CHAPTER 1

SOLVENT MICROEXTRACTION: COMPARISON WITH OTHER POPULAR SAMPLE PREPARATION METHODS

1.1 INTRODUCTION

One of the most important steps in any analytical procedure is sample preparation. Most analyses are carried out on samples containing complex mixtures of very small amounts of the chemicals that need to be identified and/or quantified. At the same time, most sample matrices, such as soils or wastewater, are also very complex. Thus, a successful sample preparation method typically has three major objectives: (1) sample matrix simplification and/or replacement, (2) analyte enrichment, and (3) sample cleanup.

Many useful sample preparation methods have been developed over the years to address specific needs for analyzing waste and drinking water, foods, medicinals, soil, and air. As an example, many of the most important methods were codified for the analysis of waste and drinking water samples, as exemplified by the U.S. Environmental Protection Agency's (EPA's) 500 and 600 methods. However, these sample preparation and analysis methodologies, which originally involved traditional laboratory liquid–liquid, liquid–solid, and gaseous extractions, suffered from a number of limitations, including the requirement for significant time-consuming manual labor or in some cases, the use of large quantities of hazardous extracting solvents. As a result, there has been a continual search for improved sample preparation procedures with the following goals: (1) reduction in the number of steps

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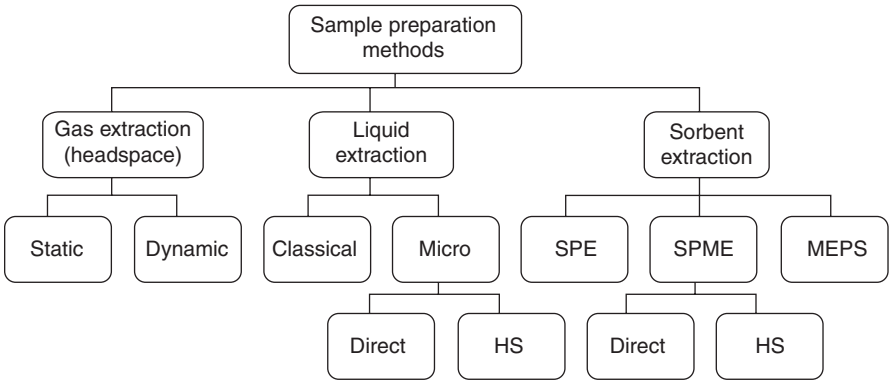

FIGURE 1.1. Sample preparation methods for aqueous and solid samples.

required for the procedure, (2) reduction or total elimination of solvents required for extraction, (3) adaptability to field sampling, and (4) automation.

The more commonly used sample preparation methods for water and solid matrix samples are represented schematically in Figure 1.1. *Solvent microextraction* (SME) is a fairly recent development in sample preparation that has the potential for meeting all four goals cited above. SME, which, in its most commonly used modifications, has also been referred to as *liquid-phase microextraction* (LPME), *single-drop microextraction* (SDME), or *dispersive liquid–liquid microextraction* (DLLME), can integrate sampling, analyte extraction and concentration, and sample introduction into a single step. Because it is the most comprehensive descriptive term available, we have decided to use the term SME originally coined by Jeannot and Cantwell[1] to cover all of the variations of this method. SME is compatible and has been used successfully with most common analytical instrumentation, including gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and atomic absorption spectroscopy (AAS). In its simplest and earliest practical variation, SME consists of a single drop of extracting solvent suspended from a GC syringe needle that is immersed in an aqueous sample solution. The same GC syringe is then used to introduce the solvent drop with extracted analytes into the GC (see Figure 1.2).

SME has its origins in traditional sample preparation methodologies, including liquid–liquid extraction, and in this chapter we not only give a brief history of the method, but also provide a general comparison with other commonly used sample preparation techniques. We include the advantages and disadvantages of each method, allowing the reader to gauge whether SME would be an appropriate method for their sample preparation needs.

1.2 COMPARISON OF SAMPLE PREPARATION METHODS

As indicated above, many of the most useful sample preparation methods have been rigorously detailed and codified into methods adopted by the U.S. EPA and other

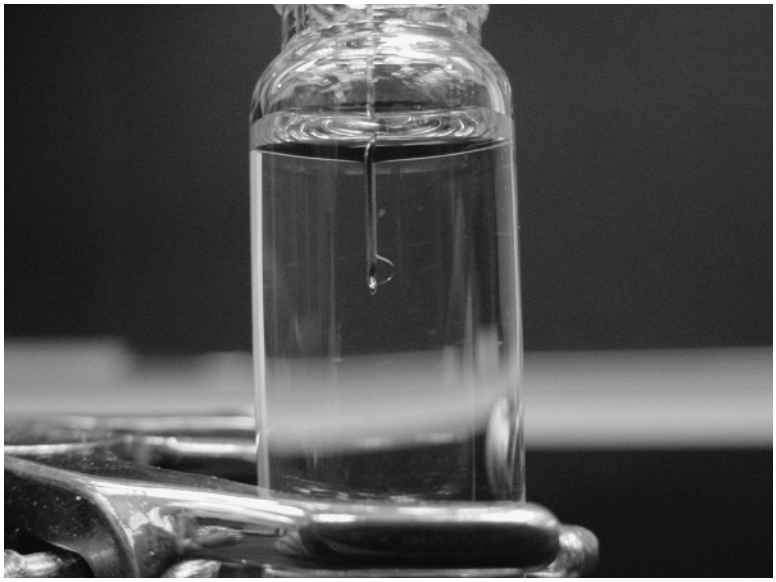

FIGURE 1.2. A 1-μL drop of octane suspended in aqueous solution on the tip of a Hamilton 701 GC syringe needle.

national and state agencies. The most commonly used procedures include variants of liquid–liquid extraction, sorbent extraction [solid-phase extraction (SPE)] and headspace extraction [including purge-and-trap (PT)], all based on classical chemical laboratory analysis techniques. More recently, solid-phase microextraction (SPME) has been developed and used for many environmental, forensic, and food analysis applications.[2,3] SME, in its most commonly implemented format, is very similar to SPME, using 1 to 2 μL of solvent at the end of a syringe needle to extract a sample rather than a liquid or porous polymer coating on a fused silica or metal fiber. Each of these techniques has advantages and disadvantages, and no one technique can be or should be used for all analyses. A number of other techniques, such as supercritical fluid extraction, cryogenic trapping, microextraction in packed syringe, and stir bar sorptive extraction are important in their own right, but are not discussed here. In addition, although SME and other techniques discussed here may be used for atmospheric sampling, we limit our discussion to extraction from liquid and solid matrices.

1.2.1 Liquid–Liquid Extraction

Traditional liquid–liquid extraction methodology was taken directly from standard techniques used for the purification of chemicals prepared in the laboratory. Thus, a solvent such as dichloromethane, pentane, or ether is used to extract analytes from water using a separatory funnel or continuous extractor, and from solids such as soil or plant material using a Soxhlet extractor. Specific examples include EPA method 625 for municipal wastewater, method 525.2 for drinking water, and method 551.1 for chlorination disinfection by-products in drinking water.

1.2.1.1 EPA Method 625: Liquid–Liquid Extraction

EPA method 625 is used for the analysis of 54 specific chemicals, including industrial by-products and pesticides, and seven chemical mixtures [chlordane, toxaphene, and the polychlorinated biphenyls (PCBs)] commonly found in municipal and industrial wastewater.[4] The method involves extracting a liter of sample with 450 mL or more of dichloromethane, which is then evaporated to a volume of 1 mL. Next, a 1-μL aliquot of the concentrate is analyzed by GC or GC-mass spectrometry (MS). A continuous extractor using 500 mL of solvent can also be used for the extraction, to limit emulsion formation.

The major advantages of this method are simplicity and the ability to extract many analytes at once into a solvent compatible for analysis by GC-MS, which in turn is capable of separating, identifying, and quantifying each analyte. The major disadvantages of the method are the large amounts of solvent used for the extraction, the manual labor involved, emulsions sometimes formed when using a separatory funnel, the time required for the extraction (up to 24 h for a continuous extractor), and the fact that the method actually does not concentrate the analytes significantly. True, 1 L of water is concentrated into 1 mL of dichloromethane, a 1000-fold enrichment, but only 1 μL is analyzed, 1/1000 of the extract. Thus, if an analyte is present at a concentration of 1 μg/L, only 1 ng is actually analyzed. A consequence of the need to evaporate the solvent is that this method is not applicable to volatile chemicals. Another major disadvantage is the relatively large amount of water sample, 1 L, that is required, making this method difficult to automate. Finally, the method will require two preparations and analyses per sample if both basic and neutral/acidic components are present, effectively doubling the analysis time. However, despite these difficulties, method 625 does yield method detection limits (MDLs) of 1 to 5 μg/L (1 to 5 ng/mL) for each analyte, and fairly good precision and accuracy.

1.2.1.2 EPA Method 551.1: Micro Liquid–Liquid Extraction

Several new methods have been developed to address the disadvantages of solvent use, manual labor, and time required for sample preparation methods such as 625. One method includes EPA method 551.1, which is a micro extraction method for analysis of the chlorination disinfection by-products, including the trihalomethanes, and several chlorinated industrial solvents and pesticides.[5] The method involves extracting 50 mL of drinking water saturated with salt with either 3 mL of methyl *tert*-butyl ether or 5 mL of pentane (compared to 500 mL of solvent for method 625, thus the term *micro*), followed by analysis of 1 to 2 μL of the extractant by GC with an electron capture detector (ECD). If pentane is the solvent of choice and 2 μL of the concentrate is injected, the analytes have been concentrated tenfold, but only 1/2500 of the analytes are then analyzed. For example, if the original sample contained 1 μg/L of an analyte, only 20 pg would be analyzed. However, because the ECD is very sensitive and selective for halogenated compounds, the effective method detection limit is approximately 0.1 μg/L (0.1 ng/mL) or less. This method does require significantly less solvent and water sample than method 625, and no solvent concentration is needed, enabling analysis of volatile as well as nonvolatile

chemicals and decreasing the preparation time significantly, to about an hour per sample. The major difficulties are that the method again is not easily fully automated and there is little analyte enrichment.

1.2.1.3 Solvent Extraction–Flow Injection Analysis Semiautomated solvent extraction (SE) approaches utilizing the technique of flow injection analysis (FIA) were first reported in 1978,[6,7] and the kinetics and mechanism of the extraction process have been described in detail.[8–10] SE-FIA involves the injection of an aqueous sample plug in a flowing aqueous stream which may or may not contain chemical reagents. The aqueous stream is ''teed'' to an organic solvent stream, resulting in a segmented flow of alternating aqueous and organic segments which flow through a sufficient length of tubing (the extraction coil). Following the extraction process, the segmented flow passes through a phase separator, which typically separates and directs the organic phase to a flow-cell type of detector for quantitation of the analyte by absorbance or fluorescence spectroscopy. The principal advantages of this approach are the reduction in volumes of sample and solvent, and the ability to achieve high sample throughput through a semiautomated approach. However, owing to the comparable volumes of aqueous and organic phases, even exhaustive extraction results in little, if any, analyte enrichment. The technique has been reviewed extensively by Kubáň.[11]

1.2.2 Liquid–Solid Extraction

Solid-phase extraction is actually based on HPLC media technology. It had been observed that very dilute organic analyte samples in water could be concentrated at the head of a reversed-phase (C18) coated silica gel chromatography column as several milliliters of the dilute water sample passed through the column. This led to the development of small cartridges containing either silica gel or alumina (to retain polar compounds), or reversed-phase adsorbent (to retain nonpolar analytes) for sample cleanup and concentration for HPLC. The success of this method led in turn to the development of cartridges and disks containing a variety of retentive adsorbents which could be used for concentrating analytes from large volumes of water.[12] More recently, the method has been transformed by the development of molecularly imprinted polymers for use as the stationary phase.[13] Molecularly imprinted polymers are polymers synthesized in the presence of a specific chemical. When the three-dimensional form of the polymer is complete and the chemical has been removed from the polymer, holes or receptor sites are left throughout the polymer which have bonding affinities for the chemical.

1.2.2.1 EPA Method 525.2: Solid-Phase Extraction Method 525 was originally a duplicate of method 625, using liquid–liquid extraction to extract analytes from drinking water. The method has been modified to use either a cartridge or a disk containing octadecylsilane (C18) bonded to silica gel to retain the organic analytes as the water is passed through.[14] The analytes are then extracted from the adsorbent with solvent, usually 10 mL of a mixture of ethyl acetate and dichloromethane. The solvent is dried, concentrated to 1 mL, and 1 μL is injected

for analysis. This method works well for relatively clean matrices such as drinking water, but less well for ''dirty'' samples since the silica adsorbents are easily plugged. Using a prefilter material ahead of the cartridge or disk is often mandatory for these samples. The filtration process takes about 1 h, and the entire preparation method about 2 h, and can be semiautomated. The sample is concentrated to the same extent and analyzed in the same manner as in method 625, yielding similar method detection limits. Although much less solvent is used, the method still requires 1 L of water sample and because the solvent is still evaporated, only nonvolatile chemicals can be determined using this method.

1.2.3 Headspace Extraction

A third general method widely used for sample preparation is headspace analysis. Two types of headspace analysis are commonly used: static headspace analysis and purge-and-trap. *Static headspace analysis* is the simplest of all sampling methods, involving sampling, usually with a gastight syringe, and injection of a portion of the headspace gas above a sample in a sealed container.[15] Only a small portion of the total analyte present in the sample is thus analyzed. This also results in a requirement for very careful calibration if this method is to be used for quantification. The method has the disadvantages that only about 10% of the headspace can be injected for analysis, and it is generally useful only for fairly volatile analytes. However, static headspace sampling has the advantage that it can be done either manually or with a standard GC autosampler. Sophisticated automated samplers are also available that can heat the sample and take headspace samples under pressure, enhancing sample enrichment in the headspace. This type of instrumentation is used for *United States Pharmacopeia* (USP) method 467 and the *European Pharmacopeia* method for the analysis of residual solvents in pharmaceutical products. In contrast to static headspace sampling, the *purge-and-trap technique*, used for EPA method 624, is a dynamic sampling procedure, capable of exhaustive extraction and concentration of all the volatile components in water or solid samples.

1.2.3.1 EPA Method 624: Purgeables Purge-and-trap method 624 was designed to analyze 31 volatile chemicals in municipal and industrial wastewater.[16] Other purge-and-trap methods extend the technique to different sample types and analytes. The main advantage of PT is the ability to effectively determine all the analytes in a 5- to 25-mL sample of water. The sample is sparged with a stream of helium gas, which carries the analytes to a solid adsorbent trap consisting of silica gel, Tenax, graphitized carbon, or layers of these materials. The trap is quickly heated and the analytes released are carried in a helium stream to a GC inlet for analysis. The high flow rate of the desorbing helium gas (10 to 30 cm^3/min) requires the use of a wide-bore analytical column and a split-column outlet flow (a jet separator), or a split inlet flow, or liquid-nitrogen cooling of a pre-column to trap the analytes in a tight sample plug which can then be released upon heating onto a narrow-bore GC analytical column at a reduced flow rate. Often, the concentrating effect of the PT method allows a simple split inlet technique to be used successfully.

Typically, method detection limits are 1 μg/L or less with a modern GC-MS and capillary column. Therefore, for a typical 5-mL sample, around 5 ng of each analyte is analyzed. The method does suffer, however, from the possibility of sample carryover from one sample to another, degradation of the trap over time, potential leaks in the plumbing, and the cost of the instrumentation. In addition, foaming of the sample due to the presence of detergents or natural products in soil can cause major contamination problems if the foam is allowed to enter the heated plumbing of the instrument. Foaming can sometimes be avoided by purging the headspace rather than by sparging the water sample with helium. The technique is fully automated, and typically, 30 to 50 samples can be run sequentially.

A variant of purge and trap is membrane extraction with a sorbent interface.[17] This technique utilizes a nonporous membrane, usually silicone, which allows selective transport of nonpolar compounds from a water sample across the membrane barrier. The extracted analyte is swept by a stream of helium and concentrated on a cold or sorbent trap and then released for analysis thermally. This technique is therefore similar to purge-and-trap in the ability to extract analytes exhaustively. Limits of detection below 0.5 ng/L have been reported for toluene and benzene.[18] The limitation for the method is the nonpolar nature of the silicone membrane. Thus, only nonpolar chemicals can be extracted.

1.2.3.2 EPA Method 524.2: Purgeables This is an updated PT method for 84 volatile chemicals ranging from dichlorodifluoromethane to naphthalene in surface waters, groundwater, and drinking water.[19] The method detection limits are in the range of 0.1 μg/L or less. In fact, PT is a very sensitive technique for volatile compounds and is so sensitive that even when using a split inlet with a narrow-bore capillary column, the sample may still need to be diluted to bring the extracted components into a concentration range compatible with the chromatography column and detector. The same advantages and disadvantages apply to method 524.2 as to method 624.

1.2.3.3 USP Method 467 and the* European Pharmacopeia *Method for Residual Solvents These methods were developed for the analysis of residual solvents present in pharmaceutical products after manufacture. The USP method, until recently, involved the analysis of 7 chemicals, but has been extended to cover 64 solvents and essentially duplicates the European method.[20] These methods involve the use of static headspace sampling of pharmaceuticals dissolved in either water, dimethylformamide–water or dimethyl sulfoxide–water. Five-milliliter solutions are heated to 80°C under pressure in a 20-mL headspace vial and 1 mL of the headspace withdrawn and injected into a GC for analysis. Static headspace analysis is not very sensitive for chemicals which are very soluble in water and chemicals with high boiling points. However, the solvents analyzed under method 467 are at relatively high concentrations, ranging from a maximum allowable concentration of 3000 ppm for the xylenes to 2 ppm for benzene. The elevated extraction temperature increases the concentration in the headspace sufficiently so that the samples can be analyzed using a GC with a flame ionization detector. The

major disadvantages of the method are the low sensitivities for some analytes and the initial expense required for the headspace autosampler.

1.2.4 Solid-Phase Microextraction

SPME is a relatively new and important sample preparation method with many advantages and some very important shortcomings. As SPE was developed using HPLC column technology, SPME was developed using GC column technology. SPME is basically an inside-out GC column. A 100- to 250-μm fused silica or metal fiber is coated with 7 to 100 μm of a coating that functions to extract analytes from a water or headspace sample. The coating is either a silicone-based polymer, identical to the polymers used for GC columns, which absorbs the analytes, or a polymer with bonded porous carbon particles (carbon molecular sieve), which adsorbs volatile chemicals. Many hundreds of application notes and papers are available for this technique, which cover samples ranging from industrial discharge waters to arson analyses to biological samples to flavorings in foods.[2,3] SPME is a true solventless method, and that is a major advantage. The technique also allows one to extract a sample and inject the extract using one device. Several fiber polymer coatings are available in various thicknesses, ranging from the nonpolar dimethylsiloxane to relatively polar polyacrylate and the porous adsorbent Carboxen (porous carbon molecular sieve). The method can be carried out manually using a special syringe-type holder or automated completely for GC or HPLC. Finally, the method can and has been used as a field sampling device for atmospheric, water, agricultural, and forensic analyses. So why hasn't SPME replaced all other sampling techniques? This method, like all others, has not only advantages but some important limitations.

One limitation often overlooked is the limited volume of the polymer extractant. One centimeter of a 100-μm-thick poly(dimethylsiloxane) (PDMS) coating on a 100-μm fiber is calculated to have a volume of approximately 0.628 μL. A 7-μm coating on a 250-μm core would have a volume of only 0.056 μL. These volumes are not severe limitations for the application, but must be considered when designing an experiment. SPME theory is almost identical to SME theory, and in Chapters 3 and 4 we show clearly that the total amount of analyte that can be extracted from a water sample, *no matter how large the volume of the sample*, is limited by the distribution coefficient between the coating and water (the water/sorbent distribution ratio) and air and water (the Henry's law constant), as well as the limited capacity of the coating to dissolve analyte. These factors may result in competitive adsorption, especially for the porous adsorbent fibers. Basically, this means that a large amount of one analyte in the sample may prevent the adsorption of components present at lower concentrations. A second limitation results from the fact that the polymer is, in fact, a very viscous, gummy material. This means that prolonged periods (up to an hour or more) are needed for the analyte concentrations in the sample and polymer to come to equilibrium. In fact, in most cases the system does not come to equilibrium. This is not a major problem if very careful manual sampling or an autosampler are used for the procedure, since the method does not rely on exhaustive extraction, and reproducible results can be achieved if extraction

conditions (extraction time, sample temperature, stirring rate, and salt concentrations) are reproduced exactly. Fiber coating thicknesses and types must be chosen carefully for a particular sample type, however. For example, if a sample contains not only relatively volatile but also relatively nonvolatile components, a problem could exist. If a thick polymer coating is used to extract the volatile components, prolonged fiber cleanup (using a heating block with helium sparge) of up to an hour may be needed to remove all the nonvolatile components from the fiber, since they will not be removed with the typical 5-min desorption time in the GC inlet normally used for SPME. Without complete thermal cleaning, carryover of nonvolatiles is likely. On the other hand, a 7-μm coating would extract and efficiently release the nonvolatiles upon injection, but might not retain the volatile analytes during the extraction.

Originally, problems with SPME involved mostly the fragility of the fibers and differences in extraction efficiencies between individual fibers. These problems have been largely overcome, especially with the advent of metal fiber cores and better production techniques. However, fiber lifetimes may still vary from only one use to approximately 100 uses. Another factor is that the fiber coatings are reported to be subject to degradation by high salt concentrations, required for maximizing extractions of very dilute volatile components with fiber–water partition coefficients less than 1000. The limited lifetime of the fibers must be taken into account when the cost per sample is considered, since each fiber costs between $85 and $170 (2007 prices).[21]

A modified SPME technique addresses the fragile fiber problem by replacing the coated fiber with a syringe needle trap. The inside of the needle is coated with an immobilized sorbent such as PDMS or even packed with a solid adsorbent such as Carboxen, with resulting sorbent volumes up to six times the extraction phase possible for SPME. Not only is the needle less fragile than a coated fiber, but dynamic sampling is possible, lending the term *solid-phase dynamic extraction* (SPDE) to this method.[22] SPDE can be used for direct (DI-SPDE) and headspace (HS-SPDE) microextractions with an autosampler.[23] In the analytical literature, this technique is also called *microextraction in a packed syringe* (MEPS).

1.2.5 Solvent Microextraction

Solvent microextraction, in its four main modes—single-drop microextraction (SDME), headspace single-drop microextraction (HS-SDME), hollow fiber–protected microextraction (HFME), and dispersive liquid–liquid microextraction (DLLME)—are the methods discussed here. The first two modes (SDME and HS-SDME) are also easily automated and can involve either static or dynamic sampling, which are explained fully later. SME is actually based on all of the methods discussed earlier in the chapter, but can best be compared directly to SPME, with which it shares the same basic operational theory. The technique can be traced to several articles: one by Liu and Dasgupta[24] which described a device for continuous monitoring of a stream of gas with a microliter volume extractant, and one by Jeannot and Cantwell describing a polymer rod device for extraction using an 8-μL drop at its tip.[1] Jeannot and Cantwell[25] and He and Lee[26] realized almost immediately, however, that replacing the polymer rod with

a standard GC syringe would not only allow for extraction from a water sample, but the microdrop could then be withdrawn into the syringe and the extract injected directly into the GC for analysis. This technique, which Jeannot and Cantwell referred to as solvent microextraction, has been referred to variously as single-drop microextraction and liquid-phase microextraction[27,28] Thus, the solvent microdrop is used to extract and concentrate the analytes, while effectively cleaning up the sample and changing the solvent to one compatible with GC. The enrichment factor for this method can range up to 1000 or more, and the extraction times range from a few seconds to 1 h. The method is easily automated, allowing for precision, reduced labor and faster extraction times, using dynamic extraction, which will be described in detail in the next chapter. Soon, publications appeared extending the method to headspace samples[29] and even biological samples.[30] The method has even been extended to the use of derivatizing agents for analytes such as aldehydes and ketones in the extraction solvent, thus increasing the sensitivity and specificity of extraction.[31]

A major advantage of SME can be illustrated by comparing extractions using headspace extraction versus HS-SDME, which was introduced through a set of papers and presentations by Przyjazny, et al.,[29,32] Theis et al.,[33] Shen and Lee,[34] and Kokosa and Przyjazny[35] (See Section 7.3 and Figures 7.1 to 7.4 for the chromatograms for the following discussion.) The standard test method for gasoline diluent in used motor oils is ASTM method D 3525-93.[36] This method involves injecting a tetradecane solution of the oil directly into a GC for analysis. Obviously, this would present major contamination problems when using a GC with a capillary column. An alternative would be to analyze a headspace sample (Figure 7.1). The problem is that whereas volatile components of the gasoline are present at high concentrations in the headspace, higher-boiling components are present in decreasing amounts, resulting in a chromatogram very different from that resulting from the ASTM procedure. However, using tetradecane or hexadecane as the extracting solvent for HS-SDME results in a chromatogram (Figure 7.4) almost identical to the ASTM results.[35] This is because the higher-boiling components have increasingly larger oil/extracting solvent partition coefficients, resulting in higher extraction efficiencies for them.

In the hollow fiber–protected mode developed by Pedersen-Bjergaard and Rasmussen in 1999 and referred to by them as liquid–phase microextraction, the extracting system consists of a porous polypropylene hollow fiber, usually sealed at one end and containing between 4 and 20 μL of the extracting solvent.[30] HFME is actually a liquid–liquid membrane extraction which is very similar in principle to supported liquid membrane (SLM) methodology reported extensively in the literature by Jönsson and Mathiasson.[37–39] The porous polymer effectively prevents the biological matrix, including proteins, from contaminating the extractant. Two modes for HFME exist: a two-phase system developed by Shen and Lee[40] [which we refer to as HF(2)ME] in which the fiber (actually, a small-diameter polymer tube) is filled with an organic extraction solvent such as 1-octanol and a three-phase system [which we refer to as HF(3)ME]. The two-phase method, which is sometimes called microporous membrane liquid–liquid extraction, may be useful for

water samples highly contaminated by solids such as silt.[41] The three-phase system, which is also called the SLM technique, has the polymeric hollow fiber saturated with an organic solvent, while the lumen of the fiber contains an aqueous phase (acceptor phase), usually acidic or basic. This method may be useful for extracting pharmaceuticals or metabolites from biological fluids.[42,43] The main disadvantage of HFME is that, at present, it is not easy to automate the method and the fiber extraction devices are constructed manually, resulting in reproducibility problems.[44,45] Despite these difficulties, however, the method has proved useful, especially for bioresearch and applications where drop stability can be a problem.[46] It may be noted that three-phase systems can also be accomplished without the use of porous hollow fibers.[47]

A recently developed mode, dispersive liquid–liquid microextraction, is actually based on the long-known technique of trituration used by synthetic chemists to purify chemicals. Thus, a contaminated chemical would be dissolved in a solvent such as ethanol or acetone and the solution rapidly pipetted into vigorously stirred water, which dispersed the water-insoluble chemical and allowed efficient extraction into the water of impurities. DLLME, first reported by Rezaee et al. in 2006,[48] involves dissolving 8.0 to 50 μL of a water-insoluble extraction solvent such as tetrachloroethene in 0.5 to 2.0 mL of a water-soluble solvent such as acetone, and rapidly injecting it into 5 mL of the water sample contained in a centrifuge tube.[49–51] The tube is then centrifuged and a portion of the extracting solvent is removed using a syringe and injected into a GC or concentrated, redissolved in acetonitrile, and injected into an HPLC. A major limitation of the technique has been that only solvents slightly soluble in water and denser than water, such as tetrachloroethene, carbon tetrachloride, carbon disulfide or chlorobenzene, could be used as extractants. This limitation has been partially circumvented in a modification developed by using a liquid extractant such as undecanol, which has a melting point close to room temperature and a density less than that of water.[52] By cooling the solution after centrifugation, the solidified drop can be removed from the vial, melted, and analyzed. The method appears not to have any major advantages over SDME or HS-SDME for extracting most samples for GC analysis. However, DLLME does have a major advantage over SDME when used for the preparation of HPLC samples for high-boiling nonpolar analytes such as polycyclic aromatic hydrocarbons (PAHs), PCBs, and pesticides, since the larger extraction volumes of water and solvent used result in the larger amounts of extracted analytes required by HPLC. The technique has also been used effectively with a complexing agent for the extraction of cadmium from water, followed by AA analysis.[53] One major disadvantage of this technique compared to SDME is the fact that several discrete steps must be taken, including centrifugation. This limits the method to semiautomation, since extraction and injection are not performed in one device.

SME theory shows that the amount of analyte extracted is almost directly proportional to the volume of the microdrop, but this has practical limitations for SDME and HS-SDME, since a drop larger than 3 μL in a stirred solution or headspace has a tendency to fall off the syringe needle. The practical drop size is therefore 1 to 2 μL, at least two to three times the volume of an SPME fiber coating.

Another major advantage of SME is that the extractant is renewed with each sampling, eliminating the problem of carryover possible with SPME, SPDE, and PT. Although SME is not a solventless technique, only microliters of solvent are actually used. Although one might think that traditional extraction solvents such as dichloromethane, chloroform, ethyl acetate, and ethyl ether would be the solvents of choice for SME, this is not the case. These solvents are too soluble in water to be used for SME in most cases. See chapter 4 for a more detailed discussion. As mentioned above, DLLME requires either a water-insoluble solvent denser than water or a high-melting liquid less dense than water. Volatile solvents such as dichloromethane cannot be used at room temperature or above for SDME, HS-SDME, or HFME, because they evaporate immediately in the headspace and/or dissolve in water. Thus, the most volatile solvents commonly used for SDME and HS-SDME extractions are toluene and the xylenes, which are used to extract analytes with higher boiling points, such as the PAHs. High-boiling solvents such as 1-octanol or tetradecane are used in turn for extracting volatile chemicals. One limitation of the method when using a solvent such as 1-octanol for extraction is that the GC injector must be set at a split from 10 : 1 to 40 : 1 to yield resolved peaks. On the other hand, this leads to sharp peaks with large signal-to-noise ratios. It has been observed that the split inlet, as with PT, tends to act somewhat as a jet separator, concentrating the analytes, and thus a 10 : 1 split does not necessarily mean that 90% of the sample is lost. If a solvent lower boiling than the extracted analytes is used, traditional splitless injections are possible, with greater chromatographic sensitivity.

One might think that using an extractant such as tetradecane or even 1-octanol would pose difficulties in extracting more polar analytes, such as alcohols and acids. In fact, extraction efficiencies are directly dependent on the water/solvent distribution ratio and polar or hydrogen-bonding-capable analytes are extracted more efficiently with 1-octanol rather than tetradecane. However, even analytes containing polar functional groups have significant solubility in tetradecane at the very low concentrations typically encountered in SME. The water/1-octanol distribution coefficient, K_{ow}, is easily calculated (references and examples are given in Chapters 3 and 4) or available in the literature and distribution coefficients for other solvents, such as toluene or tetradecane, are also available in the literature or can be estimated fairly accurately from K_{ow}.[54] Before this technique is attempted, however, the reader is strongly advised to carry out the calculations illustrated in Chapter 4 to determine whether enough analyte will be extracted to meet the requirements needed for its quantitative determination.

One approach often taken to increase the amount of analyte extracted and thus analyzed is to increase the volume or mass of sample. This may not work for methods such as SME and SPME. The theory for SME clearly shows that the amount of analyte extracted depends on the size of the drop (~ 1 to 2 μL) (or fiber volume for SPME), the solvent, the salt concentration, the sample temperature, and most importantly, the water/solvent distribution coefficient (K_{ow}) and Henry's law constant (K_{aw}) for headspace extractions. For analytes with K_{ow} values below about 1000, the amount extracted does *not* increase linearly with sample volume and quickly reaches a maximum. See Chapters 4 and 5 for example calculations and

plots. This means that for all practical purposes, the typical amount of water sample that should normally be extracted for chemicals up to the boiling point of naphthalene is 1 to 4 mL. Larger sample volumes (10 to 40 mL) often seen in the literature simply will not give better results. This is not the case for chemicals such as the PAHs, PCBs, and nonpolar pesticides, however, since theory shows that the extraction for chemicals with large K_{ow} values is essentially exhaustive, and larger sample volumes can be extracted effectively. However, it should be realized that the larger the volume extracted, the longer it will take the system to come to equilibrium. In addition, there is a practical limit to the actual amount of analyte that can be dissolved in 1 to 2 μL of the extracting solvent. This is a common limitation not only for SME, but also SPME, that is often forgotten. For example, in one comprehensive study, the experiment was designed to extract 1 μg of each of several PAH analytes into 1 μL of undecane.[55] Calculations for the study above might show that the PAHs should be extracted exhaustively, but this ignores the fact that as a practical matter, PAHs cannot dissolve completely in undecane at these concentrations.

One remaining major disadvantage of SME has recently been overcome. No new sampling method will be widely accepted unless it is sensitive, accurate, and precise and amenable to automation. SME has similar sensitivity, accuracy, and precision to the other methods listed in this chapter, but until recently it was not automated. This has been overcome by the development of computer programs allowing a commercial autosampler to carry out the SME extraction and injection of the sample into a GC or HPLC.[56] Similar programming should allow interfacing to commercial CE and AA systems. Automation of the extraction is important, not just because it removes the need for intense manual labor, but also because it allows the use of dynamic SME. As discussed above, SME, like SPME, is often not an exhaustive extraction process but an equilibrium process. Manual extraction, with practice, does give very reproducible results. However, to maximize the extraction of analytes, it is necessary for the extraction system to come to equilibrium, and this often can take 10 to 30 min or more. If, however, a dynamic extraction is used, equilibrium can be achieved in 10 min or less. For example, one variant of dynamic extraction involves depositing the drop at the tip of the needle withdrawing it into the needle, and repeating the process 10 to 30 times. This repeated extraction increases the flux of analyte through the surface of the drop into the interior and thus decreases equilibration time. Dynamic extraction is, however, practical only when using a computer-controlled autosampler, due to the need for accurate and precise timing and syringe plunger movement. The ability to conduct dynamic sampling, an option not available for SPME, decreases the time required to bring the extraction to equilibrium, thus increasing sample throughput.

1.3 SUMMARY

In this chapter we have presented a summary of some of the most commonly used sample preparation techniques. Each method has distinct advantages and disadvantages. For instance, liquid–liquid extraction is simple, straightforward, relatively sensitive, and technically simple. The method can be tedious and requires

sizable amounts of sample as well as large amounts of hazardous and expensive solvents, which are its major limitations. Solid-phase extraction decreases the manual labor and solvent requirements dramatically and has good sensitivity, selectivity, accuracy, and precision. However, sample preparation is only semi-automated, requires 1 to 2 h per sample, and if analyte concentration through solvent evaporation is necessary, the method is limited to less volatile analytes.

Static headspace analysis is technically the simplest sample preparation method and fairly sensitive for volatile chemicals. However, it requires careful calibration to be useful for quantification. In addition, the method is of limited usefulness for the less volatile chemicals. Purge-and-trap, on the other hand, is one of the most sensitive sample preparation methods available for volatile chemicals, and has good accuracy and precision. The major disadvantages of PT are the possibilities of sample carryover and potential leaks in the system, as well as the expense of the instrumentation.

Solid-phase microextraction and solvent microextraction are very similar in both operational theory and practical method development, and potentially can replace or supplement the sample preparation methods discussed above. In many ways these two techniques are complementary. For instance, SPME may be the method of choice for sensitivity when analyzing very volatile chemicals if a Carboxen extraction fiber is used. On the other hand, SME may be the method of choice for sensitivity when analyzing nonpolar PAHs, PCBs, and pesticides. SME may also be the method of choice when analyzing samples containing a complex matrix or analytes with wide differences in boiling points, as discussed in Section 1.2.4.

In the remaining chapters we help the analyst to decide whether SME is an appropriate method to use for a sample preparation. In Chapter 2 we give a more detailed view of each SME mode and instrumentation requirement. Chapter 3 is a comprehensive examination of the theory for SME. Chapter 4 is a practical chapter, intended to introduce the analyst quickly to the basic example calculations resulting from SME theory and the suggested experimental conditions needed to develop an SME method. In Chapter 5 we then look in detail at each step of SME method development. In Chapter 6 we summarize the literature on SME, including recent developments in the field. Finally, in Chapter 7 we present a number of detailed, validated SME experimental procedures that the analyst can use as a starting point for a specific sample.

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