1.4.4 Extraction

The sample preparation techniques previously discussed are used for inorganic samples or for the determination of inorganic components in organic materials by removing the organic matrix. Obviously, they cannot be used if we want to determine organic analytes. The most common approach for organic analytes is to extract the analytes out of the sample matrix using a suitable solvent. Solvents are chosen with the polarity of the analyte in mind, since "like dissolves like." That is, polar solvents dissolve polar compounds, while nonpolar solvents dissolve nonpolar compounds. Common extraction solvents include hexane, methylene chloride, methyl isobutyl ketone (MIBK), and xylene.

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1.4.4.1 Solvent Extraction

Solvent extraction is based on preferential solubility of an analyte in one of two immiscible phases. There are two common situations that are encountered in analysis: extraction of an organic analyte from a solid phase, such as soil, into an organic solvent for subsequent analysis and extraction of an analyte from one liquid phase into a second immiscible liquid phase, such as extraction of PCBs from water into an organic solvent for subsequent analysis.

Liquid–liquid extraction is similar to what happens when you shake oil and vinegar together for salad dressing. If you pour the oil and vinegar into a bottle carefully, you will have two separate clear layers because the oil and vinegar are not soluble in each other (they are *immiscible*). You shake the bottle of oil and vinegar vigorously, and the "liquid" gets cloudy and you no longer see the two separate phases. On standing, the two immiscible phases separate into clear liquids again. Our two immiscible solvents will be called solvent 1 and solvent 2. The analyte, which is a solute in solvent 2, will distribute itself between the two phases on vigorous shaking. After allowing the phases to separate, the ratio of the concentration of analyte in the two phases is approximately a constant, K_D:

$$K_D = \frac{[A]_1}{[A]_2} \tag{1.16}$$

K_D is called the distribution coefficient and the concentrations of A, the analyte, are shown in solvent 1 and solvent 2. A large value of K_D means that the analyte will be more soluble in solvent 1 than in solvent 2. If the distribution coefficient is large enough, most of the analyte can be extracted quantitatively out of solvent 2 into solvent 1. The liquid containing the analyte and the extracting solvent are usually placed into a separatory funnel, shaken manually, and the desired liquid phase drawn off into a separate container. The advantages of solvent extraction are to remove the analyte from a more complex matrix, to extract the analyte into a solvent more compatible with the analytical instrument to be used, and to preconcentrate the analyte. For example, organic analytes can be extracted from water using solvents such as hexane. Typically, 1 L of water is extracted with 10–50 mL of hexane. Not only is the analyte extracted, but it is also now more concentrated in the hexane than it was in the water. The analyte percent extracted from solvent 2 into solvent 1 can be expressed as

$$\%E = \frac{[A]_1 V_1}{[A]_2 V_2 + [A]_1 V_1} \times 100 \tag{1.17}$$

where

$\%E$ is the percent of analyte extracted into solvent 1, the concentration of analyte in each solvent is expressed in molarity

V_1 and V_2 are the volumes of solvents 1 and 2, respectively

The percent extracted is also related to K_D:

$$\%E = \frac{100 K_D}{K_D + (V_2 / V_1)} \tag{1.18}$$

The percent extracted can be increased by increasing the volume of solvent 1, but it is more common to use a relatively small volume of extracting solvent and repeat the extraction more than once. The multiple volumes of solvent 1 are combined for analysis. Multiple small extractions are more efficient than one large extraction.

Liquid–liquid extraction is used extensively in environmental analysis to extract and concentrate organic compounds from aqueous samples. Examples include the extraction of pesticides, PCBs,

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and petroleum hydrocarbons from water samples. Extraction is also used in the determination of fat in milk. Liquid–liquid extraction can be used to separate organometallic complexes from the matrix in clinical chemistry samples such as urine. For example, heavy metals in urine can be extracted as organometallic complexes for determination of the metals by flame AAS. The chelating agent and a solvent such as MIBK are added to a pH-adjusted urine sample in a separatory flask. After shaking and being allowed to stand, the organic solvent layer now contains the heavy metals, which have been separated from the salts, proteins, and other components of the urine matrix. In addition to now having a "clean" sample, the metals have been extracted into a smaller volume of solvent, increasing the sensitivity of the analysis. An added benefit is that the use of the organic solvent itself further increases the sensitivity of flame AAS measurement (as discussed in Chapter 6).

Extraction of organic analytes such as pesticides, PCBs, and fats from solid samples such as food, soil, plants, and similar materials can be done using a Soxhlet extractor. A Soxhlet extractor consists of a round-bottom flask fitted with a glass sample/siphon chamber in the neck of the flask. On top of the sample chamber is a standard water-cooled condenser. The solid sample is placed in a cellulose or fiberglass porous holder, called a thimble; the solvent is placed in the round-bottom flask. Using a heating mantle around the flask, the solvent is vaporized, condensed, and drips or washes back down over the sample. Soluble analytes are extracted and then siphoned back into the round-bottom flask. This is a continuous extraction process as long as heat is applied. The extracted analyte concentrates in the round-bottom flask.

As you can imagine, performing these extractions manually is time-consuming and can be hard work (try shaking a 1 L separatory funnel full of liquid for 20 min and imagine having to do this all day!). In addition, a lot of bench space is needed for racks of 1 L flasks or multiple heating mantles. There are several instrumental advances in solvent extraction that have made extraction a more efficient process. These advances generally use sealed vessels under elevated pressure to improve extraction efficiency and are classified as pressurized fluid (or pressurized solvent) extraction methods. One approach is the Accelerated Solvent Extraction (ASE®) system, from Dionex, now part of Thermo Fisher Scientific (www.dionex.com, www.thermofisher.com). This technique is used for extracting solid and semisolid samples, such as food, with liquid solvents. The technique is shown schematically in Figure 1.7. ASE uses conventional solvents and mixtures of solvents at elevated temperature and pressure to increase the efficiency of the extraction process. Increased temperature, up to 200°C compared with the 70°C–80°C normal boiling points of common solvents, accelerates the extraction rate, while elevated pressure keeps the solvents liquid at temperatures above their normal boiling points, enabling safe and rapid extractions. Extraction times for many samples can be cut from hours using a conventional approach to minutes, and the amount of solvent used is greatly reduced. Table 1.13 presents a comparison of the use of a commercial ASE system with conventional Soxhlet extraction. Dozens of application examples, ranging from fat in chocolate through environmental and industrial applications, can be found at the Dionex website. The SpeedExtractor from Büchi Corporation, introduced in 2009, also has applications available at www.mybuchi.com. The US EPA has recognized ASE and other instruments that use pressure and temperature to accelerate extraction of samples for environmental analysis by issuing US EPA Method 3545A (SW-846 series) for Pressurized Fluid Extraction of samples. The method can be found at www.usepa.gov.

A second approach also using high pressure and temperature is that of microwave-assisted extraction. The sample is heated with the extraction solvent in a sealed vessel by microwave energy, as was described for microwave digestion. The temperature can be raised to about 150°C with the already described advantages of high temperature and high pressure. One limitation of microwave-assisted extraction is that some solvents are "transparent" to microwave radiation and do not heat; pure nonpolar solvents such as the normal alkanes are examples of such transparent solvents. Several instrument companies manufacture microwave extraction systems. Milestone Inc. (www. milestonesci.com) has numerous applications on their website as well as video compact disks (CDs) of microwave extraction, ashing, and digestion available. CEM (www.cem.com) also has application notes available for

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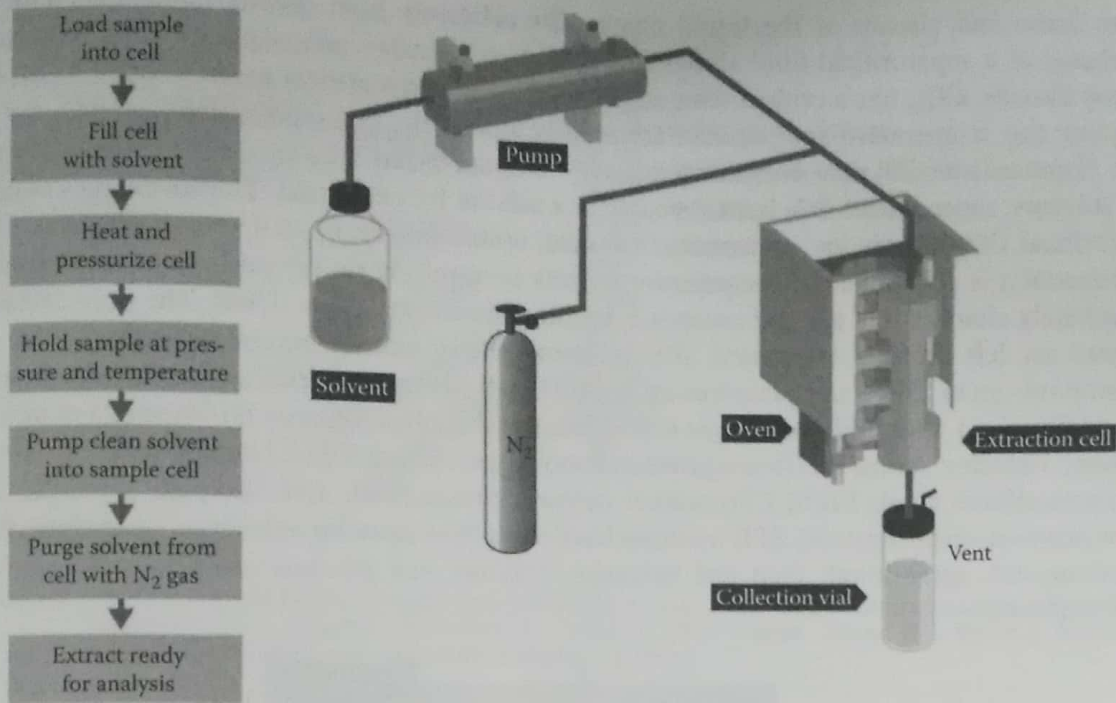


Figure 1.7 ASE technique. (Courtesy of Dionex Corp., Sunnyvale, CA, www.dionex.com; © Thermo Fisher Scientific (www.thermofisher.com). Used with permission.)

Table 1.13 Comparison of Soxhlet Extraction with ASE

Extraction Method	Average Solvent Used per Sample (mL)	Average Extraction Time per Sample	Average Cost per Sample (US $)
Manual Soxhlet	200–500	4–48 h	27
Automated Soxhlet	50–100	1–4 h	16
Accelerated solvent extraction	15–40	12–18 min	14

Source: Courtesy of Dionex Corp., Sunnyvale, CA. www.dionex.com; © Thermo Fisher Scientific (www.thermofisher.com). Used with permission.

Table 1.14 Comparison of Microwave-Assisted Extraction with Conventional Solvent Extraction for Herbicides in Soil Samples

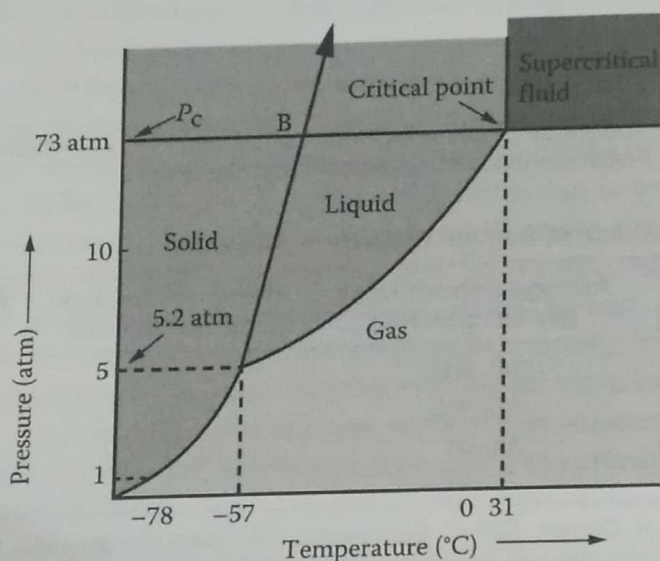
Extraction Method	Time (min)	Volume of Solvent (mL)	% Recovery
Separatory funnel	15	25	42–47
Soxhlet	90	40	51–52
Microwave extraction	10 (90°C)	20	66–78

Source: Data in table courtesy of Milestone Inc., Shelton, CT, www.milestonesci.com.

microwave extraction. An example of improved performance from a microwave extraction system versus conventional extraction is shown in Table 1.14.

The third instrumental approach is the use of supercritical fluid extraction (SFE). A **supercritical fluid** is a substance at a temperature and pressure above the critical point for the substance. You may want to review phase diagrams and the critical point on the phase diagram in your general chemistry text or see the Applied Separations, Inc. website at www.appliedseparations.com. Supercritical fluids are more dense and viscous than the gas phase of the substance but

not as dense and viscous as the liquid phase. The relatively high density (compared with the gas phase) of a supercritical fluid allows these fluids to dissolve nonvolatile organic molecules. Carbon dioxide, CO_2, has a critical temperature of 31.3°C and a critical pressure of 72.9 atm (see diagram); this temperature and pressure are readily attainable, making supercritical CO_2 easy to form. Supercritical CO_2 dissolves many organic compounds, so it can replace a variety of common solvents; supercritical CO_2 is used widely as a solvent for extraction. The advantages of using supercritical CO_2 include its low toxicity, low cost, nonflammability, and ease of disposal. Once the extraction is complete and the pressure returns to atmospheric pressure, the carbon dioxide immediately changes to a gas and escapes from the opened extraction vessel. The pure extracted analytes are left behind. Automated SFE instruments can extract multiple samples at once at temperatures up to 150°C and pressures up to 10,000 psi (psi, pounds per square inch, is not an SI unit; 14.70 psi = 1 atm). SFE instrument descriptions and applications can be found at a number of company websites: Jasco, Inc. (www.jascoinc.com); Supercritical Fluid Technologies, Inc. (www.supercriticalfluids.com); Büchi Corporation (www.mybuchi.com); and Newport Scientific, Inc. (www.newport-scientific.com). SFE methods have been developed for extraction of analytes from environmental, agricultural, food and beverage, polymer and pharmaceutical samples, among other matrices.



1.4.4.2 *Solid-Phase Extraction*

In **solid-phase extraction** (SPE), the "extractant" is not an organic liquid but a solid-phase material. Organic compounds are chemically bonded to a solid substrate such as silica beads or polymer beads. The bonded organic layer interacts with organic analytes in the sample solution and extracts them from the sample solution as it is poured through a bed or disk of the solid extractant. The excess solution is allowed to drain away, and interfering compounds are washed off the extractant bed with a solution that does not remove the target analytes. The extracted organic analytes are then **eluted** from the solid-phase extractant by passing a suitable organic solvent through the bed. The interactions that cause the analytes to be extracted are those intermolecular attractive forces you learned about in general chemistry: van der Waals attractions, dipole–dipole interactions, and electrostatic attractions.

The types of organic compounds that can be bonded to a solid substrate vary widely. They can be hydrophobic nonpolar molecules such as C_8 and C_{18} hydrocarbon chains, chains with highly polar functional groups such as cyano (—C≡N), amine (—NH_2), and hydroxyl (—OH) groups and with

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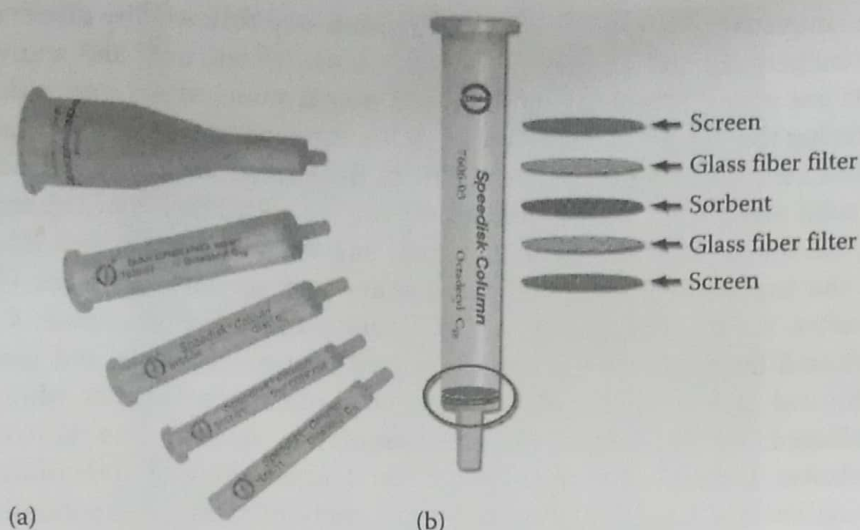

Figure 1.8 (a) Commercial plastic syringe-type cartridges and (b) schematic of sorbent packing. (Permission to reprint from Avantor Performance Materials, Inc., Center Valley, PA, formerly known as Mallinckrodt Baker, Inc., www.avantormaterials.com.)

ionizable groups like sulfonic acid anions ($—SO_3^-$) and carboxylic acid anions ($—CO_2^-$), to extract a wide variety of analytes. The term **sorbent** is used for the solid-phase extractant. Commercial SPE cartridges have the sorbent packed into a polymer syringe body or disposable polymer pipette tip. Figure 1.8a shows commercial plastic syringe-type cartridges with a variety of sorbents, including several of those just mentioned, while Figure 1.8b shows a schematic of how the sorbent is packed and held in the cartridge. These are used only once, preventing cross-contamination of samples and allowing the cleanup of extremely small sample volumes (down to 1 μL), such as those encountered in clinical chemistry samples. Specialized sorbents have been developed for the preparation of urine, blood, and plasma samples for drugs of abuse, for example. Automated SPE systems that can process hundreds of samples simultaneously are now in use in the pharmaceutical and biotechnology industries.

SPE is used widely for the cleanup and concentration of analytes for analysis using LC, HPLC, and LC-MS, discussed in Chapter 13. As you will see, the phases used in HPLC for the separation of compounds are in many cases identical to the bonded solid-phase extractants described here. Detailed examples and application notes are available from a number of SPE equipment suppliers: Avantor Performance Materials, Inc. (www.avantormaterials.com), Supelco (www.sigma-aldrich.com/supelco), Phenomenex (www.phenomenex.com), and Büchi Corporation (www.mybuchi.com) are a few of the companies that supply these products.

1.4.4.3 QuEChERS

A very important analytical method is the determination of pesticide residues in fruits, vegetables, and processed foods such as cereal. The method used to require complex extraction and cleanup of samples prior to HPLC and MS analysis. In 2003, US Department of Agriculture scientists (Anastassiades et al.) developed a simplified method for food samples, called QuEChERS (pronounced "ketchers" or "catchers"), which stands for quick, easy, cheap, effective, rugged, and safe. QuEChERS has now been applied to food samples, dietary supplements, and other matrices for pesticides, veterinary drugs, and other compounds and is recognized as a sample preparation method in AOAC and European standards. The QuEChERS

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approach uses a simple two-step approach, an extraction step followed by dispersive SPE. First, the sample is homogenized and weighed into a 50 mL centrifuge tube and appropriate organic solvent and salts are added. The salts may include magnesium sulfate and sodium acetate for drying and buffering the sample. The sample is shaken and centrifuged. The supernatant is further extracted and cleaned using dispersive SPE. In dispersive SPE, the SPE sorbent(s) is(are) in a centrifuge tube along with a small amount of $MgSO_4$. An aliquot of the supernatant from step 1 is added, the tube is shaken and centrifuged, and the sorbent removes interfering matrix materials from the sample. Sorbents include primary and secondary amine (PSA) exchange materials to remove sugars, fatty acids, organic acids, and some pigments; C18 (a common octadecyl-substituted hydrophobic LC material) used to remove lipids and nonpolar interferences and graphitized carbon black (GBC) to remove pigments and sterols. Multiple companies now market packaged QuEChERS kits that meet regulatory standards in the United States and Europe. The websites www.quechers.com, www.restek.com, www.gerstel.com/en/applications.htm, and www.agilent.com/chem/Quechers offer a variety of tutorials, application notes, audio slideshows, webinars, and video demonstrations of the QuEChERS process.

A recent environmental application of the QuEChERS method was demonstrated by researchers from Gerstel, Inc. and the Arkansas Public Health Laboratory to deal with the huge 2010 oil well spill in the Gulf of Mexico (Whitecavage et al.). Estimates of the number of samples to be analyzed for petroleum hydrocarbons such as polyaromatic hydrocarbons (PAHs) in seafood ran as high as 10,000 samples per month. Standard regulatory assays took 1 week to analyze 14–25 samples. Using Gerstel's Twister™ stir bar sorptive extraction (SBSE), sample throughput for PAHs in seafood could be increased to 40 samples/day. The Twister™ is a glass-coated magnetic stir bar with an external layer of polydimethylsiloxane (PDMS). The stir bar is added to an aliquot of the supernatant from the QuEChERS step, and while stirring the solution, organic compounds are extracted into the PDMS phase. The Twister is removed from the sample, rinsed with DI water, dried with a lint-free cloth, and placed in a thermal desorption tube for automated direct thermal desorption into a GC-MS system. The SBSE step provides a concentration of the analytes as well as an additional cleanup of the supernatant, resulting in limits of detection 10–50 times lower than the regulatory assay while using significantly less solvent.

The SPE field is still developing, with the introduction in 2009 of an automated SPE instrument from Dionex designed to be used with large-volume samples (20 mL to 4 L) for the isolation of trace organics in water and other aqueous matrices.

1.4.4.4 Solid-Phase Microextraction

Solid-phase microextraction (SPME, pronounced "spee-mee" by some users) is a sampling technique developed first for analysis by GC; the use of SPME for GC and related applications is discussed in greater detail in Section 12.3. The solid phase in this case is a coated fiber of fused silica. The coatings used may be liquid polymers like PDMS, which is a silicone polymer. Solid sorbents or combinations of both solid and liquid polymers are also used. Figure 1.9a shows a commercial SPME unit with the coated fiber inserted into a sample vial; the coated fiber tip is shown in Figure 1.9b. No extracting solvent is used when the sample is analyzed by GC. The coated fiber is exposed to a liquid or gas sample or to the vapor over a liquid or solid sample in a sealed vial (this is called sampling the **headspace**) for a period of time. Analyte is adsorbed by the solid coating or absorbed by the liquid coating on the fiber and then thermally desorbed by insertion into the heated injection port of the gas chromatograph. The process is shown schematically in Figure 1.10.

Unlike solvent extraction, the entire amount of analyte is not extracted. The amount of analyte extracted by the coated fiber is proportional to the concentration of analyte in the sample. This will be true if equilibrium between the fiber and the sample is achieved or before equilibrium is achieved

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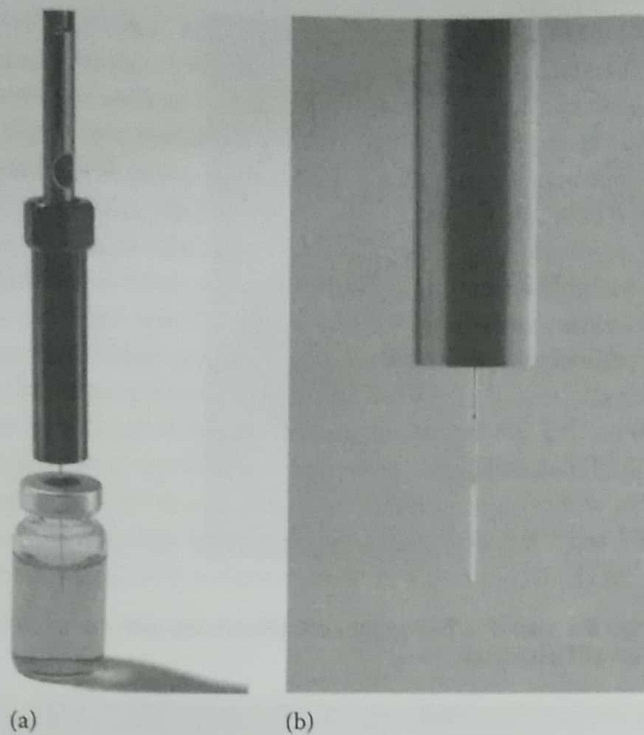

Figure 1.9 (a) A commercial SPME unit with (b) the coated fiber and inserted into a sample vial. (Reprinted with permission of Supelco, Bellefonte, PA, www.sigma-aldrich.com.)

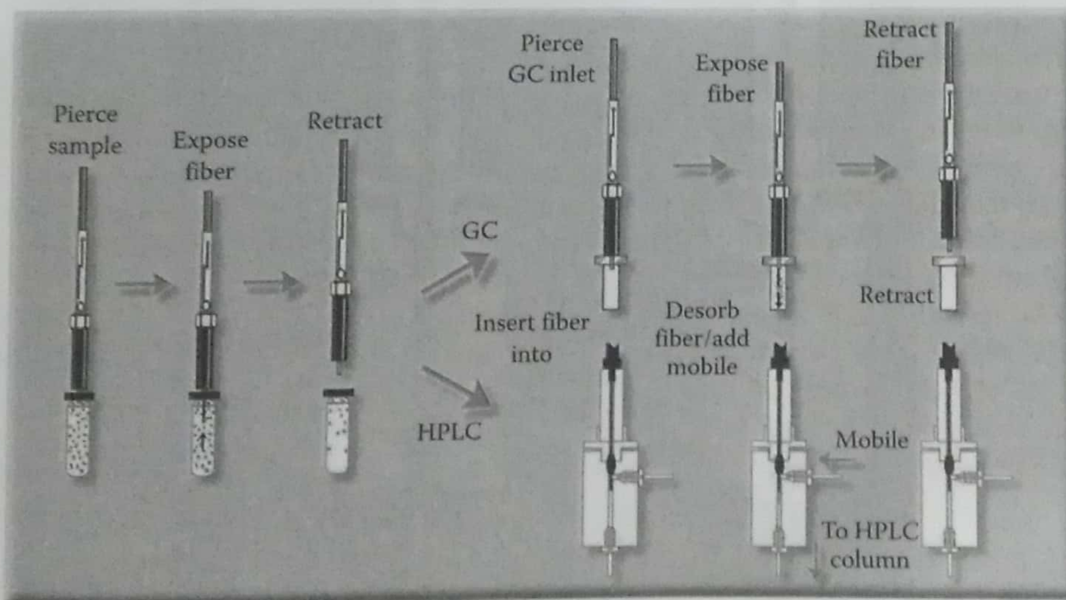

Figure 1.10 Schematic of the SPME process. (Reprinted with permission of Supelco, Bellefonte, PA, www.sigma-aldrich.com.)

if the sampling conditions are controlled carefully. SPME sampling and desorption can be used for qualitative and quantitative analyses. Quantitative analysis using external calibration, internal standard calibration, and the method of standard additions (MSA) are all possible with SPME. Calibration is discussed in Section 1.5.2 and at greater length in Chapter 2.

SPME sampling is used for a wide variety of analytes, including environmental pollutants, volatiles from botanical samples (e.g., used to identify tobacco species), explosives, and chemical

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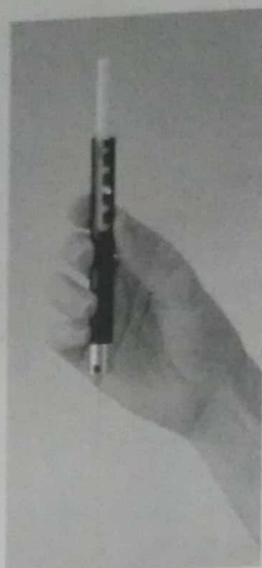

Figure 1.11 An SPME probe the size of a ball-point pen. (Reprinted with permission of Supelco, Bellefonte, PA. www.sigma-aldrich.com.)

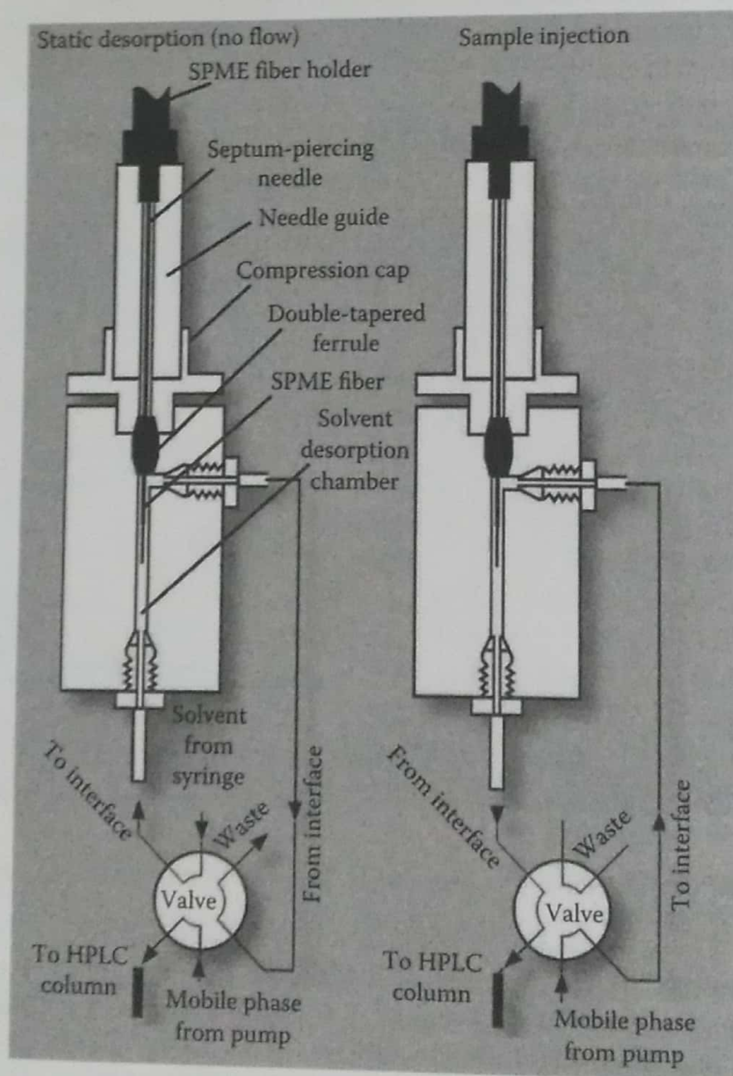

Figure 1.12 An SPME–HPLC interface. (Reprinted with permission of Supelco, Bellefonte, PA. www.sigma-aldrich.com.)

agent residues. Gasoline and other accelerants in the headspace over fire debris can be sampled with SPME to determine whether arson may have caused the fire. As little as 0.01 μL of gasoline can be detected. Gas samples such as indoor air and breath have been sampled using SPME. Liquid samples analyzed by either immersion of the fiber into the sample or sampling of the headspace vapor include water, wine, fruit juice, blood, milk, coffee, urine, and saliva. Headspace samplings of the vapors from solids include cheese, plants, fruits, polymers, pharmaceuticals, and biological tissue. These examples and many other application examples are available in pdf format and on CD from Supelco at www.sigma-aldrich.com/supelco. SPME probes that are about the size of a ball-point pen (Figure 1.11) are available for field sampling (e.g., see www.fieldforensics.com or www.sigma-aldrich.com/supelco). These can be capped and taken to an on-site mobile lab or transported back to a conventional laboratory for analysis.

While SPME started as a solvent-free extraction system for GC analysis, it can now also be used to introduce samples into an HPLC apparatus. An SPME–HPLC interface, Figure 1.12, allows the use of an SPME fiber to sample nonvolatile analytes such as nonionic surfactants in water and elute the analyte into the solvent mobile phase used for the HPLC analysis. The sampling process and elution are shown schematically in Figure 1.10. HPLC and its applications are covered in Chapter 13.

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