, selective extraction that can be followed immediately by chromatographic analysis becomes increasingly important in routine analysis of environmental samples.

While the solvent strength of CO_2 can be altered by changing temperature and pressure, selective extraction of organics with similar polarities from complex environmental matrices has not been very successful. Fractionation of compounds is, however, possible with CO_2 provided the compounds are adequately different. This has been shown by King [2] who fractionated DDT from lard at 207 bar and 60°C yielding only a fraction of the initial lard in the extract but >75% of the DDT. David et al. [16] showed the selective extraction of PCBs from seagull eggs. At conditions suitable for extraction of PCBs they only extracted ~1% of the total fat in the sample. Similar selectivity was obtained for PCBs from a certified milk powder. Bøwadt et al. selectively and quantitatively extracted PCBs from lyophilized fish tissues with large differences in fat content [19]. With a higher lipid content (>8–9%) co-extracted lipids in the extract were too high for on-column injection into a GC–ECD. However, if either splitless injection was employed or a fast clean-up over acid silica was performed, routine analysis of fish with higher lipid content could be performed without interference.

When the selectivity of SFE using CO_2 is not adequate, selective sorbents have been added to the extraction cell to reduce the extraction of unwanted matrix components. France et al. [78] has shown that Al_2O_3 can be used as an additive to samples to retain lipids when extracting organochlorine pesticides from fats. These findings have been confirmed by Johansen et al. [79,80] who used basic Al_2O_3 (up to two times the sample weight) for the extraction of PCBs from biological tissues. Fig. 4 shows the chromatogram of a selective extraction of PCBs from a certified milk powder. The 2-g sample that contained 25% lipids was mixed with 2 g of basic Al_2O_3 prior to extraction with pure CO_2 and was injected on-column without clean-up. The selective extraction of PCBs from sulfur containing sediments, by the addition of Cu powder to the extraction cell to retain elemental sulfur, has been shown by Bøwadt and Johansson [18]. A number of sediment samples containing up to 2.5% sulfur were extracted and analyzed in 60 min without any clean-up before on-column

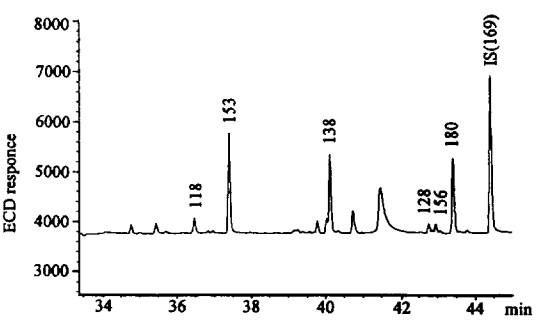

Fig. 4. GC–ECD chromatogram (DB-17) of a selective extraction of native PCBs from certified milk powder (CRM 450) containing ca. 25% lipids. The on-column injected amounts of PCB range from ca. 0.5 pg (PCB 156) to ca. 7 pg (PCB 169, internal standard).

injection on GC–ECD. In all cases the sulfur was completely retained in the extraction cell.

4. Collection of analytes

Apart from the extraction process, the single most important process in SFE is the efficient trapping of extracted material. Two different approaches are commonly used for off-line SFE, liquid solvent collection and solid phase trapping. Both systems have their advantages and disadvantages with respect to ease of handling, choice of restrictor type, maximum gas flow, and compatibility with the various types of supercritical fluids, modifiers and analytes [3,50,81].

4.1. Solvent collection

Liquid solvent collection is mechanically simple and has been the most widely used approach for environmental samples. Two common approaches have been used. In the first approach, the end of the flow restrictor is placed directly into the collection solvent, and the CO_2–analyte mixture is depressurized directly in contact with the solvent. Since depressurization forms aerosols (clusters of the extraction fluid and analytes) [82], it seems likely that collection of the analytes occurs by the aerosol clusters impacting the liquid solvent. In the second approach, the CO_2–analyte effluent is first depressurized to the gas phase in a glass transfer tube before contacting the solvent. For this system, efficient collection appears to depend on efficient transfer of the analytes from the gas phase to the collection solvent. Somewhat surprisingly, the first approach has been shown to yield better collection efficiencies of more volatile components, and efficient collection using the second approach has often required the addition of a second solid phase trap [57,83] or a glass wool insert in the glass liner [84]. For example, Burford et al. tested the two different methods and discovered that while expansion directly into methylene chloride yielded $>90\%$ collection efficiencies for PAHs as volatile as naphthalene, expansion in the glass transfer tube yielded only ca. 50% collection efficiencies under identical extraction conditions [85].

The collection efficiencies achieved using liquid solvent trapping can also be affected by several experimental factors. Most obvious, the selection of collection solvent is important. For example, Langenfeld et al. investigated the collection of 66 organics from the U.S. Environmental Protection Agency's semi-volatile pollutant list (including phenols, amines, and aromatics) by direct depressurization in several different collection solvents [86]. In general, collection efficiencies in methylene chloride, chloroform, and acetone were much better than collection efficiencies in methanol and hexane. When methylene chloride was used as a collection solvent and thermostatted at 5°C (to avoid restrictor plugging from ice formation), all 66 test compounds were quantitatively (90 to 104%) collected.

Compared to the study of Langenfeld et al., much lower collection efficiencies for the same (or similar) organics have been reported [61,82,83,87]. While some of the differences in efficiencies may be a result of the depressurization directly in the solvent versus depressurization into the gas phase prior to contacting the solvent (as discussed above), it is clear that several other experimental factors can affect collection efficiencies. These factors include the height and volume of the collection solvent [85,88], the SFE flow-rate (remember that 1 ml/minute of supercritical CO_2 expands to ca. 500 ml/minute of gaseous CO_2), and the method used to heat the restrictor and the restrictor temperature [27]. The variation in collection efficiencies experienced by different investigators accentuates the need to reconfirm high collection efficiencies any time the analyst encounters new analytes or makes any significant changes to the SFE procedure.

Further development and standardization of SFE systems will also help to make liquid solvent collection efficiencies less operator-dependent. For example, Yang et al. have shown that recent commercial instrumentation using 15 ml of collection solvent can yield collection efficiencies

>90% for species as volatile as *n*-heptane and benzene [88].

4.2. Solid phase trapping

Solid phase trapping is normally performed by depressurizing the CO_2 and analytes prior to the trap, and collecting analytes from the gas phase onto sorbents such as silica gel, Florisil, or bonded phase packings or onto cooled inert surfaces such as glass or stainless steel beads [10,11,43,50,84,89,90]. After trapping, the analytes are eluted with liquid solvents for subsequent analysis. This approach has been used less frequent than liquid trapping, but as several new commercial instruments utilize this principle (either exclusively or as an extra feature), it is expected to gain more attention in the future. The packing material provides two trapping mechanisms (i.e., cryogenic trapping and adsorption to active sites on the trapping material) and a clean-up mechanism (i.e., liquid chromatography during the elution step) [84]. Solid phase trapping can be more complicated to optimize than liquid solvent collection since the analyst must choose the trapping material, trapping temperature, and elution solvent. However, sorbent trapping has the advantage over liquid solvent trapping by allowing class fractionation (e.g., clean-up) during elution of the trap [43], and by yielding a relatively concentrated extract. This technique is, therefore, well suited for automation and routine analysis of samples requiring class fractionation prior to analysis.

Selection of trapping material should be made both to yield high trapping efficiencies, and for the potential to perform selective elution of target analytes from complex extracts. The use of cryogenic trapping on inert materials (e.g., glass or stainless steel beads) is attractive from a standpoint of simplicity, but is often a poor choice since only very non-volatile analytes will be effectively collected. For example, Mulcahey et al. [11,50] reported only ca. 25% collection efficiency for decanoic acid on stainless steel beads with a trap temperature of 5°C, despite the fact that the boiling point of decanoic acid is 270°C. Similarly, Bøwadt et al. [43] has reported losses of the more volatile PCB congeners using stainless steel beads, particularly when modifiers were present in the SFE fluid.

While lowering trapping temperature increases collection efficiencies for traps filled either with sorbents or inert materials, two experimental realities limit the use of cryogenic trapping. First, water from wet samples will freeze in (and plug) the collection trap if the temperature is held below 0°C. For environmental samples this effectively means that traps should not be used below 0°C unless samples are dry. An additional trapping problem occurs when organic modifiers are added to the CO_2 since, upon depressurization, the modifiers can condense on the trap and elute analytes during the remaining SFE time (thus resulting in poor trapping efficiencies). The normal solution to this problem is to raise the trap temperature above the boiling point of the modifier to ensure that no modifier condenses in the trap [11,43]. Unfortunately, raising the trap temperature can adversely affect the collection of more volatile analytes. For this reason, Lee et al. [44] have used a two-step extraction procedure for PAHs. The more volatile PAHs (e.g., naphthalene) were extracted with pure CO_2 and trapped at −5°C on ODS. The trap was then eluted and the sample was extracted a second time to recover the higher molecular weight PAHs with 1% methanol/4% methylene chloride as a modifier with a trapping temperature of 80°C.

Bøwadt et al. [43] have also reported that keeping the concentration of modifier low enhances trapping efficiencies. For example, with 2 vol% of methanol modifier, all PCB congeners were efficiently trapped on silica, but the more volatile PCB congeners were lost from a silica trap if 5% methanol modifier was used even with the trap temperature at the boiling point of methanol (65°C). However, both Florisil and ODS efficiently trapped all of the PCB congeners at 65°C even with 5% methanol modifier.

Despite the need to optimize sorbent trapping conditions based on the extraction conditions needed for high extraction efficiencies, properly designed sorbent trapping system can efficiently trap very volatile components. For example, in a

direct comparison between sorbent (Poropak Q at 5°C) and solvent trapping (15 ml methylene chloride), Yang et al. [88] reported quantitative (>90%) trapping efficiency of benzene using both systems, and quantitative trapping of *n*-hexane with the sorbent trap held at 5°C. However, it is impossible to overemphasize that the analyst must understand the trapping system used, and perform trapping efficiency studies whenever a change in the SFE conditions (such as adding a modifier) is made that may affect the trapping efficiencies.

4.3. On-line collection

Nearly all quantitative uses of SFE for environmental samples have used off-line collection of the SFE extracts. However, on-line collection (where the analytes are transferred directly to the instrument used to analyze the extract without an intervening collection step) can also be a viable approach to quantitative analyses. On-line techniques generally couple the SFE step to a chromatographic system, most often GC, followed by SFC, and occasionally LC. Since multidimensional coupled SFE is the subject of other papers in this special issue, only brief mention of on-line techniques in terms of analyte collection will be made here. The interested reader is referred to those papers as well as recent reviews [4,22,91–94].

One major reason for performing on-line SFE with environmental samples is to increase the collection efficiencies of very volatile analytes. As discussed above, properly designed off-line collection systems using either sorbent or solvent traps can efficiently trap analytes as volatile as benzene. However, the quantitative trapping of more volatile analytes is quite difficult. While sorbent traps are likely capable of trapping more volatile species, the solvent used to recover the analytes from the trap would obscure very volatile components during the chromatographic determination. A recent example of trapping very volatile compounds after SFE has been reported by Burford et al. based on a simple interface using a split/splitless injection port [92]. The SFE effluent was depressurized directly in the injection port in the split mode, and the analytes were collected in a cooled DB-1 capillary column (5 μm film thickness). With this approach, alkanes as volatile as *n*-butane and solvents such as ethanol, acetone, and methylene chloride were quantitatively collected in the GC column and exhibited reasonable chromatographic peak shapes.

In terms of analyte collection, a second reason to utilize on-line techniques is the potential to transfer every extracted analyte molecule to the chromatographic system, and thus obtain maximum sensitivity from small sample sizes. Unfortunately, methods that can transfer every target analyte also transfer every unwanted matrix component that is in the SFE effluent. For example, SFE–GC methods that use a cooled trap (whether an external trap or using the GC column as the trap) can have the trapping system plugged with ice if wet samples are extracted and the trapping temperature is below 0°C. A more difficult problem is when the SFE effluent contains high concentrations of matrix organics that are not compatible with the chromatographic system, for example, the fat extracted from biological samples. For fat-containing samples, elegant solutions to such problems have been reported using on-line class fractionation techniques which remove the interfering fats prior to transfer of the target analytes to the GC. For example, Johansen et al. [79,80] used an alumina adsorbent to selectively retain fat components so that the SFE effluent could be transferred directly to the GC interface. Stalling et al. used on-line SFE–GPC to seperate fat and environmental contaminants [95]. Similarly, Nam et al. used sequential on-line SFE–packed column SFC–GC to allow pesticides to be determined by on-line GC in fatty food samples [96].

Although most environmental analyses for organics require chromatographic separation, some determinations can be performed without separation of individual components, e.g., using detection systems based on spectroscopy or immunochemistry. For example, the proposed U.S. Environmental Protection Agency SFE method for total petroleum hydrocarbons (TPH) uses infrared spectroscopy to determine TPH content

in the off-line SFE extracts. For such analyses, the coupling of a high-pressure spectroscopic cell with SFE would be an ideal on-line system, since no flow control is needed (and therefore no restrictor plugging could occur). Heglund et al. [97] has recently reported a simple fiber-optic interface based on an inexpensive stainless steel cross for on-line IR determinations of SFE extracts up to 400 bar. Even though the supercritical CO_2 causes spectral interference in the range of 3800 to 3500 cm^{-1} and from 2500 to 2150 cm^{-1}, the IR spectra is sufficiently clean to allow determination of several important organic compound classes. They utilized this system to determine TPH for real soil samples, and obtained good quantitative agreement with both off-line SFE–IR and Soxhlet extraction–IR. The system has the additional advantage that, after IR determination, the SFE effluent can be collected off-line for subsequent chromatographic measurement. Thus, coupled SFE–IR (or other spectroscopic detectors) provide a useful tool for rapidly surveying samples, with the extracts from contaminated samples being collected for further analysis. Although the use of on-line SFE–spectroscopic approaches for environmental analyses has been very limited, high pressure systems using similar approaches have been used for UV and fluorescence studies in supercritical fluids, and should have potential for environmental analysis [98].

5. Comparison to conventional methods

5.1. Spiked versus native analytes in SFE experiments

In the infancy of SFE, many experiments were conducted on spiked or fortified samples (as is often done with other extraction techniques) under the assumption that spiked analytes provided a good model for so-called "native" analytes that were formed with the solid (e.g., PAHs in soot) or had been aged under environmental conditions. The results from spiked samples indicated that SFE was a truly revolutionary technique facilitating easy, fast and effective extraction of a whole variety of compounds. Unfortunately, when SFE was performed with real environmental samples it became clear that SFE conditions developed on spiked samples often yielded poor recoveries from real samples [16,87]. Even under conditions that facilitated the solubility of high concentrations of analytes in the extraction fluid it was often not possible to achieve good recoveries of native analytes. The frequent inability of methods developed on spiked analytes to yield good recoveries of native analytes from environmental matrices seems, in retrospect, quite reasonable. Spiked analytes are situated on the surface of sample matrices and have little time to migrate to strong binding sites. In contrast, native pollutants are often in contact with the matrix for years (in the case of soils or sediments) or are formed as part of the matrix (e.g., soots), and have the potential to be associated with much stronger binding sites than recently spiked organics [15].

Despite the fact that the recovery of spiked analytes has traditionally been used to develop conventional liquid solvent extraction methods, the assumption that spiked analytes show extraction behavior that is the same as native analytes has never been tested until recent evaluations of SFE. In the first comprehensive study to evaluate the ability of spiked analytes to mimic the extraction behavior of native analytes, Burford et al. [15] determined the relative extraction rates of native PAHs versus spiked deuterated PAHs from air particulates, soil, and petroleum waste sludge. Regardless of the spiking method or aging time (up to 14 hours), the extraction rates of most of the spiked deuterated PAHs were substantially faster than those of the same native PAHs. Differences in extraction rates between spiked and native PAHs were most dramatic for the lower molecular weight PAHs, indicating that relatively volatile species such as naphthalene must be tightly bound in order to remain associated with an environmental sample. In most cases, 30 min extractions with pure CO_2 recovered more than 90% of the spiked deuterated PAHs, while only 25–80% of the native PAHs were extracted. Conventional sonication in methylene chloride also showed substantial differences in the extraction of spiked versus native PAHs, demonstrating that spike

recovery studies are not valid for the development of quantitative extraction methods for heterogeneous environmental samples regardless of the extraction method. From these studies, Burford et al. concluded that quantitative extraction methods should be evaluated based on multiple extractions with different techniques (e.g., SFE, SFE with modifier, and finally by liquid solvent extraction) to best ensure that all native analytes are extracted [15]. More recently, Langenfeld et al. [27] has determined the relative extraction rates of spiked and native chlorinated dioxins from fly ash and PAHs from sediment and soil at 40 and 200°C. They found that the spiked analytes were always extracted more rapidly than the native pollutants [27]. An example of this behavior is shown in Fig. 5, where the extraction behavior of spiked and native PAHs at different temperatures can be seen for a marine sediment [27].

Similar problems with developing SFE methods based on spike recoveries have been reported by other investigators. Alexandrou et al. [99] reported that pure CO_2 could efficiently extract spiked chlorinated dioxins from incinerator fly ash, but could not extract native chlorinated dioxins without the addition of toluene as a modifier. David et al. [16] developed a method for SFE of PCBs optimizing the parameters using spiked sediment samples. The average recovery from spiked samples was 98%. When this method was applied to a sewage sludge the mean recovery was found to be ~30% relative to the certified values. Moreover, there was a discrimination against PCBs with a high degree of chlorination, for which only 10% was extracted. These examples clearly illustrate that SFE methods (or any other extraction method) developed on spiked samples will not necessarily yield high recoveries of native analytes when significant analyte–matrix interactions exist.

5.2. Defining quantitative recovery

As discussed above, the use of spike recovery studies to determine the extraction efficiency of an SFE method (or for that matter, any other extraction method) is often not valid for complex

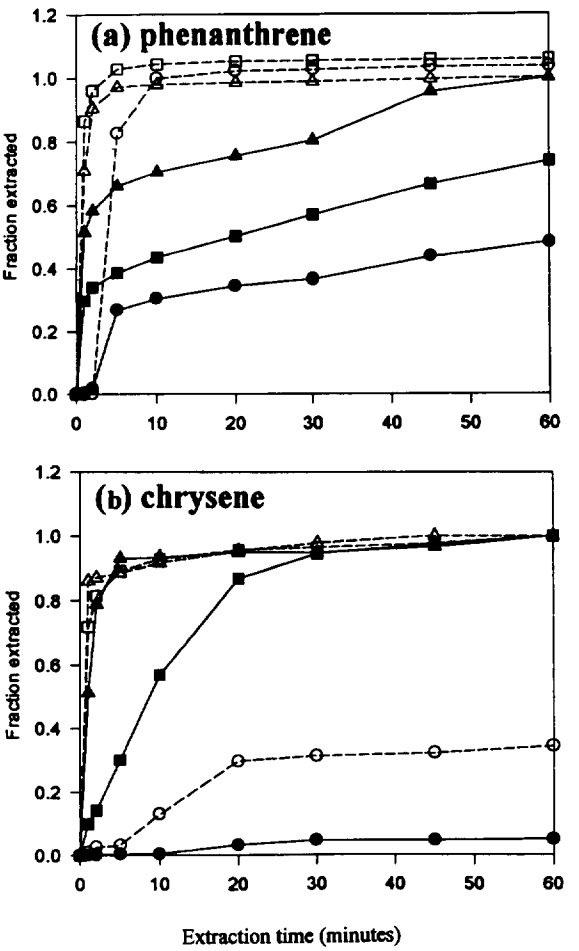

Fig. 5. SFE extraction rates for spiked [$^2H_{10}$]-phenanthrene and native phenanthrene (a), and spiked [$^2H_{12}$]-chrysene and native chrysene (b) from marine sediment at 40°C (○,●), 120°C (□,■), and 200°C (△,▲) and constant density (d = 0.67 g/ml). Open symbols: spiked, filled symbols: native. Adapted from Ref. [27].

environmental solids because spiked analytes typically extract more easily than native analytes that were formed with the sample matrix or have been aged for several years under environmental conditions. These observations emphasize the need to develop extraction methods (whether SFE or liquid solvent extractions) using real samples that represent the type of matrix and analyte concentrations expected. It is important to note that spike recovery studies definitely should be performed to demonstrate collection efficiencies in SFE, or to demonstrate that no

losses of target analytes occur during conventional liquid solvent extraction. However, spike recoveries should not be used to determine the efficiency of the extraction process. This leaves the analyst with a seemingly unsolvable dilemma, i.e., since the concentration of native organics cannot be known on a real sample, there is no direct way to determine extraction efficiencies. Therefore, recovery data for real samples is generally based on comparisons with standard extraction methods (usually Soxhlet extraction or sonication). When the goal is to develop an SFE method to directly replace an existing standard method, direct comparison of the extraction efficiencies can be based on the amounts of target analytes extracted using the standard method. However, it should be recognized that the standard extraction method itself may not yield truly quantitative recoveries [100]. For example, several recent studies have demonstrated higher recoveries using SFE (in typically 30 to 60 minutes) than using several hours of Soxhlet extraction [6,56,58,59,61,64,88,101]. Two different reasons may account for these higher SFE recoveries. First, both Soxhlet and SFE may extract the same quantity of analytes, but the collection efficiencies of the SFE method can result in much lower losses of volatile components than the Soxhlet process. For example, Yang et al. [88] reported higher recoveries of the more volatile components of gasoline including so-called "BTEX" (benzene, toluene, ethylbenzene, and xylenes) using SFE for 30 minutes than obtained using Soxhlet extraction for 4 hours. They concluded that the apparent higher efficiencies obtained using SFE were a result of losses during the Soxhlet extraction (not poor extraction of BTEX from the soil samples by the Soxhlet process). Such losses would be expected from Soxhlet extraction since volatile organics are typically determined using purge and trap (dynamic headspace) methods [102].

As SFE methodology continues to develop, an increasing number of investigators have demonstrated higher extraction efficiencies (as opposed to simply better collection efficiencies) when using optimized SFE methods than those obtained using conventional liquid solvent extraction [6,56,58,59,61,64,101]. Examples of higher recoveries by SFE include heavy petroleum hydrocarbons compared to the ASTM Soxhlet procedure with Freon-113 [59], aromatic amines from soil compared to 18-hour Soxhlet extractions [56], PAHs from soil compared to Soxhlet extraction and sonication [6], and PAHs extracted from sediment and urban air particulates using a reactive solvent modifier [66].

Such results emphasize the need to optimize the Soxhlet extraction conditions as well as the SFE conditions. For example, standard Soxhlet extraction methods often use only one solvent, while the use of an azeotrophic mixture of two solvents having different polarities can yield better extraction efficiencies [103]. Since the recoveries from both SFE and conventional solvent extractions can often be improved by optimizing extraction conditions, it is conceivable that no extraction method can successfully remove all of the target analytes from a particular matrix. Such considerations have lead one of the leading producers of reference materials, the Community Bureau of Reference (BCR) to advocate the use of sequential liquid solvent extraction using solvents having different polarities to better estimate the true concentration of analytes on real samples [104]. Similarly, it has recently been suggested that SFE extractions be evaluated by performing conventional liquid solvent extractions on the SFE residue (and similarly, to evaluate a liquid solvent extraction by performing an optimized SFE extraction on the residue) [15,19,23].

Consideration of these problems leads to some generalized suggestions for developing and evaluating any extraction method for complex environmental samples:

1. Spike recoveries should be used to determine collection efficiencies for SFE (and to determine any losses that may occur from volatilization for liquid solvent extractions), but can only be used to determine initial extraction conditions for real samples.
2. Development of extraction conditions should be performed with real samples having analyte concentrations and matrix components representative of the samples of interest. The use of

certified reference materials should be used whenever appropriate materials are available as they provide a source of common material for various investigators as well as "bench-mark" concentrations for target analytes.

3. If the goal is to obtain similar results to a standard method, SFE recoveries can be compared directly to that method, however:

4. If the goal is to obtain the highest possible recovery (i.e., the concentration closest to the true value present in the sample), both SFE and liquid solvent procedures should be optimized as discussed above, and the determination of "100% recovery" should be based on sequential extractions using different procedures (e.g., SFE followed by liquid solvent extraction and vice versa).

5.3. SFE used in intercomparison and certification exercises

Being a relatively new technique, SFE has only recently been used for the certification of environmental reference materials, as well as for interlaboratory comparisons. Initial attempts at interlaboratory comparisons were conducted by the U.S. National Institute of Standards and Technology and yielded results varying from quite good recoveries for PAHs to only ca. 50% recoveries for PCBs from sediment [105]. However, these studies were performed using SFE conditions that have been demonstrated to be less than efficient by several subsequent SFE studies [6,16,18,19,38]. More recent round robin studies have met with better success. For example, the proposed U.S. Environmental Protection Agency SFE method for total petroleum hydrocarbons (TPH) was tested in 14 different laboratories. Recoveries were typically 76 to 107%, overall method accuracy (recovery) was 83%, and intralaboratory reproducibilities typically ca. 11 to 18% (including the RSD of the GC analyses), while interlaboratory method precisions that appeared to be matrix dependent ranged from 17% to 48% [106]. A separate mini-round-robin between three laboratories on the extraction of PAHs from soil also demonstrated that SFE has a future for intercomparison with conventional solvent extraction methods [107]. Three real samples were extracted in triplicate by each laboratory at 355 bar and 90°C using 2 ml of methylene chloride added to the extraction cells before a 20 min. dynamic extraction with a flow-rate of ~1 ml/min [40,107]. The SFE method accuracy (percent recovery) was determined relative to sonication extraction since the true levels of PAHs in the samples were unknown. The PAH recoveries were typically 80 to 120% by SFE when present at concentrations of 1 μg/g (1 ppm) or higher and ca. 60% for the one sample with concentrations <1 μg/g. The interlaboratory precisions (overall RSDs) appeared to be concentration-dependent; concentrations above 1 μg/g RSDs (including the RSD of the GC analyses) were typically 10–20% while at concentrations below 1 μg/g RSDs typically ranged from 20 to 40%. Intralaboratory RSDs were usually less than 1/2 the interlaboratory RSDs.

A recent certification exercise on PCBs from an industrially polluted soil (CRM 481) was conducted independently with 21 participating laboratories of which 18 used Soxhlet extraction for sample preparation while 3 used SFE [108]. All three laboratories using SFE had independently developed methods as well as different analytical setups for the GC–ECD and GC–MS analyses. The results from the preliminary certification meeting for this highly contaminated soil (7 to 136 μg/g of individual congeners) are shown in Table 3[1]. It is noteworthy that the results from the SFE laboratories are very close to the average results from all laboratories when considering the use of different sample preparation methods. The standard deviations between mean results of the participating laboratories for the different PCB congeners is quite similar for the whole BCR group and for the SFE group. Since the BCR group represents 12–18 laboratories (whose values were selected for the certification) while the SFE group represents 3 laboratories, this standard deviation reflects a much

[1] The data are subject to minor changes upon a final certification meeting late in 1994. This will, however, not change the comparison between the SFE and Soxhlet data.

Table 3
Independent determination of PCBs in a certified reference material (CRM 481)

PCB No.	BCR Group (12–18 Labs)[a]		SFE Group (3 Labs)	
	Mean (μg/g)	SD (μg/g)	Mean (μg/g)	SD (μg/g)
101	37	4.4	37	4.0
118	9.4	1.3	9.5	1.2
128	8.7	1.5	9.0	1.1
149	96	10	99	11
153	136	13	135	11
156	7.1	1.0	7.5	0.7
170	52	5.7	51	7.8
180	123	10	128	15

SD = Interlaboratory standard deviation.
[a] The values represent the certified values.

higher consensus among the SFE laboratories than for the whole BCR group. To further validate the SFE efficiencies, four of the SFE-extracted samples were pooled and Soxhlet extracted for an additional 48 hours as suggested above in Section 5.2. The amount of PCBs extracted from the SFE residue was less than 0.4% of the SFE values, further demonstrating the efficiency of the SFE step [108].

6. Summary

SFE for environmental sample preparation has undergone a very rapid growth supported by the need to perform more rapid and selective extractions, while reducing the volumes of potentially hazardous solvents required. The strong desire for new sample extraction methods is made evident by the fact that analytical-scale SFE instrumentation was almost non-existent only a few years ago, while several fully automated SFE instruments are now available. The development and evaluation of SFE for environmental samples has proved to be more complicated than initially thought, primarily because the strong matrix–analyte interactions that may occur in environmental matrices frequently make the development of quantitative extraction conditions based solely on solubility considerations and spike recoveries invalid for real samples. However, as the understanding of the factors controlling SFE processes (including factors controlling the partitioning of analytes from matrix active sites and controlling the collection of extracted analytes) is improved, the number of samples which can be rapidly and quantitatively extracted using SFE is increasing. SFE is also being viewed more favorably by regulatory agencies, as evidenced by the proposed acceptance of SFE methods both in the United States and in the European Union. While SFE has been used almost exclusively with relatively non-polar (e.g., "GC-able") analytes, the reports on extracting polar molecules (and even metal ions) with suitable modifiers, derivatizing reagents, and chelating reagents demonstrate the potential for much wider applications in environmental extractions. As SFE methods improve, the abilities of well-established liquid solvent extraction methods to yield truly quantitative recoveries of tightly bound environmental analytes are now being questioned, and it is clear that the abilities of both conventional extraction methods and SFE to extract every molecule of a target analyte must be evaluated in more detail. Further development of SFE can be expected to increase our understanding of the interactions between environmental pollutants and environmental ma-

trices, as well as to better define what are truly 100% recoveries.

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