CHAPTER

2

PRINCIPLES OF EXTRACTION AND THE EXTRACTION OF SEMIVOLATILE ORGANICS FROM LIQUIDS

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2.1. PRINCIPLES OF EXTRACTION

This chapter focuses on three widely used techniques for extraction of semivolatile organics from liquids: liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME). Other techniques may be useful in selected circumstances, but these three techniques have become the extraction methods of choice for research and commercial analytical laboratories. A fourth, recently introduced technique, stir bar sorptive extraction (SBSE), is also discussed.

To understand any extraction technique it is first necessary to discuss some underlying principles that govern all extraction procedures. The chemical properties of the analyte are important to an extraction, as are the properties of the liquid medium in which it is dissolved and the gaseous, liquid, supercritical fluid, or solid extractant used to effect a separation. Of all the relevant solute properties, five chemical properties are fundamental to understanding extraction theory: *vapor pressure*, *solubility*, *molecular weight*, *hydrophobicity*, and *acid dissociation*. These essential properties determine the transport of chemicals in the human body, the transport of chemicals in the air–water–soil environmental compartments, and the transport between immiscible phases during analytical extraction.

Extraction or separation of dissolved chemical component X from liquid phase A is accomplished by bringing the liquid solution of X into contact with a second phase, B, given that phases A and B are immiscible. Phase B may be a solid, liquid, gas, or supercritical fluid. A distribution of the com-

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ponent between the immiscible phases occurs. After the analyte is distributed between the two phases, the extracted analyte is released and/or recovered from phase B for subsequent extraction procedures or for instrumental analysis.

The theory of chemical equilibrium leads us to describe the reversible distribution reaction as

$$X_A \rightleftharpoons X_B \tag{2.1}$$

and the equilibrium constant expression, referred to as the *Nernst distribution law* [1], is

$$K_D = \frac{[X]_B}{[X]_A} \tag{2.2}$$

where the brackets denote the concentration of X in each phase at constant temperature (or the activity of X for nonideal solutions). By convention, the concentration extracted into phase B appears in the numerator of equation (2.2). The equilibrium constant is independent of the rate at which it is achieved.

The analyst's function is to optimize extracting conditions so that the distribution of solute between phases lies far to the right in equation (2.1) and the resulting value of K_D is large, indicating a high degree of extraction from phase A into phase B. Conversely, if K_D is small, less chemical X is transferred from phase A into phase B. If K_D is equal to 1, equivalent concentrations exist in each phase.

2.1.1. Volatilization

Volatilization of a chemical from the surface of a liquid is a partitioning process by which the chemical distributes itself between the liquid phase and the gas above it. Organic chemicals said to be *volatile* exhibit the greatest tendency to cross the liquid–gas interface. When compounds volatilize, the concentration of the organic analyte in the solution is reduced. *Semivolatile* and *nonvolatile* (or *involatile*) describe chemicals having, respectively, less of a tendency to escape the liquid they are dissolved in and pass into the atmosphere above the liquid.

As discussed in this book, certain sample preparation techniques are clearly more appropriate for volatile compounds than for semivolatile and nonvolatile compounds. In this chapter we concentrate on extraction methods for semivolatile organics from liquids. Techniques for extraction of volatile organics from solids and liquids are discussed in Chapter 4.

Henry's Law Constant

If the particular extracting technique applied to a solution depends on the volatility of the solute between air and water, a parameter to predict this behavior is needed to avoid trial and error in the laboratory. The volatilization or escaping tendency (fugacity) of solute chemical X can be estimated by determining the gaseous, G, to liquid, L, distribution ratio, K_D, also called the *nondimensional*, or *dimensionless*, *Henry's law constant*, H'.

$$H' = K_D = \frac{[\mathrm{X}]_\mathrm{G}}{[\mathrm{X}]_\mathrm{L}} \tag{2.3}$$

The larger the magnitude of the Henry's law constant, the greater the tendency for volatilization from the liquid solvent into the gaseous phase [2–4].

According to equation (2.3), the Henry's law constant can be estimated by measuring the concentration of X in the gaseous phase and in the liquid phase at equilibrium. In practice, however, the concentration is more often measured in one phase while concentration in the second phase is determined by mass balance. For dilute neutral compounds, the Henry's law constant can be estimated from the ratio of vapor pressure, P_{vp}, and solubility, S, taking the molecular weight into consideration by expressing the molar concentration:

$$H = \frac{P_{\mathrm{vp}}}{S} \tag{2.4}$$

where P_{vp} is in atm and S is in mol/m^3, so H is in atm·m^3/mol.

Vapor Pressure

The vapor pressure, P_{vp}, of a liquid or solid is the pressure of the compound's vapor (gas) in equilibrium with the pure, condensed liquid or solid phase of the compound at a given temperature [5–9]. Vapor pressure, which is temperature dependent, increases with temperature. The vapor pressure of chemicals varies widely according to the degree of intermolecular attractions between like molecules: The stronger the intermolecular attraction, the lower the magnitude of the vapor pressure. Vapor pressure and the Henry's law constant should not be confused. *Vapor pressure* refers to the volatility from the pure substance into the atmosphere; the *Henry's law constant* refers to the volatility of the compound from liquid solution into the air. Vapor pressure is used to estimate the Henry's law constant [equation (2.4)].

Solubility

Solubility is also used to estimate the Henry's law constant [equation (2.4)]. *Solubility* is the maximum amount of a chemical that can be dissolved into another at a given temperature. Solubility can be determined experimentally or estimated from molecular structure [6,10–12].

The Henry's law constant, H, calculated from the ratio of vapor pressure and solubility [equation (2.4)] can be converted to the dimensionless Henry's law constant, H', [equation (2.3)] by the expression

$$H' = \frac{P_{\text{vp}}(\text{MW})}{0.062ST} \tag{2.5}$$

where P_{vp} is the vapor pressure in mmHg, MW the molecular weight, S the water solubility in mg/L, T the temperature in Kelvin, and 0.062 is the appropriate universal gas constant [9].

For the analyst's purposes, it is usually sufficient to categorize the escaping tendency of the organic compound from a liquid to a gas as high, medium, or low. According to Henry's law expressed as equation (2.4), estimating the volatilization tendency requires consideration of both the vapor pressure and the solubility of the organic solute. Ney [13] ranks vapor pressures as

- *Low:* 1×10^{-6} mmHg
- *Medium:* between 1×10^{-6} and 1×10^{-2} mmHg
- *High:* greater than 1×10^{-2} mmHg

while ranking water solubilities as

- *Low:* less than 10 ppm
- *Medium:* between 10 and 1000 ppm
- *High:* greater than 1000 ppm

However, note that in Ney's approach, concentration expressed in parts per million (ppm) does not incorporate molecular weight. Therefore, it does not consider the identity or molecular character of the chemical.

Rearranging equation (2.4) produces

$$P_{\text{vp}} = HS \tag{2.6}$$

In this linear form, a plot (Figure 2.1) of vapor pressure (y-axis) versus solubility (x-axis) yields a slope representing the Henry's law constant at values

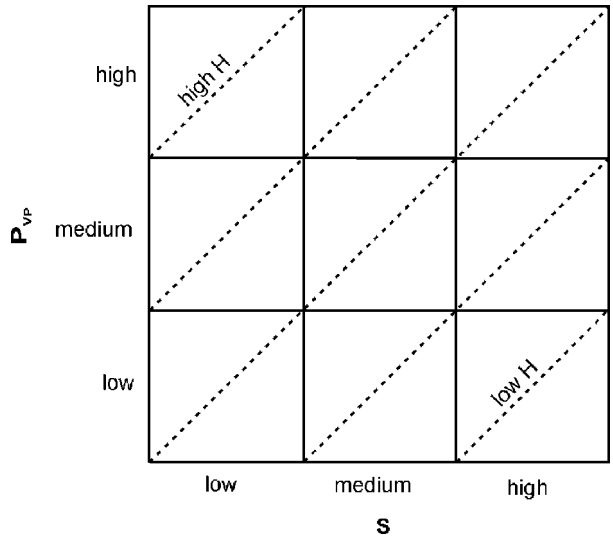


Figure 2.1. Henry's law constant at values of constant H conceptually represented by diagonal (dotted) lines on a plot of vapor pressure (P_{vp}) versus solubility, S.

of constant H. From this figure it can be deduced that low volatility from liquid solution is observed for organic chemicals with low vapor pressure and high solubility, whereas high volatility from liquid solution is exhibited by compounds with high vapor pressure and low solubility. Intermediate levels of volatility result from all other vapor pressure and solubility combinations. H is a ratio, so it is possible for compounds with low vapor pressure and low solubility, medium vapor pressure and medium solubility, or high vapor pressure and high solubility to exhibit nearly equivalent volatility from liquid solution.

The Henry's law constant can be used to determine which extraction techniques are appropriate according to solute volatility from solution. If the Henry's law constant of the analyte (solute) is less than the Henry's law constant of the solvent, the solute is nonvolatile in the solvent and the solute concentration will increase as the solvent evaporates. If the Henry's law constant of the analyte (solute) is greater than the Henry's law constant of the solvent, the solute is semivolatile to volatile in the solvent. In a solution open to the atmosphere, the solute concentration will decrease because the solute will evaporate more rapidly than the solvent.

Mackay and Yuen [2] and Thomas [4] provide these guidelines for organic solutes in water (Figure 2.2):

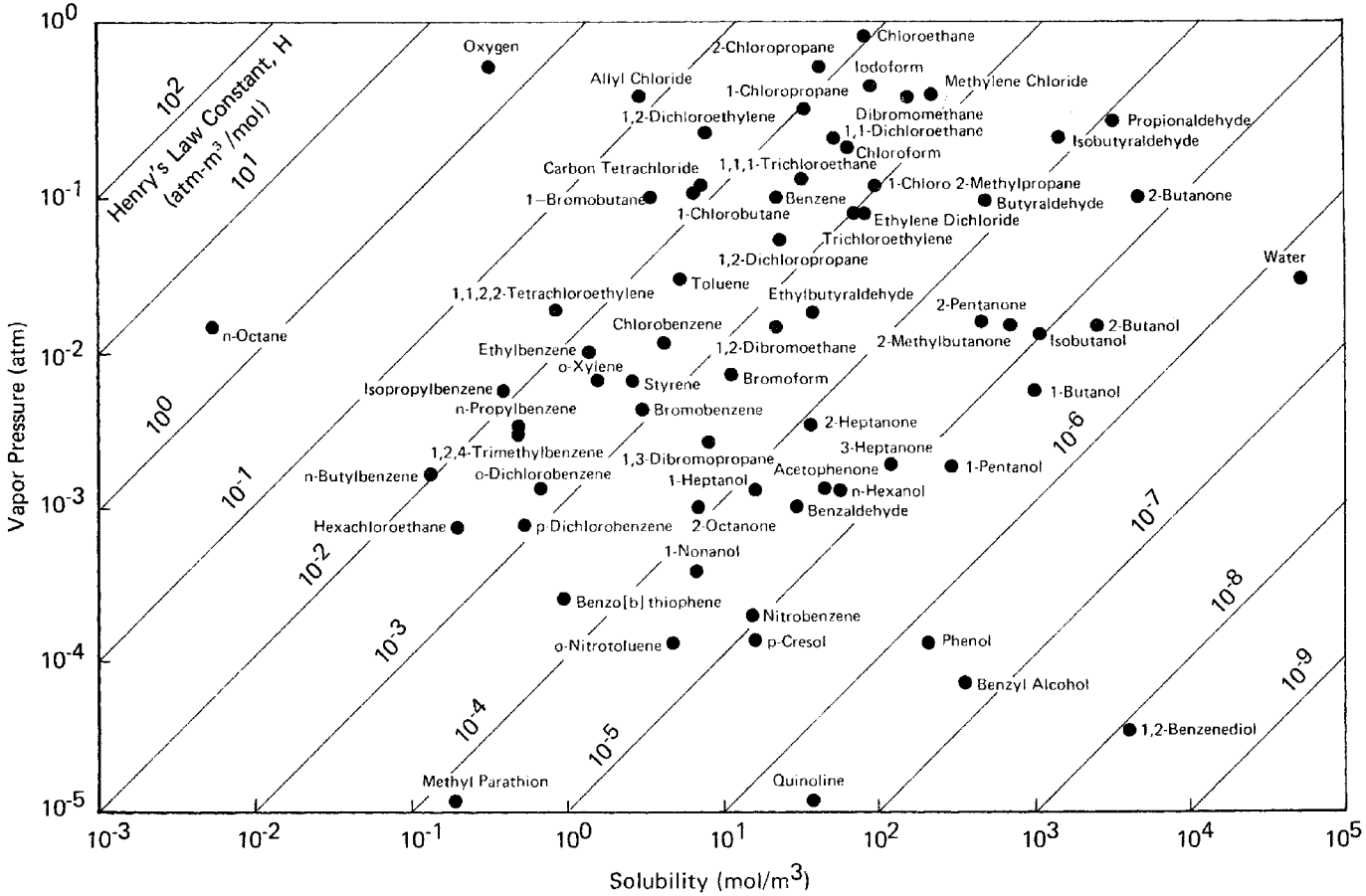


Figure 2.2. Solubility, vapor pressure, and Henry's law constant for selected chemicals [2,4]. (Reprinted with permission from Ref. 2. Copyright © 1980 Elsevier Science.)

- *Nonvolatile:* volatilization is unimportant for $H < 3 \times 10^{-7}$ atm·m^3/mol (i.e., H for water itself at 15°C)
- *Semivolatile:* volatilizes slowly for $3 \times 10^{-7} < H < 10^{-5}$ atm·m^3/mol
- *Volatile:* volatilization is significant in the range $10^{-5} < H < 10^{-3}$ atm·m^3/mol
- *Highly volatile:* volatilization is rapid if $H > 10^{-3}$ atm·m^3/mol

Schwarzenbach et al. [8] illustrate the Henry's law constant (Figure 2.3*c*) for selected families of hydrocarbons in relation to vapor pressure (Figure 2.3*a*) and solubility (Figure 2.3*b*). Vapor pressure (Figure 2.3*a*) and solubility (Figure 2.3*b*) tend to decrease with increasing molecular size. In Figure 2.3*c*, the Henry's law constant is expressed in units of atm·L/mol, whereas the Henry's law constant in Figure 2.2 is expressed in units of atm·m^3/mol. Applying Mackay and Yuen's, and Thomas's volatility guidelines to the units in Figure 2.3*c*, the Henry's law constant for semivolatile compounds in water lies between $3 \times 10^{-4} < H < 10^{-2}$ atm·L/mol (since 1 L = 0.001 m^3). Highly volatile compounds lie above a Henry's law constant of 1 atm·L/mol. For example, Figure 2.3*c* illustrates that a high-molecular-weight polycyclic aromatic hydrocarbon (PAH) such as benzo[*a*]pyrene ($C_{20}H_{12}$) is semivolatile in its tendency to escape from water according to the Henry's law constant, whereas a low-molecular-weight PAH, naphthalene ($C_{10}H_8$), is volatile.

The most common gas–liquid pair encountered in analytical extractions is the air–water interface. The extraction methods discussed in this chapter are most applicable to organic solutes that are considered nonvolatile and semivolatile. However, it is possible to extend these techniques to more volatile chemicals as long as careful consideration of the tendency of the solute to volatilize is made throughout the extraction process.

2.1.2. Hydrophobicity

Studies about the nature of the hydrophobic effect have appeared in the literature since the early work of Traube in 1891 [14]. According to Tanford, a hydrophobic effect arises when any solute is dissolved in water [15]. (*Hydrophobic effects*, *hydrophobic bonds*, and *hydrophobic interactions* are used synonymously in the literature.) *A hydrophobic bond* has been defined as one "which forms when non-polar groups in an aqueous solvent associate, thereby decreasing the extent of interaction with surrounding water molecules, and liberating water originally bound by the solutes" [16]. In the past, the hydrophobic effect was believed to arise from the attraction of nonpolar groups for each other [17]. Although a "like-attracts-like" interaction certainly plays a role in this phenomenon, current opinion views the strong

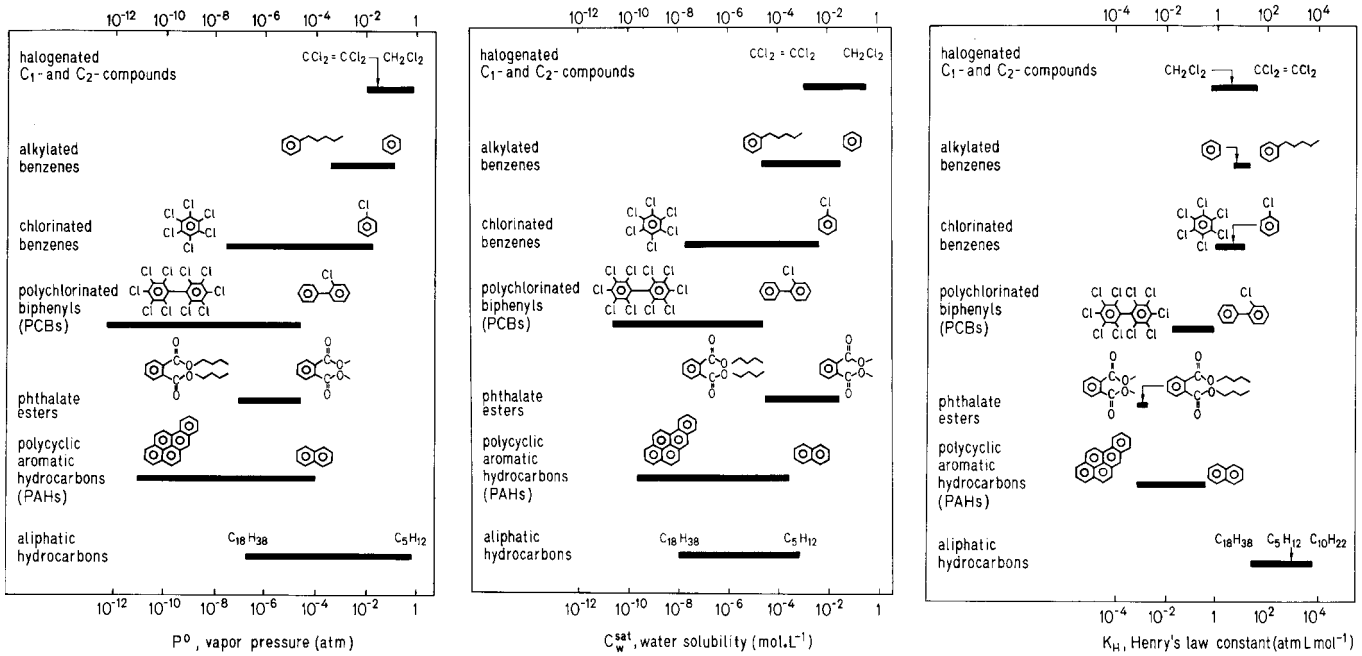


Figure 2.3. Ranges of (*a*) saturation vapor pressure (P°) values at 25°C, (*b*) water solubilities (C_W^{sat}), and (*c*) Henry's law constants (K_{H}) for some important classes of organic compounds. (Reprinted with permission from Ref. 8. Copyright © 1993 John Wiley & Sons, Inc.)

forces between water molecules as the primary cause of the hydrophobic effect. The detailed molecular structure of liquid water is complex and not well understood [18]. Many of the unusual properties of water are the result of the three-dimensional network of hydrogen bonds linking individual molecules together [19].

The attractive forces between water molecules are strong, and foreign molecules disrupt the isotropic arrangement of the molecules of water. When a nonpolar solute is dissolved in water, it is incapable of forming hydrogen bonds with the water, so some hydrogen bonds will have to be broken to accommodate the intruder. The breaking of hydrogen bonds requires energy. Frank and Evans [20] suggested that the water molecules surrounding a nonpolar solute must rearrange themselves to regenerate the broken bonds. Thermodynamic calculations indicate that when this rearrangement occurs, a higher degree of local order exists than in pure liquid water. Tanford [15] concludes that the water molecules surrounding a nonpolar solute do not assume one unique spatial arrangement, but are capable of assuming various arrangements, subject to changes in temperature and hydrocarbon chain length. The first layer of water molecules surrounding the solute cavity and subsequent layers are often termed *flickering clusters* [20–22].

An intruding hydrocarbon must compete with the tendency of water to re-form the original structure and is "squeezed" out of solution [23]. This hydrophobic effect is attributed to the high cohesive energy density of water because the interactions of water with a nonpolar solute are weaker than the interactions of water with itself [24]. Leo [22] notes that "part of the energy 'cost' of creating the cavity in each solvent is 'paid back' when the solvent interacts favorably with parts of the solute surface."

Recognizing that the *hydrophobic effect* (or more generally, a *solvophobic effect*) exists when solutes are dissolved in water leads to considering the influence of this property on the distribution of a solute between immiscible phases during extraction. A parameter that measures hydrophobicity is needed. This parameter is considered important to describe transport between water and hydrophobic biological phases (such as lipids or membranes), between water and hydrophobic environmental phases (such as organic humic substances), and between water and hydrophobic extractants (such as methylene chloride or reversed-phase solid sorbents). Although the earliest attempts to quantitate hydrophobicity used olive oil as the immiscible reference phase [25,26], since the 1950s, *n*-octanol has gained widespread favor as the reference solvent [27].

The general equilibrium constant expression in equation (2.2) can be rewritten to express the distribution of solute chemical X between water (W) and *n*-octanol (O) as

$$K_{OW} = K_D = \frac{[X]_O}{[X]_W} \tag{2.7}$$

The *n*-octanol/water partition coefficient, K_{OW} (also referred to as P_{OW}, P, or P_{oct}), is a dimensionless, "operational" [21] or "phenomenological" [24] definition of hydrophobicity based on the *n*-octanol reference system [28]. The amount of transfer of a solute from water into a particular immiscible solvent or bulk organic matter will not be identical to the mass transfer observed in the *n*-octanol/water system, but K_{OW} is often directly proportional to the partitioning of a solute between water and various other hydrophobic phases [8]. The larger the value of K_{OW}, the greater is the tendency of the solute to escape from water and transfer to a bulk hydrophobic phase. When comparing the K_{OW} values of two solutes, the compound with the higher number is said to be the more hydrophobic of the two.

The *n*-octanol/water reference system covers a wide scale of distribution coefficients, with K_{OW} values varying with organic molecular structure (Figure 2.4). The magnitude of the *n*-octanol/water partition coefficient generally increases with molecular weight. The differences in K_{OW} cover several orders of magnitude, such that hydrophobicity values are often reported on a logarithmic scale (i.e., log K_{OW} or log P), in the range -4.0 to $+6.0$ [21].

The distribution coefficient refers to the hydrophobicity of the entire molecule. Within a family of organic compounds it is sometimes useful to deal with hydrophobic substituent constants that relate the hydrophobicity of a derivative, log P_X, to that of the parent molecule, log P_H. Therefore, a substituent parameter, π, has been defined [21] as

$$\pi_X = \log P_X - \log P_H \tag{2.8}$$

where a positive value means the substituent is more hydrophobic (i.e., prefers *n*-octanol to water) relative to hydrogen, and a negative value indicates that the substituent prefers the water phase and is more hydrophilic than hydrogen (Table 2.1). The hydrophobic contribution of a substituent such as CH_3, Cl, OH, or NO_2 varies according to the molecular subenvironment of the substituent [21,30].

In order to use the value of the distribution coefficient between *n*-octanol and water as a guide for methodology to use when extracting organic compounds from water, the effect of variation in the degree of hydrophobicity must be considered. If a solute has low hydrophobicity, according to equation (2.7), it will prefer to remain in the aqueous phase relative to *n*-octanol. If a solute has very high hydrophobicity, it will prefer to be in the *n*-octanol phase. Intuitively, highly hydrophobic organic chemicals are easier to extract from water by a second immiscible, hydrophobic phase, but analyti-

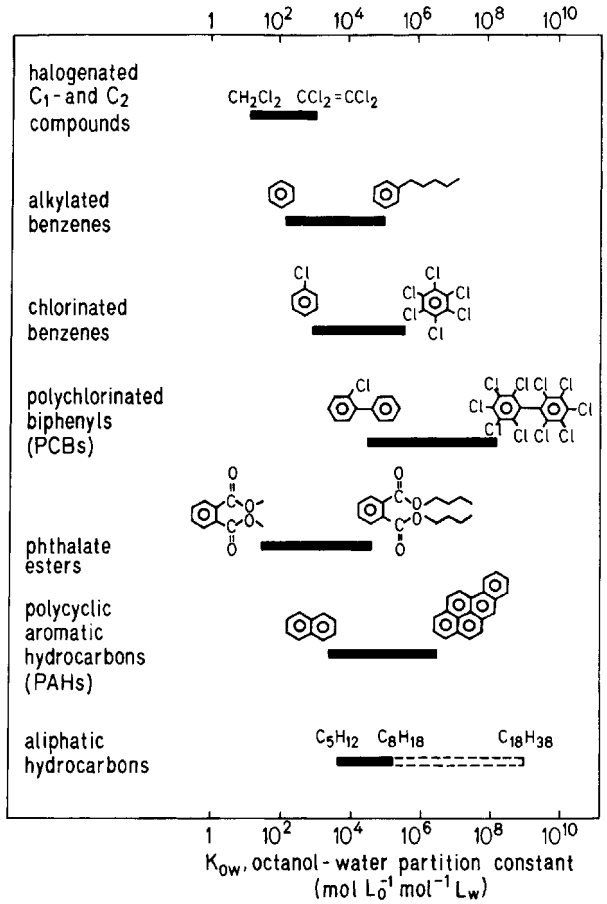


Figure 2.4. Ranges in octanol–water partition constants (K_{OW}) for some important classes of organic compounds. (Reprinted with permission from Ref. 8. Copyright © 1993 John Wiley & Sons, Inc.)

cally they can subsequently be difficult to remove from the immiscible phase. Ney [13] defines low K_{OW} as values less than 500 ($\log K_{OW} = 2.7$), midrange values as $500 \leq K_{OW} \leq 1000$ ($2.7 \leq \log K_{OW} \leq 3.0$), and high K_{OW} values as greater than 1000 ($\log K_{OW} > 3.0$). Others [31,32] found it useful to consider compounds with a $\log K_{OW}$ less than 1 as highly hydrophilic, and compounds with a $\log K_{OW}$ above 3 to 4 (depending on the nature of the immiscible phase) as highly hydrophobic.

The relationship between water solubility and the *n*-octanol/water partition coefficient must be addressed. Why are both parameters included in

Table 2.1. Substituent Constants Derived from Partition Coefficients

	Aromatic Para-Substituted Systems (π)									
Functional Group	Monobenzenes	Phenoxyacetic Acid	Phenylacetic Acid	Benzoic Acid	Benzyl Alcohol	Nitrobenzenes	Benzamides	Phenols	Anilines	Acetanilides
OCH_3	−0.02	−0.04	0.01	0.08	0	0.18	0.21	−0.12		−0.02
CH_3	0.56	0.52	0.45	0.42	0.48	0.52	0.53	0.48	0.49	0.24
NO_2	−0.28	0.24	−0.04	0.02	0.16	−0.39	0.17	0.50	0.49	0.50
Cl	0.71	0.70	0.70	0.87	0.86	0.54	0.88	0.93		0.71

Source: Data from Refs. 21, 29, and 30.

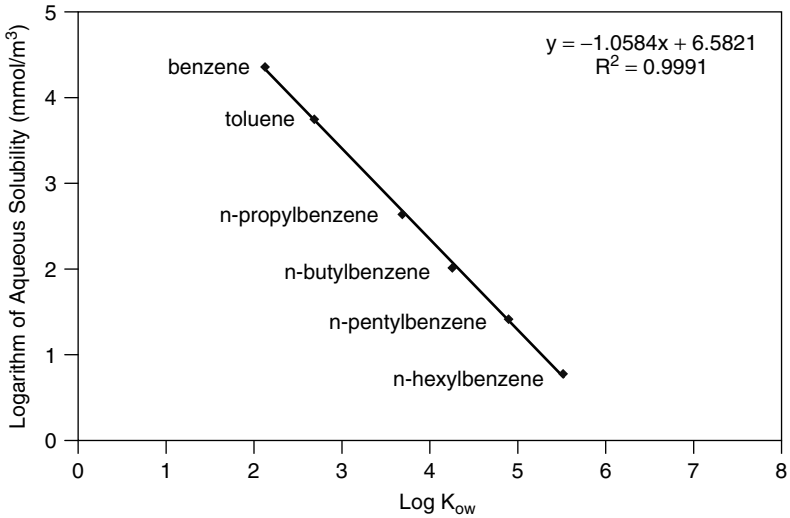


Figure 2.5. Comparison of hydrophobicity and aqueous solubility for a series of *n*-alkylbenzenes. (Data from Ref. 33.)

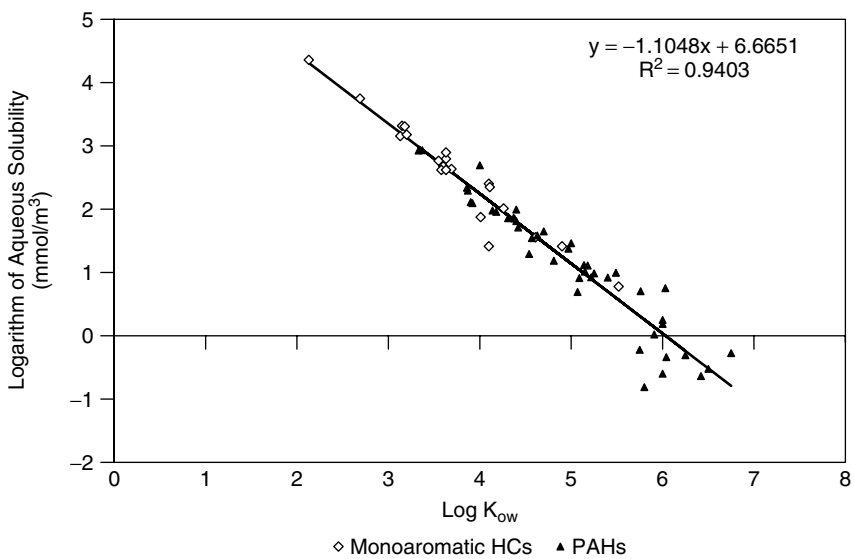


Figure 2.6. Comparison of hydrophobicity and aqueous solubility for monoaromatic hydrocarbons (HCs) and polycyclic aromatic hydrocarbons (PAHs). (Data from Ref. 6 and 33.)

the list of key chemical properties? In general, there is a trend toward an inverse relationship between these parameters such that high water solubility is generally accompanied by low hydrophobicity, and vice versa. Many authors use this relationship to estimate one of these parameters from the other. However, it is this author's opinion that the *n*-octanol/water partition coefficient and water solubility are not interchangeable (via inverse relationships) because they measure different phenomena. Water solubility is a property measured at maximum capacity or saturation. The *n*-octanol/water partition coefficient measures distribution across an interface. While the relationship between water solubility and the *n*-octanol/water partition coefficient may be highly correlated for closely related families of congeners (Figure 2.5), as the diversity of the compounds compared increases, the correlation between these two parameters decreases (Figure 2.6). However, solubility should remain on the list of essential chemical properties because if the value of the octanol–water partition coefficient is unavailable, water solubility can be used as a surrogate. Also, solubility is used to estimate the Henry's law constant.

2.1.3. Acid–Base Equilibria

The acid–base character of a chemical and the pH of the aqueous phase determine the distribution of ionized–nonionized species in solution. Starting from the equilibrium dissociation of a weak acid, HA,

$$\mathrm{HA} \rightleftharpoons \mathrm{H^+} + \mathrm{A^-} \tag{2.9}$$

the equilibrium constant for dissociation of a weak acid can be written as

$$K_a = \frac{[\mathrm{H^+}][\mathrm{A^-}]}{[\mathrm{HA}]} \tag{2.10}$$

Analogously, the dissociation of the conjugate acid, $\mathrm{BH^+}$, of a base, B, is described as

$$\mathrm{BH^+} \rightleftharpoons \mathrm{H^+} + \mathrm{B} \tag{2.11}$$

and the related constant is

$$K_a = \frac{[\mathrm{H^+}][\mathrm{B}]}{[\mathrm{BH^+}]} \tag{2.12}$$

Ionizable compounds' K_a values (Figure 2.7) have an orders-of-magnitude

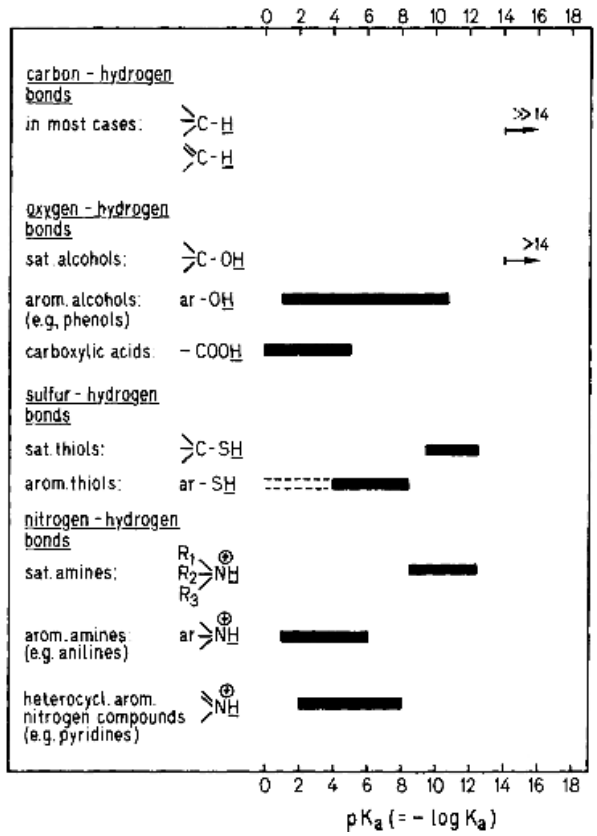


Figure 2.7. Ranges of acid dissociation constants (pK_a) for some important classes of organic compounds. (Reprinted with permission from Ref. 8. Copyright © 1993 John Wiley & Sons, Inc.)

range. This makes it useful to describe K_a values in terms of logarithms; that is, $pK_a = -\log K_a$.

Two graphical methods described here, a master variable (pC–pH) diagram and a distribution ratio diagram, are extremely useful aids for visualizing and solving acid–base problems. They help to determine the pH at which an extraction should be performed. Both involve the choice of a *master variable*, a variable important to the solution of the problem at hand. The obvious choice for a master variable in acid–base problems is $[H^+]$ [equations (2.9)–(2.12)], or pH when expressed as the negative logarithm of $[H^+]$.

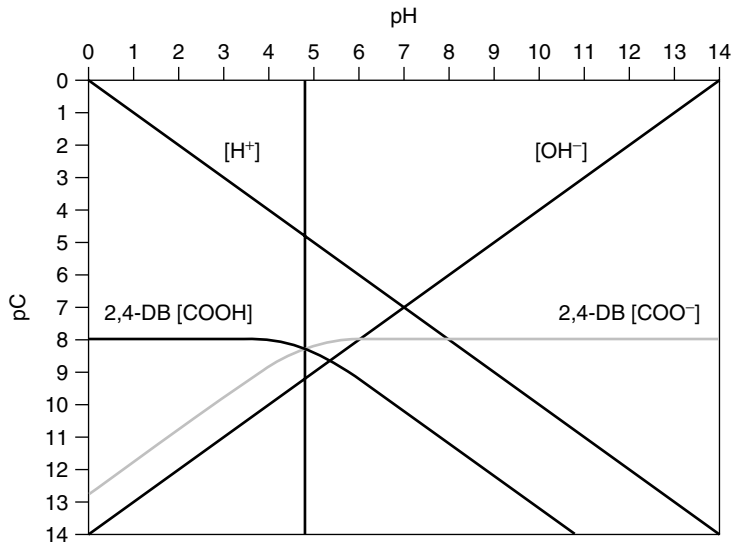


Figure 2.8. Master variable (pC–pH) diagram for 2,4-DB; p$K_a = 4.8$, $C_T = 1 \times 10^{-8}$ M.

To prepare a *pC–pH diagram*, the master variable, pH, is plotted on the x-axis. On the y-axis, the concentration of chemical species is plotted as a function of pH. The concentration, C, of each chemical species is expressed as a logarithm (log C), or more often as the negative logarithm of its concentration, that is pC (analogous to pH). The pC–pH diagram (Figure 2.8) for a representative acidic solute, 4-(2,4-dichlorophenoxy)butanoic acid or 2,4-DB, is prepared by first determining that the pK_a for this compound is 4.8. A reasonable concentration to assume for trace levels of this compound in water is 2.5 ppm or 1×10^{-8} M, since the molecular weight of 2,4-DB is 249.1. Based on the molar concentration of 1×10^{-8}, pC has a value of 8. By mass balance, the total concentration at any given pH value, C_T, is the sum of all species. That is,

$$C_T = [\mathrm{HA}] + [\mathrm{A}^-] \tag{2.13}$$

for a monoprotic acid, as in the example in Figure 2.8. The diagonal line connecting pH, pC values $(0, 0)$ with $(14, 14)$ represents the hydrogen ion concentration, and the diagonal line connecting pH, pC values $(0, 14)$ with $(14, 0)$ represents the hydroxide ion concentration, according to the expression

$$[\mathrm{H}^+][\mathrm{OH}^-] = K_W = 10^{-14} \tag{2.14}$$

where K_W is the ion product of water. The vertical line in Figure 2.8 indicates data at which the $pH = pK_a$.

To graph the curves representing [HA] and [A^-], a mathematical expression of each as a function of [H^+] (a function of the master variable) is needed. The appropriate equation for [HA] is derived by combining the equilibrium constant for dissociation of a weak acid [equation (2.10)] with the mass balance equation [equation (2.13)] to yield

$$[HA] = \frac{[H^+]C_T}{[H^+] + K_a} \tag{2.15}$$

Analogously, solving for [A^-] yields

$$[A^-] = \frac{K_a C_T}{[H^+] + K_a} \tag{2.16}$$

Point-by-point plotting of equations (2.15) and (2.16) produces the curves for the nonionized, 2,4-DB[COOH], and ionized, 2,4-DB[COO^-], species in Figure 2.8. This approach can be expanded to generate master variable diagrams of more complex polyprotic systems (Figure 2.9) such as phosphoric

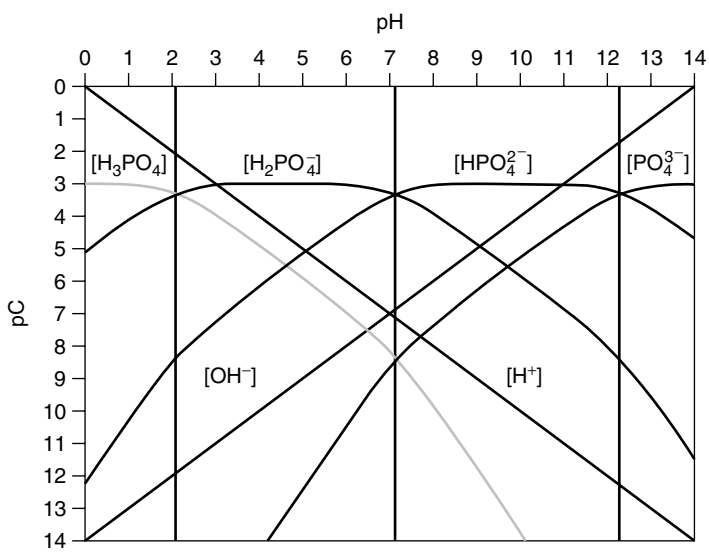


Figure 2.9. Master variable (pC–pH) diagram for phosphoric acid: $pK_{a1} = 2.15$, $pK_{a2} = 7.20$, and $pK_{a3} = 12.35$, $C_T = 1 \times 10^{-3}$ M.

acid. Figure 2.9 was generated by using the acid dissociation constants of phosphoric acid, $pK_{a1} = 2.15$, $pK_{a2} = 7.20$, and $pK_{a3} = 12.35$. Additionally, a total phosphate concentration of 0.001 M was assumed. In this case, $C_T = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]$. Figures 2.8 and 2.9 were produced using a free software package, EnviroLand version 2.50, available for downloading from the Internet [34]. Alternatively, equations (2.15) and (2.16) can be input to spreadsheet software to produce pC–pH diagrams.

A second graphical approach to understanding acid–base equilibria is preparation of a *distribution ratio diagram*. The fraction, α, of the total amount of a particular species is plotted on the y-axis versus the master variable, pH, on the x-axis, where

$$\alpha_{HA} = \frac{[HA]}{[A^-] + [HA]} \tag{2.17}$$

and

$$\alpha_{A^-} = \frac{[A^-]}{[A^-] + [HA]} \tag{2.18}$$

By combining equations (2.15), (2.16), and (2.18), a distribution diagram (Figure 2.10) for acetic acid can be prepared given that the acid dissociation constant is 1.8×10^{-5} with an assumed concentration of 0.01 M. The vertical line in Figure 2.10, positioned at $x = 4.74$, is a reminder that when the pH of the solution is equal to the pK_a of the analyte, the α value is 0.5, which signifies that the concentration of HA is equal to the concentration of A^-. The distribution diagram can be used to determine the fraction of ionized or nonionized acetic acid at any selected pH.

Another way of understanding the distribution of species as a function of pH is to apply the *Henderson–Hasselbach equation*:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{2.19}$$

which is derived by taking the negative logarithm of both sides of equation (2.10). The Henderson–Hasselbach equation provides a useful relationship between system pH and acid–base character taking the ratio of ionized to nonionized species into consideration.

To calculate the relative amount of A^- present in a solution in which the pH is 1 unit above the pK_a (i.e., $pH = pK_a + 1$), apply the Henderson–

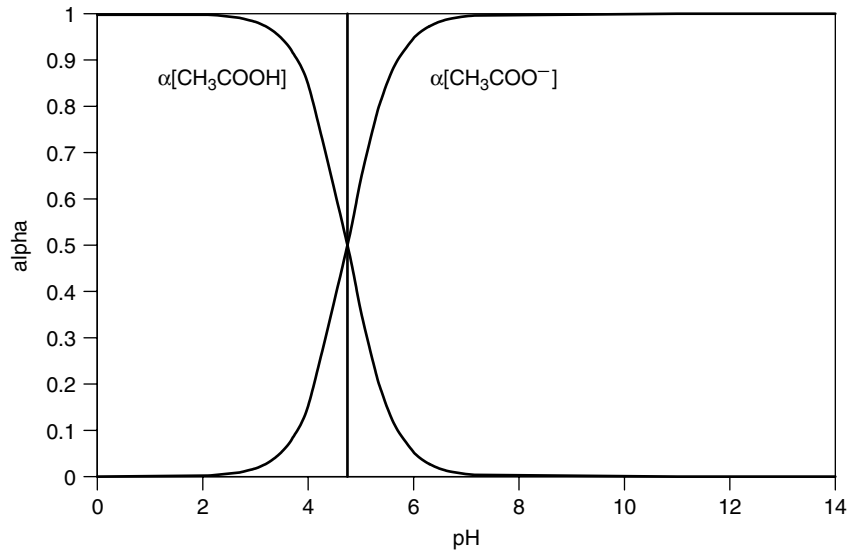


Figure 2.10. Distribution diagram for acetic acid; $pK_a = 4.74$, $C_T = 1 \times 10^{-2}$ M.

Hasselbach equation such that

$$1 = \log \frac{[A^-]}{[HA]} \tag{2.20}$$

and taking the antilogarithm of both sides yields

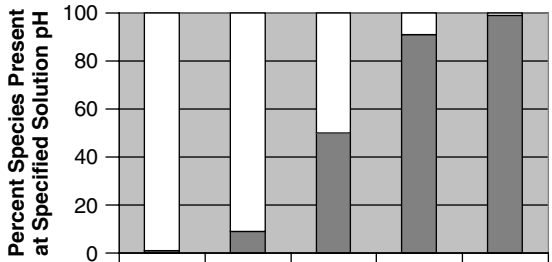
$$10 = \frac{[A^-]}{[HA]} \tag{2.21}$$

Assume that the only species present are HA and A^- such that

$$[HA] + [A^-] = 1 \tag{2.22}$$

Rearranging equation (2.22) to solve for [HA] and substituting into equation (2.21) gives

$$10 = \frac{[A^-]}{1 - [A^-]} \tag{2.23}$$



	pH = $pK_a - 2$	pH = $pK_a - 1$	pH = pK_a	pH = $pK_a + 1$	pH = $pK_a + 2$
□ nonionized acid (HA) or ionized conjugate acid (BH+)	99	91	50	9	1
■ ionized acid (A–) or unionized base (B)	1	9	50	91	99

Figure 2.11. Percent of ionogenic (ionizable) species present for weak acids and bases when solution pH is 2 units above or below the acid dissociation constant.

and therefore $[A^-] = 0.909$. In an analogous manner, it is possible to calculate that the fraction of $[A^-]$ present in a solution in which the pH is 2 units above the pK_a (i.e., pH = $pK_a + 2$) is 0.990. According to the Henderson–Hasselbach equation, 50% of each species is present when the pH is equal to the pK_a. Therefore, depending on whether the compound is an acid or a base (Figure 2.11), an analyte is either 99% nonionized or ionized when the pH value is 2 units above or below the pK_a.

The purpose of applying master variable diagrams, distribution diagrams, and the Henderson–Hasselbach equation to ionizable organic chemicals is to better understand the species present at any solution pH. Organic compounds can be extracted from liquids in either the ionized or nonionized form. Generally, however, for ionizable compounds, it is best to adjust the solution pH to force the compound to exist in the ionized state or in the nonionized state as completely as possible. Less than optimal results may be obtained if the ionizable compound is extracted within the window of the $pK_a \pm 2$ log units. When the pH is equal to the pK_a, half of the compound is ionized and half of the compound is nonionized. Mixed modes of extraction are required to transfer the compound completely from one phase to another. The "2 units" rule of thumb is very important for an analyst to understand and apply when developing extraction protocol for acidic or basic compounds. More information concerning graphical methods for

solving acid–base equilibrium problems can be found in Bard [1], Snoeyink and Jenkins [35], and Langmuir [36].

2.1.4. Distribution of Hydrophobic Ionogenic Organic Compounds

Some highly hydrophobic weak acids and bases exhibit substantial hydrophobicity even in the ionized state. For highly hydrophobic ionogenic organic compounds, not only is transfer of the neutral species between the aqueous phase and the immiscible phase important, but the transfer of the hydrophobic, ionized, organic species as free ions or ion pairs may also be significant [37]. Mathematically, this is described by refining the *n*-octanol/water partition coefficient, as defined in equation (2.7), to reflect the pH-dependent distribution between water (W) and *n*-octanol (O) of chemical X in both the ionized and nonionized forms. If chemical X is a weak acid, HA, the distribution ratio is

$$D_{\mathrm{OW}}(\mathrm{HA}, \mathrm{A}^-) = \frac{[\mathrm{HA}]_{\mathrm{O,\,total}}}{[\mathrm{HA}]_{\mathrm{W}} + [\mathrm{A}^-]_{\mathrm{W}}} \tag{2.24}$$

where $[\mathrm{HA}]_{\mathrm{O,\,total}}$ is the sum of all neutral species, free ions, and ions paired with inorganic counterions that transfer to octanol [8,37].

For example, the ratio of the *n*-octanol/water distribution coefficient of the nondissociated species to that of the ionic species is nearly 10,000 for 3-methyl-2-nitrophenol, but only about 1000 for pentachlorophenol because of the greater significance of the hydrophobicity of the ionized form of pentachlorophenol. The logarithm of the *n*-octanol/water distribution coefficient of pentachlorophenol as the phenolate is about 2 (determined at pH 12, and 0.1 *M* KCl), which indicates significant distribution of the ionized form into the *n*-octanol phase [8,37]. Extraction of such highly hydrophobic ionogenic organic compounds can result from mixed-mode mechanisms that incorporate both the hydrophobic and ionic character of the compound.

2.2. LIQUID–LIQUID EXTRACTION

In liquid–liquid extraction (LLE), phases A and B are both liquids. The two liquid phases must be immiscible. For that reason, LLE has also been referred to as *immiscible solvent extraction*. In practice, one phase is usually aqueous while the other phase is an organic solvent. An extraction can be accomplished if the analyte has favorable solubility in the organic solvent. Chemists have used organic solvents for extracting substances from water since the early nineteenth century [38].

Miscibility

Solvent manufacturer Honeywell Burdick & Jackson [39] defines solvents as *miscible* if the two components can be mixed together in all proportions without forming two separate phases. A solvent miscibility chart (Figure 2.12) is a useful aid for determining which solvent pairs are immiscible and would therefore be potential candidates for use in LLE. More solvent combinations are miscible than immiscible, and more solvents are immiscible with water than with any other solvent. Solvents miscible with water in all proportions include acetone, acetonitrile, dimethyl acetamide, *N*,*N*-dimethylformamide, dimethyl sulfoxide, 1,4-dioxane, ethyl alcohol, glyme, isopropyl alcohol, methanol, 2-methoxyethanol, *N*-methylpyrrolidone, *n*-propyl alcohol, pyridine, tetrahydrofuran, and trifluoroacetic acid [40].

Density

Another consideration when selecting an extraction solvent is its density [41]. Solvents that are more dense than water will form the lower layer of the pair when mixed together, while solvents that are less dense than water will form the upper layer or "float" on water. For example, ethyl ether has a density of 0.7133 g/mL at 20°C and would constitute the upper phase when combined with water, which has a density of 0.9982 g/mL at that temperature. On the other hand, the density of chloroform is 1.4892 at 20°C. Therefore, water would form the top layer in a water–chloroform solvent pair.

Solubility

Although solvents may form two visibly distinct phases when mixed together, they are often somewhat soluble in each other and will, in fact, become mutually saturated when mixed with each other. Data on the solubility of various solvents in water (Table 2.2) and on the solubility of water in other solvents (Table 2.3) should be consulted when selecting an extraction solvent pair. For example, 1.6% of the solvent dichloromethane (or methylene chloride) is soluble in water. Conversely, water is 0.24% soluble in dichloromethane. According to Table 2.3, when the phases are separated for recovery of the extracted analyte, the organic solvent layer will contain water. Similarly, according to Table 2.2, after extraction the depleted aqueous phase will be saturated with organic solvent and may pose a disposal problem. (*Author's note:* I previously recounted [43] my LLE experience with disposal of extracted aqueous samples that were cleaned of pesticide residues but saturated with diethyl ether. Diethyl ether is 6.89% soluble in water at 20°C.)

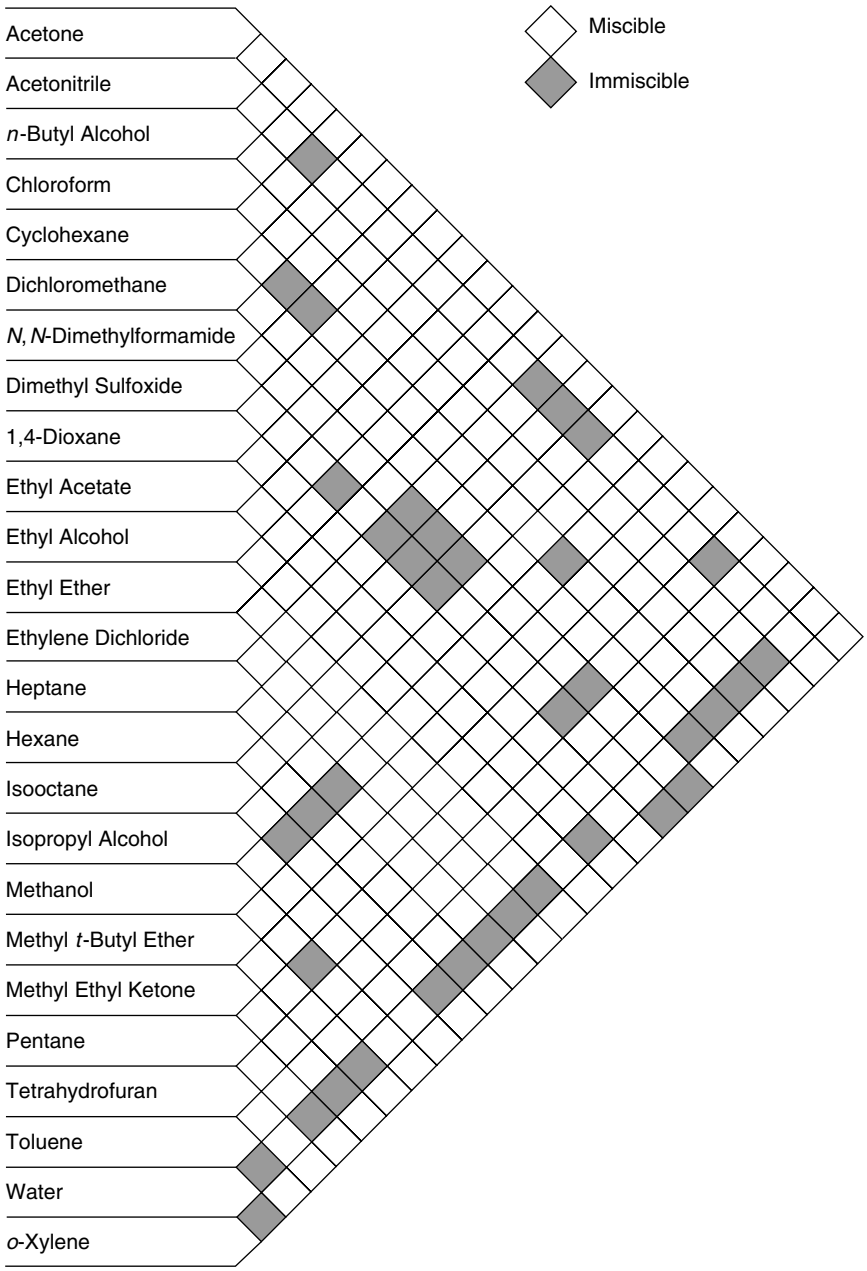


Figure 2.12. Solvent miscibility chart. (Reprinted with permission from Ref. 39. Copyright © 2002 Honeywell Burdick & Jackson.) Available online at *http://www.bandj.com/BJProduct/SolProperties/Miscibility.html*

Table 2.2. Solubility in Water

Solvent	Solubility (%)[a]
Isooctane	0.0002 (25°C)
Heptane	0.0003 (25°C)
1,2,4-Trichlorobenzene	0.0025
Cyclohexane	0.006 (25°C)
Cyclopentane	0.01
Hexane	0.014
o-Dichlorobenzene	0.016 (25°C)
1,1,2-Trichlorotrifluoroethane	0.017 (25°C)
o-Xylene	0.018 (25°C)
Pentane	0.04
Chlorobenzene	0.05
Toluene	0.052 (25°C)
n-Butyl chloride	0.11
Methyl isoamyl ketone	0.54
n-Butyl acetate	0.68
Ethylene dichloride	0.81
Chloroform	0.815
Dichloromethane	1.60
Methyl isobutyl ketone	1.7
Methyl *t*-butyl ether	4.8
Triethylamine	5.5
Methyl *n*-propyl ketone	5.95
Ethyl ether	6.89
n-Butyl alcohol	7.81
Isobutyl alcohol	8.5
Ethyl acetate	8.7
Propylene carbonate	17.5 (25°C)
Methyl ethyl ketone	24.0

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[a] Solvents are arranged in order of increasing solubility in water, the maximum weight percent (w/w) of each solvent that can be dissolved in water (at 20°C unless otherwise indicated).

2.2.1. Recovery

As defined earlier,

$$K_D = \frac{[\mathrm{X}]_\mathrm{B}}{[\mathrm{X}]_\mathrm{A}} \tag{2.2}$$

Table 2.3. Solubility of Water in Each Solvent

Solvent	Solubility (%)[a]
Isooctane	0.006
Pentane	0.009
Cyclohexane	0.01
Cyclopentane	0.01
Heptane	0.01 (25°C)
Hexane	0.01
1,1,2-Trichlorotrifluoroethane	0.011 (25°C)
1,2,4-Trichlorobenzene	0.020
Toluene	0.033 (25°C)
Chlorobenzene	0.04
Chloroform	0.056
n-Butyl chloride	0.08
Ethylene dichloride	0.15
Dichloromethane	0.24
o-Dichlorobenzene	0.31 (25°C)
n-Butyl acetate	1.2
Ethyl ether	1.26
Methyl isoamyl ketone	1.3
Methyl *t*-butyl ether	1.5
Methyl isobutyl ketone	1.9 (25°C)
Ethyl acetate	3.3
Methyl *n*-propyl ketone	3.3
Triethylamine	4.6
Propylene carbonate	8.3 (25°C)
Methyl ethyl ketone	10.0
Isobutyl alcohol	16.4
n-Butyl alcohol	20.07

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[a]Solvents are arranged in order of increasing solubility of water in each solvent, the maximum weight percent (w/w) of water that can be dissolved in the solvent (at 20°C unless otherwise indicated).

Analytes distribute themselves between aqueous and organic layers according to the Nernst distribution law, where the distribution coefficient, K_D, is equal to the analyte ratio in each phase at equilibrium.

The analyte distributes itself between the two immiscible liquids according to the relative solubility in each solvent [1,38,44,45]. To determine the effect of the distribution coefficient on an extraction, consider the following example.

Example

A 1-L aqueous sample containing 100 parts per billion (ppb) of a compound having a molecular weight of 250 g/mol is extracted once with 150 mL of organic extracting solvent. Assume that the K_D value is 5. Given this information, the molarity of the original sample is 4.0×10^{-10} M. Calculate the percent of the analyte extracted into the organic extracting solvent at equilibrium.

Step 1. Calculate the moles of analyte in the original sample.

$$\text{moles in original sample} = \text{molarity of sample (in mol/L)} \times \text{volume extracted (in L)}$$

Therefore,

$$\text{moles in original sample} = 4.0 \times 10^{-10}\ M \times 1\ \text{L} = 4.0 \times 10^{-10}\ \text{mol} \quad (2.25)$$

Step 2. Calculate the moles of analyte left in the aqueous phase after extraction.

$$K_D = \frac{(\text{moles in original sample} - \text{moles left in water after extraction})/\text{extraction solvent volume (in L)}}{\text{moles left in water after extraction}/\text{volume of original sample (in L)}} \quad (2.26)$$

Therefore,

$$\frac{\text{moles left in water}}{\text{after extraction}} = \frac{\text{moles in original sample}}{\{[K_D \times \text{extraction solvent volume (in L)}]/\text{volume of original sample (in L)}\} + 1}$$

such that,

$$\text{moles left in water after extraction} = \frac{4.0 \times 10^{-10}\ \text{mol}}{[(5 \times 0.150\ \text{L})/1\ \text{L}] + 1} = 2.2857 \times 10^{-10}\ \text{mol}$$

Step 3. Calculate the moles of analyte extracted into layer B (i.e., the extracting solvent) at equilibrium.

moles of analyte extracted into organic solvent

= moles of analyte in original sample − moles left in water after extraction

$$= 4.0 \times 10^{-10}\ \text{mol} - 2.2857 \times 10^{-10}\ \text{mol} = 1.7143 \times 10^{-10}\ \text{mol} \quad (2.27)$$

Step 4. Calculate the percent of analyte extracted into the organic solvent at equilibrium. The recovery factor, R_X, is the fraction of the analyte extracted divided by the total concentration of the analyte, multiplied by 100 to give the percentage recovery:

$$\begin{aligned} \%\, R_X &= \text{percent of analyte extracted into organic solvent} \\ &= \frac{\text{moles of analyte extracted into organic solvent}}{\text{moles of analyte in original sample}} \times 100 \\ &= \frac{1.7143 \times 10^{-10}\ \text{mol}}{4.0 \times 10^{-10}\ \text{mol}} \times 100 = 42.857\% \end{aligned} \tag{2.28}$$

If the problem is reworked such that the volume of the extracting solvent is 50 mL instead of 150 mL, the percent of analyte extracted into the organic solvent, calculated by repeating steps 1 through 4, is determined to be only 20% (Table 2.4) as compared to 42.857% if an extracting solvent of 150 mL is used. If after separating the phases, the aqueous sample is extracted with a second sequential extraction volume of 50 mL, again 20% of what remained available for extraction will be removed. However, that represents only 16% additional recovery, or a cumulative extraction of 36% after two sequential extractions (i.e., 2×50 mL). If after separating the phases, the aqueous sample is extracted with a third sequential extraction volume of 50 mL, again 20% of what remained available for extraction will be removed. That represents only 12.8% of additional recovery or a cumulative extraction of 48.8% after three sequential extractions (i.e., 3×50 mL). Analogous to a hapless frog that jumps halfway out of a well each time it jumps, never to escape the well, LLE recovery is an equilibrium procedure in which exhaustive extraction is driven by the principle of repeated extractions.

The percent recovery obtained with a single extraction of 150 mL of organic solvent is compared to that for three sequential extractions of 50 mL each for K_D values of 500, 250, 100, 50, and 5 (Table 2.4). In sequential extractions, the same percent recovery is extracted each time (i.e., the frog jumps the same percentage of the distance out of the well each time). That is, at a K_D value of 500, 96.154% is extracted from the original sample using an organic solvent volume of 50 mL; 96.154% of the analyte remaining in solution after the first extraction is removed during the second sequential extraction by 50 mL; and 96.154% of the analyte remaining in solution after the second extraction is removed during the third sequential extraction by 50 mL.

When K_D is equal to 500, the first extraction using 50 mL recovers 96.154% of the original analyte; the second sequential extraction produces

Table 2.4. Distribution Coefficient Effects on Single and Repeated Extractions

	Single Extraction 1 × 150 mL	Single Extraction 1 × 50 mL	Second Sequential Extraction			Third Sequential Extraction		
			1 × 50 mL	1 × 50 mL	2 × 50 mL	1 × 50 mL	1 × 50 mL	3 × 50 mL
K_d	Percent Extracted	Percent Extracted	Repeat Percent Extracted	Additional Recovery	Cumulative Extraction	Repeat Percent Extracted	Additional Recovery	Cumulative Extraction
500	98.684	96.154	96.154	3.697	99.851	96.154	0.142	99.993
250	97.403	92.593	92.593	6.859	99.451	92.593	0.508	99.959
100	93.750	83.333	83.333	13.890	97.223	83.333	2.315	99.538
50	88.235	71.429	71.429	20.411	91.839	71.429	5.832	97.671
5	42.857	20.000	20.000	16.000	36.000	20.000	12.800	48.800

additional recovery of 3.697% of the original analyte; and the third sequential extraction produces further recovery of 0.142% of the original analyte, for a cumulative recovery after three sequential extractions (3×50 mL) of 99.993%. The cumulative recovery after three extractions of 50 mL each is greater than that calculated for recovery from a single extraction of 150 mL of organic solvent (i.e., 98.684%).

The effect of concentration on recovery by single or repeated extractions can be examined. Instead of assuming a concentration of 4.0×10^{-10} M for the aqueous sample to be extracted as stated in the original problem, the values in Table 2.4 can be recalculated after substitution with a concentration of 0.01 M. If the same four steps outlined previously are followed, it can be demonstrated that the recovery values in Table 2.4 are identical regardless of concentration. The most desirable analytical protocols are independent of sample concentration in the range of samples to be analyzed.

The operation conducted in steps 1 through 4 above can be summarized by the following equation such that the recovery factor of analyte X, expressed as a percent, is

$$\% \, R_{\mathrm{X}} = \frac{100 K_D}{K_D + (V_O/V_E)} \tag{2.29}$$

where V_O is the volume of the original sample and V_E is the extraction solvent volume. (Note that the recovery factor is independent of sample concentration.) The recovery factor can also be expressed in the equivalent form

$$\% \, R_{\mathrm{X}} = 100\left[\frac{K_D(V_E/V_O)}{1 + K_D(V_E/V_O)}\right] = 100\left[\frac{K_D(V)}{1 + K_D(V)}\right] \tag{2.30}$$

where $V = V_E/V_O$ is known as the *phase ratio*.

Therefore, applying equation (2.29) to the previous example in which a 1-L aqueous sample containing 100 ppb of a compound having a molecular weight of 250 g/mol is extracted once with 150 mL of organic extracting solvent, and assuming that K_D is 5, substitution yields.

$$R_{\mathrm{X}} = \frac{100 \times 5}{5 + (1.0 \text{ L}/0.150 \text{ L})} = 42.857\%$$

If the analyte is partially dissociated in solution and exists as the neutral species, free ions, and ions paired with counterions, the distribution ratio, D, analogous to equation (2.24), would be

$$D = \frac{\text{concentration of X in all chemical forms in the organic phase}}{\text{concentration of X in all chemical forms in the aqueous phase}} \tag{2.31}$$

In this instance, the value for D would be substituted for K_D in equation (2.29).

The formula for expressing repeated extractions is

$$\% R_X = \left\{1 - \left[\frac{1}{1 + K_D(V_E/V_O)}\right]^n\right\} \times 100 \tag{2.32}$$

Applying equation (2.32) to the previous calculation having three successive multiple extractions where $K_D = 5$, $V_E = 50$ mL, $V_O = 1$ L, and $n = 3$, the cumulative recovery is calculated to be 48.8% (Table 2.4).

Repeated extractions may be required to recover the analyte sufficiently from the aqueous phase. Neutral compounds can have substantial values of K_D. However, organic compounds that form hydrogen bonds with water, are partially soluble in water, or are ionogenic (weak acid or bases) may have lower distribution coefficients and/or pH-dependent distribution coefficients. Additionally, the sample matrix itself (i.e., blood, urine, or wastewater) may contain impurities that shift the value of the distribution coefficient relative to that observed in purified water.

Investigation of the principle of repeated extractions demonstrates that:

- The net amount of analyte extracted depends on the value of the distribution coefficient.
- The net amount of analyte extracted depends on the ratio of the volumes of the two phases used.
- More analyte is extracted with multiple portions of extracting solvent than with a single portion of an equivalent volume of the extracting phase.
- Recovery is independent of the concentration of the original aqueous sample.

2.2.2. Methodology

The LLE process can be accomplished by shaking the aqueous and organic phases together in a separatory funnel (Figure 2.13*a*). Following mixing, the layers are allowed to separate. Flow from the bottom of the separatory funnel is controlled by a glass or Teflon stopcock and the top of the separatory funnel is sealed with a stopper. The stopper and stopcock must fit tightly and be leakproof. Commonly, separatory funnels are globe, pear, or cylindrically shaped. They may be shaken mechanically, but are often shaken manually.

With the stopcock closed, both phases are added to the separatory funnel. The stopper is added, and the funnel is inverted without shaking. The stop-

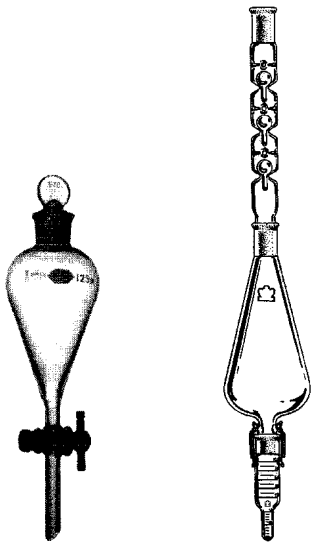


Figure 2.13. Liquid–liquid extraction apparatus: (*a*) separatory funnel and (*b*) evaporative Kuderna–Danish sample concentrator. (Reprinted with permission from Ref. 46. Copyright © 2002 Kimble/Kontes.)

cock is opened immediately to relieve excess pressure. When the funnel is inverted, the stem should be pointed away from yourself and others. The funnel should be held securely with the bulb of the separatory funnel in the palm of one hand, while the index finger of the same hand is placed over the stopper to prevent it from being blown from the funnel by pressure buildup during shaking. The other hand should be positioned to hold the stopcock end of the separatory funnel, and for opening and closing the stopcock.

The separatory funnel should be gently shaken for a few seconds, and frequently inverted and vented through the stopcock. When pressure builds up less rapidly in the separatory funnel, the solvents should be shaken more vigorously for a longer period of time while venting the stopcock occasionally. The separatory funnel should be supported in an upright position in an iron ring padded with tubing to protect against breakage.

When the layers are completely separated (facilitated by removing the stopper), the lower layer should be drawn off through the stopcock, and the upper layer should be removed through the top of the separatory funnel. The relative position of each layer depends on the relative densities of the two immiscible phases. During an extraction process, all layers should be saved until the desired analyte is isolated. A given solvent layer can easily be determined to be aqueous or organic by testing the solubility of a few drops in water.

Once the analyte has been extracted into phase B, it is usually desirable to reduce the volume of the extracting solvent. This can be accomplished with specialized glassware such as a Kuderna–Danish sample concentrator (Figure 2.13*b*), which is widely used for concentrating semivolatile compounds dissolved in volatile solvents. The concentrator consists of three primary components held together by hooks and/or clamps: a central flask with sufficient capacity to hold the extracting solvent, a tapered receiving vessel to contain the concentrated extract, and a distilling–condensing column that allows the solvent vapor to pass while retaining the analyte. The apparatus should be placed over a vigorously boiling water bath to bathe the central flask in steam. The solvent should then be allowed to escape into a hood or recovered via an additional solvent recovery system. Alternatively, a mechanical rotary evaporator may be used to evaporate excess extracting solvent, or other evaporating units that evaporate solvent with an inert gas should be used.

Performing LLE of analytes from drinking water is relatively straightforward. However, if your "aqueous" sample is blood, urine, or wastewater, the extraction process can become more tedious. Quite often in such samples, a scum forms at the layer interface, due to the presence of nonsoluble debris and the formation of emulsions. Analysts overcome this difficulty using techniques such as adding salts, chilling the sample, or centrifugation. Applying a continuous LLE technique can be useful also.

Continuous LLE is a variant of the extraction process that is particularly applicable when the distribution coefficient of the analyte between phases A and B is low. Additionally, the apparatus for conducting continuous LLE (Figures 2.14 and 2.15) automates the process somewhat. The analyst is freed from manually shaking the phases in a separatory funnel to effect a separation allowing multiple extractions to be performed simultaneously. Since the phases are not shaken to mix them, this procedure also helps avoid the formation of emulsions. The apparatus can be assembled to perform extraction alone (Figure 2.14), or extraction and concentration (Figure 2.15). The extractor performs on the principle that organic solvent cycles continuously through the aqueous phase, due to constant vaporization and condensation of the extracting solvent. Continuous LLE apparatus designed for heavier-than-water or lighter-than-water extracting solvents is available.

2.2.3. Procedures

A general extraction scheme (Figure 2.16) can be devised to extract semivolatile organics from aqueous solution such that important categories of organic compounds (i.e., bases, weak acids, strong acids, and neutrals) are fractionated from each other and isolated in an organic solvent. Many

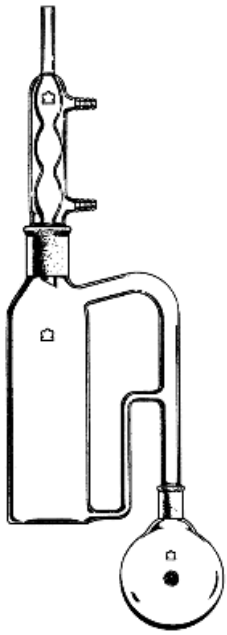


Figure 2.14. Continuous liquid–liquid extraction apparatus designed for samples where the extracting solvent is heavier than water. (Reprinted with permission from Ref. 46. Copyright © 2002 Kimble/Kontes.)

pharmaceuticals and pesticides are ionogenic or neutral compounds, and could be recovered by this procedure. Such a scheme is based on pH control of the aqueous sample. The K_D value of a base in acidic conditions is low as is the K_D value of an acid in basic conditions, because in each instance the compound would be ionized. In these situations, the ionized base or acid would therefore tend to remain in the aqueous solution when mixed with an organic extracting solvent. Neutral compounds tend to transfer to the organic extracting phase regardless of solution pH.

If an aqueous sample hypothetically containing inorganics and organics, including bases, strong acids, weak acids, and neutrals, is adjusted to pH 2 and extracted with an organic solvent (Figure 2.16, step 1), a separation in which the inorganics and bases will remain in the aqueous phase is effected. The inorganics prefer the aqueous phase, due to charge separation in ionic bonds, and at pH 2, the ionogenic organic bases will be positively charged and thereby prefer the aqueous phase. The neutral, strongly acidic, and weakly acidic organic compounds will have higher K_D values under these conditions and will prefer to transfer to the organic phase from the aqueous phase.

To isolate the organic bases from inorganic compounds and to recover the organic bases in an organic solvent, the acidified aqueous solution from

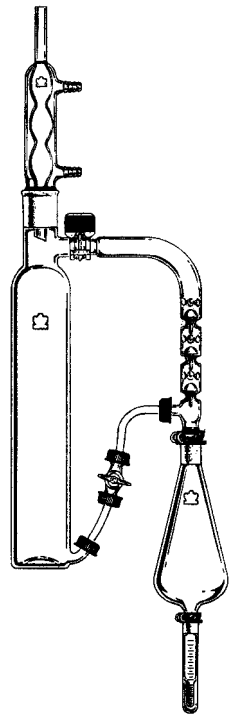


Figure 2.15. Continuous liquid–liquid extraction apparatus designed for samples where the extracting solvent is heavier than water in which both extraction and concentration are performed with the same apparatus. (Reprinted with permission from Ref. 46. Copyright © 2002 Kimble/Kontes.)

which the neutral and acidic compounds were removed is adjusted to pH 10 and extracted with an organic solvent (Figure 2.16, step 2). At pH 10, the K_D values of nonionized organic bases should be favorable for extraction into an organic solvent, while inorganic compounds preferentially remain in the aqueous solution.

To separate strongly acidic organic compounds from weakly acidic and neutral compounds, the organic phase containing all three components is mixed with a sodium bicarbonate (pH 8.5) solution (Figure 2.16, step 3). This seeming reversal of the process, that is, extracting compounds back into an aqueous phase from the organic phase, is called *washing*, *back-extraction*, or *retro-extraction*. Under these pH conditions, the organic phase retains the nonionized weakly acidic and neutral compounds, while ionized strong acids transfer into the aqueous washing solution.

The organic solvent phase containing only weakly acidic and neutral compounds is sequentially back-extracted with an aqueous (pH 10) solution of sodium hydroxide (Figure 2.16, step 4). Neutral compounds remain in the organic solvent phase, while weak organic acids, ionized at this pH, will be extracted into the aqueous phase.

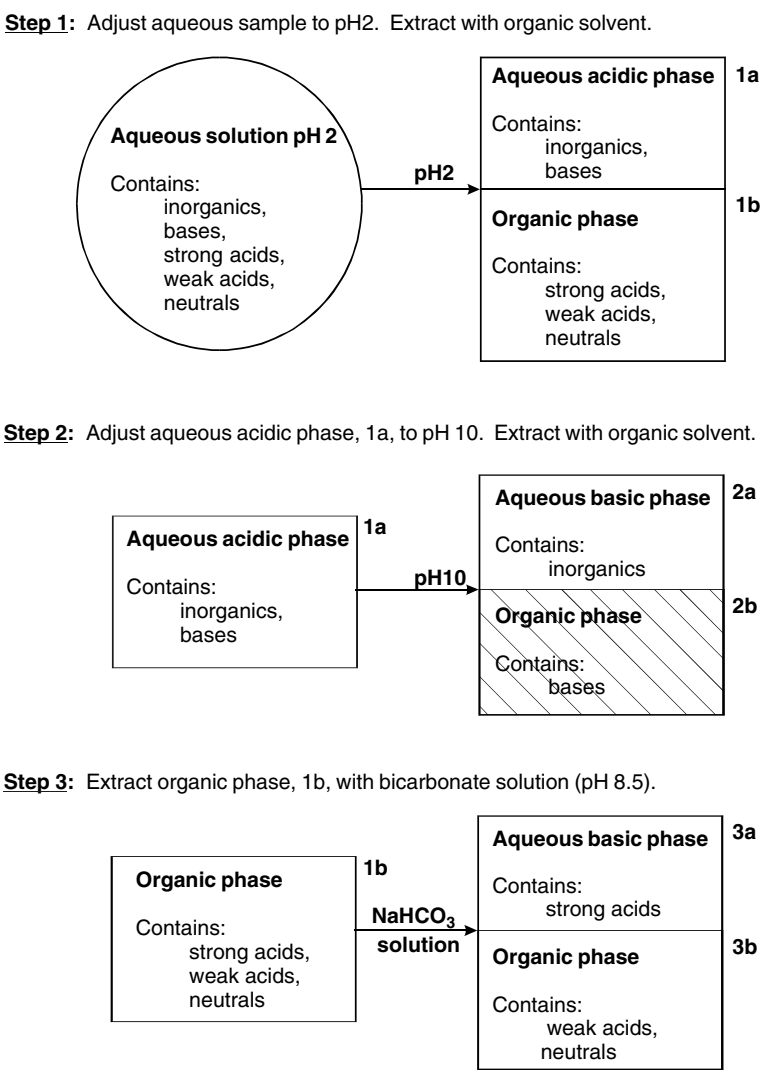
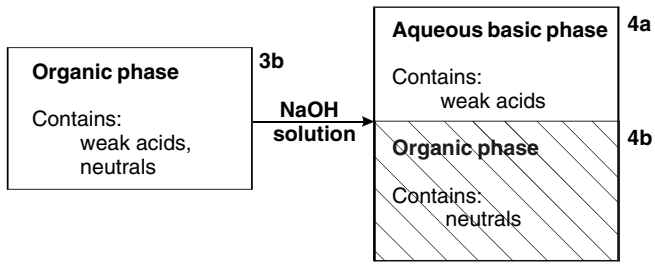


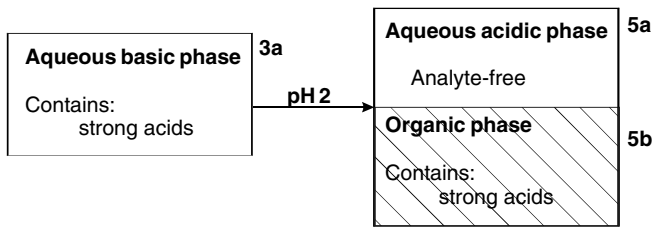
Figure 2.16. General extraction scheme. Hatched boxes represent isolation of organic compound categories in an organic phase.

The aqueous basic phase containing strong acids (Figure 2.16, step 5) and the aqueous basic phase containing weak acids (Figure 2.16, step 6) are each separately adjusted to pH 2 and extracted with organic solvent. Two organic solutions result: one containing recovered strong organic acids and the other containing weak organic acids.

Step 4: Extract organic phase, 3b, with hydroxide solution (pH 10).



Step 5: Adjust aqueous basic phase, 3a, to pH 2. Extract with organic solvent.



Step 6: Adjust aqueous basic phase, 4a, to pH 2. Extract with organic solvent.

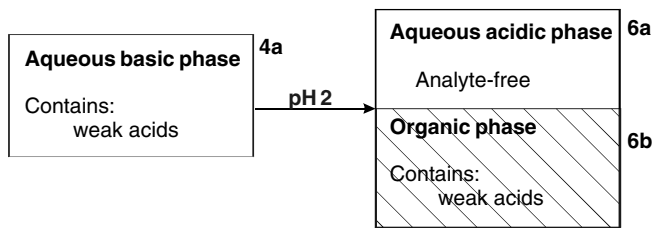


Figure 2.16. *(Continued)*

2.2.4. Recent Advances in Techniques

Historically, analysts performing LLE have experienced difficulties such as exposure to large volumes of organic solvents, formation of emulsions, and generation of mountains of dirty, expensive glassware. To address these problems, other sample preparation techniques, such as solid-phase extraction (SPE) and solid-phase microextraction (SPME), have experienced increased development and implementation during the previous two decades. However, advances in microfluidics amenable to automation are fueling a resurgence of LLE applications while overcoming some of the inherent difficulties associated with them.

Fujiwara et al. [47] devised instrumentation for online, continuous ion-pair formation and solvent extraction, phase separation, and detection. The procedure was applied to the determination of atropine in synthetic urine, and of atropine and scopolamine in standard pharmaceuticals. Aqueous sample solution was pumped at a flow rate of 5 mL/min. The organic extracting solvent, dichloromethane, was pumped at a flow rate of 2 mL/min and mixed with the aqueous sample stream to produce an aqueous-to-organic volume ratio of 2.5. The mixture was passed through an extraction coil composed of a 3-m PTFE tube [0.5 mm inside diameter (ID)] where associated ion pairs were transferred from the aqueous into the organic phase. The phases were separated using a Teflon membrane. The organic phase transversed the phase-separating membrane and passed onward in the stream to the detector while the aqueous stream was wasted.

Tokeshi et al. [48] performed an ion-pair solvent extraction successfully on a microchannel-fabricated quartz glass chip. An aqueous Fe complex (Fe–4,7-diphenyl-1,10-phenanthrolinedisulfonic acid) and a chloroform solution of capriquat (tri-*n*-octylmethylammonium chloride) were introduced separately into a microchannel (250 μm) to form a parallel two-phase laminar flow producing a liquid–liquid aqueous–organic interface (Figure 2.17). The authors noted that in the microchannel, the aqueous–organic interface did not attain the upper–lower arrangement produced by differences in specific gravity normally observed in LLE. In the microchannel environment, surface tension and frictional forces are stronger than specific gravity, resulting in an interface that is side by side and parallel to the sidewalls of the microchannel. The ion-pair product extracted from aqueous solution into

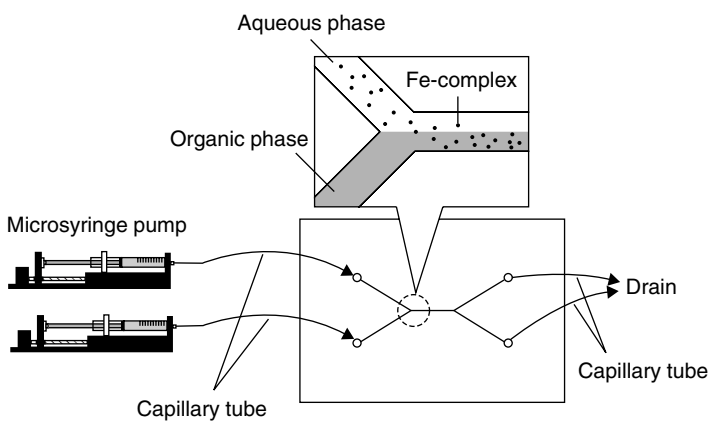


Figure 2.17. Schematic diagram of microextraction system on a glass chip. (Reprinted with permission from Ref. 48. Copyright © 2000 American Chemical Society.)

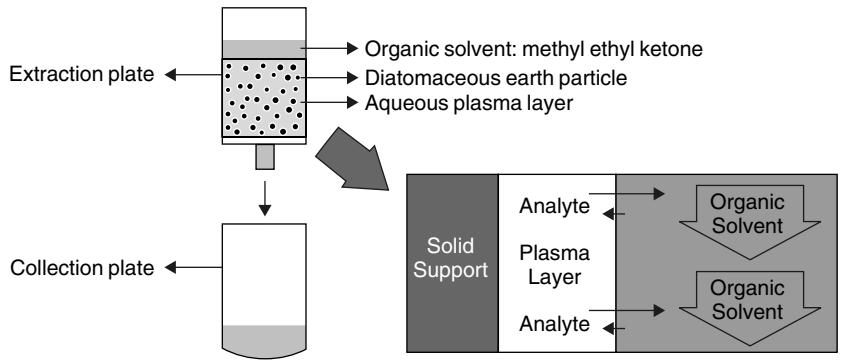


Figure 2.18. Schematic representation of automated liquid–liquid extraction. (Reprinted with permission from Ref. 50. Copyright © 2001 American Chemical Society.)

chloroform within 45 seconds when the flow was very slow or stopped, corresponding with molecular diffusion time. The extraction system required no mechanical stirring, mixing, or shaking.

Solid-supported LLE is a new approach reported by Peng et al. [49,50]. They exploited the efficiency of 96-channel, programmable, robotic liquid-handling workstation technology to automate methodology for this LLE variation. A LLE plate was prepared by adding inert diatomaceous earth particles to a 96-well plate with hydrophobic GF/C glass fiber bottom filters. Samples and solvents were added to the plate sequentially. LLE occurred in the interface between the two liquid phases and on the surface of individual particles in each well (Figure 2.18). The organic phase extracts were eluted under gentle vacuum into a 96-well collection plate. The approach was used for initial purification of combinatorial library samples and for quantitative analysis of carboxylic acid–based matrix metalloprotease inhibitor compounds in rat plasma.

2.3. LIQUID–SOLID EXTRACTION

When a liquid is extracted by a solid, phase A of the Nernst distribution law [equation (2.2)] refers to the liquid sample, and phase B, the extracting phase, represents the solid (or solid-supported liquid) phase:

$$K_D = \frac{[\mathrm{X}]_\mathrm{B}}{[\mathrm{X}]_\mathrm{A}} \tag{2.2}$$

Classically, batch-mode liquid–solid extractions (LSEs), were used to con-

centrate semivolatile organic compounds from liquids into the solid phase. The liquid sample was placed in contact with the flowable, bulk solid extracting phase, an equilibrium between the two phases was allowed to occur, followed by physical separation (by decanting or filtering) of the solid and liquid phases. During the past quarter century, different approaches to solid-phase extractions of semivolatile organic compounds have emerged, including three described here: solid-phase extraction (SPE), solid-phase microextraction (SPME), and stir bar sorptive extraction (SBSE). Like LLE, SPE is designed to be a total, or exhaustive, extraction procedure for extracting the analyte completely from the entire sample volume via the sorbent. Unlike LLE, SPE is a nonequilibrium or pseudoequilibrium procedure. Unlike SPE, SPME is an equilibrium procedure that is not intended to be an exhaustive extraction procedure. SPME is an analytical technique in its own right that is inherently different from SPE or LLE. SBSE is physically a scaled-up version of SPME, but in principle it is more closely related to LLE (as it has been applied to date), in that it is an equilibrium partitioning procedure that unlike SPME more easily presents the opportunity to achieve exhaustive extraction. Each variation on the theme of liquid–solid extraction is an important addition to the analyst's arsenal of procedures for recovering semivolatile organics from liquids.

2.3.1. Sorption

To understand any of the solid-phase extraction techniques discussed in this chapter, it is first necessary to understand the physical–chemical processes of sorption. Schwarzenbach et al. [8] make the distinction between *absorption* (with a "b") meaning *into* a three-dimensional matrix, like water uptake in a sponge, and *adsorption* (with a "d") as meaning *onto* a two-dimensional surface (Figure 2.19). Absorption, also referred to as *partitioning*, occurs when analytes pass into the bulk of the extracting phase and are retained. Adsorption is the attraction of an analyte to a solid that results in accumulation of the analyte's concentration at porous surfaces of the solid. Absorption results from weaker interactive forces than adsorption. Because adsorption and/or absorption processes are sometimes difficult to distinguish experimentally [52] and often occur simultaneously, the general term *sorption* will be used here when referring to these processes. The term *sorbent* will refer to the solid extracting phase, including certain solid-supported liquid phases. To predict and optimize extraction, it is important for the analyst to be aware of the nature of the sorbent used.

Although different processes may dominate in different situations, it can be assumed that multiple steps occur during sorption of an organic compound from liquids "into" or "onto" a solid phase. Any of the steps may

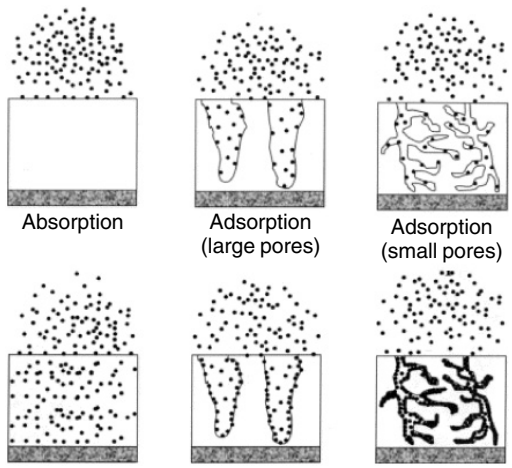


Figure 2.19. Schematic representation of absorptive versus adsorptive extraction and adsorption in small versus large pores. (Reprinted with permission from Ref. 51. Copyright © 2000 Elsevier Science.)

become a rate-limiting process in controlling sorption of an analyte. The analyte may interact with a solid-phase sorbent in at least four ways:

1. Through absorption, the analyte may interact with the sorbent by penetrating its three-dimensional structure, similar to water being absorbed by a sponge. Three-dimensional penetration into the sorbent is a particularly dominating process for solid-supported liquid phases. In the absorption process, analytes do not compete for sites; therefore, absorbents can have a high capacity for the analyte.
2. The analyte may interact two-dimensionally with the sorbent surface through adsorption due to intermolecular forces such as van der Waals or dipole–dipole interactions [53]. Surface interactions may result in displacement of water or other solvent molecules by the analyte. In the adsorption process, analytes may compete for sites; therefore, adsorbents have limited capacity. Three steps occur during the adsorption process on porous sorbents: *film diffusion* (when the analyte passes through a surface film to the solid-phase surface), *pore diffusion* (when the analyte passes through the pores of the solid-phase), and *adsorptive reaction* (when the analyte *binds*, *associates*, or *interacts* with the sorbent surface) [54].
3. If the compound is ionogenic (or ionizable) in aqueous solution (as discussed earlier), there may be an electrostatic attraction between the

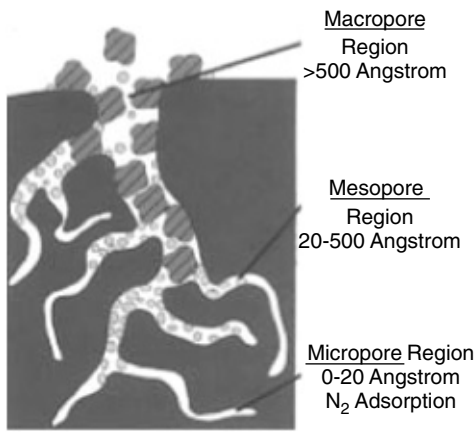


Figure 2.20. Micro-, macro-, and mesopores in a porous sorbent. (Reprinted with permission from Ref. 56. Copyright © 1996 Barnebey Sutcliffe Corporation.)

analyte and charged sites on the sorbent surface. Sorbents specifically designed to exploit these types of ionic interactions are referred to as *ion-exchange* (either anion- or cation-exchange) *sorbents*.

4. Finally, it is possible that the analyte and the sorbent may be chemically reactive toward each other such that the analyte becomes covalently bonded to the solid-phase sorbent. This type of sorption is generally detrimental to analytical recovery and may lead to slow or reduced recovery, also termed *biphasic desorption*. All of these interactions have the potential of operating simultaneously during sorption [8,54,55].

For porous sorbents, most of the surface area is not on the outside of the particle but on the inside pores of the sorbent (Figure 2.20) in complex, interconnected networks of *micropores* (diameters smaller than 2 nm), *mesopores* (2 to 50 nm), also known as *transitional pores*, and *macropores* (greater than 50 nm) [57]. Most of the surface area is derived from the small-diameter micropores and the medium-diameter transitional pores [56]. Porous sorbents vary in pore size, shape, and tortuosity [58] and are characterized by properties such as particle diameter, pore diameter, pore volume, surface areas, and particle-size distribution.

Sorption tendency is dependent on the characters of the sorbent, the liquid sample (i.e., solvent) matrix, and the analyte. Much of the driving force for extracting semivolatile organics from liquids onto a solid sorbent results from the favorable energy gains achieved when transferring between phases.

For some of the sorbents discussed in this section on liquid–solid extraction, the solid-supported liquid sorbent phase performing the extraction may appear to the naked eye to be a solid when it is actually a liquid. The chromatographic method of employing two immiscible liquid phases, one of which is supported on a solid phase, was introduced by Martin and Synge in 1941 [59]. The liquid sorbent phase was mechanically added to the solid support material, which can lead to problems with *bleeding*, or *stripping*, of the liquid phase from the supporting solid material. Therefore, in the 1960s, covalently bonded phases were developed that overcame some of these problems by actually anchoring the liquid phase to the solid support. When the liquid extracting phase merely coats a solid support instead of bonding to the surface, it continues to behave primarily like a liquid; that is, the solid-supported liquid phase still has three-dimensional freedom of motion and the sorptive behavior observed is dominated by absorption processes. When the liquid extracting phase is covalently bonded to the surface, it no longer acts primarily like a bulk liquid, since there is freedom of movement in two dimensions only; translational and rotational movement are restricted; and retention on this type of phase can no longer be described solely by absorption processes. Retention on a liquid phase covalently bonded to a porous solid support does not result from a pure absorption or a pure adsorption mechanism.

Is analyte recovery using a solid-supported liquid phase classified as LLE or LSE? In Section 2.2.4, a process described as solid-supported LLE [49,50] was discussed in which the liquid sorbent phase was distributed on the surfaces of individual particles (Figure 2.18). The solid-supported phases in the LSE section have been arbitrarily distinguished as liquids mechanically supported on solid devices, such as the liquid-coated fused silica fibers used for SPME or the liquid-coated glass sheath of a stirring bar in used SBSE, rather than liquids supported on finely divided solid particles.

2.4. SOLID-PHASE EXTRACTION

The historical development of solid-phase extraction (SPE) has been traced by various authors [60,61]. After a long latency period (from biblical times to 1977) when the theoretical "science" of SPE was known but not frequently practiced, technological breakthroughs in sorbents and devices fueled the growth of SPE use that continues today. The modern era of SPE, which resulted in today's exponential growth in applications of this technique, began in 1977 when the Waters Corporation introduced commercially available, prepackaged disposable cartridges/columns containing bonded silica sorbents. The term *solid-phase extraction* was coined in 1982 by employees of the J.T. Baker Chemical Company [62–65].

The most commonly cited benefits of SPE that led to early advances relative to LLE are reduced analysis time, reduced cost, and reduced labor (because SPE is faster and requires less manipulation); reduced organic solvent consumption and disposal [66–68], which results in reduced analyst exposure to organic solvents; and reduced potential for formation of emulsions [43]. The potential for automation of SPE increased productivity because multiple simultaneous extractions can be accomplished [43]. SPE provides higher concentration factors (i.e., K_D) than LLE [68] and can be used to store analytes in a sorbed state or as a vehicle for chemical derivatization [69]. SPE is a multistaged separation technique providing greater opportunity for selective isolation than LLE [66,68,70,71], such as fractionation of the sample into different compounds or groups of compounds [69]. The use of SPE for all of these objectives is being exploited by today's SPE researchers.

Solid-phase extraction refers to the nonequilibrium, exhaustive removal of chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent recovery of selected constituents by elution from the sorbent [72]. The introduction of sorbents exhibiting a very strong affinity for accumulating semivolatile organic compounds from water was the primary advance in the 1970s that propelled the technique into widespread use. The affinity, which was strong enough to be analytically useful from sorbents that were inexpensive enough to be economically feasible, was useful in both pharmaceutical and environmental applications. Mathematically, a *strong affinity* equates to a large K_D value in equation (2.2) because the concentration in the sorbent extracting phase, $[X]_B$, is large relative to the sample extracted. For this reason, SPE is sometimes referred to as *digital chromatography*, indicating the all-or-nothing extremes in the sorptive nature of these sorbents, caused by the strong attraction for the analyte by the sorbent. SPE drives liquid chromatographic mechanisms to their extreme, such that K_D approaches infinity, representing total accumulation of the analyte during retention, and K_D approaches zero during subsequent elution or release of the analyte.

Some analysts mistakenly refer to SPE sorbents as "filters" and the SPE process as "filtration" because of the porous character of many of the sorbents used for SPE. The molecules of the analyte that exist in true homogeneous solution in the sample are not filtered; they become associated with the solid phase through sorption. However, sorbent particles do act as *depth filters* toward particulate matter that is not in true homogeneous solution in the sample. Particulate matter can become lodged in the interstitial spaces between the sorbent particles or in the intraparticulate void volume, or pore space, within sorbent particles. The filtering of particulate matter is generally detrimental to the analysis and can lead to *plugging* of the extraction sorbent or channeling the flow through the sorbent. Fritz [73] summarizes that the

severity of a plugging problem in SPE depends on (1) the concentration, type, and size of the particulates in the sample; (2) the pore size of the sorbent; and (3) the surface area of the sorbent bed.

While particulate matter can cause plugging and channeling of the sorbent in SPE as described above, analysts performing SPE extraction and other analytical procedures must also be concerned with the potential for the analyte's association with particulate and colloidal matter contamination in the sample. Complex equilibria govern partitioning of organic analytes among the solution phase, colloidal material, and suspended particulate matter. Depending on the chemical nature of the analyte and the contamination, some of the analyte molecules can become sorbed to the contaminating particulate and/or colloidal matter in the sample [74]. Analytes can adhere to biological particulates such as cellular debris or bind to colloidal proteins. Similarly, analytes can adhere to environmental particulates or associate with colloidal humic substances. If the sample is not filtered, particulates can partially or entirely elute from the sorbent, leading to both a dissolved and particulate result when the sample is analyzed [75]. In addition to concern about the potential for suspended solids in the water sample plugging the SPE sorbent and analytes of interest adsorbing onto particulates, loss of the analyte may occur if small particulates pass through the pores of the sorbent bed [73].

To avoid these problems and ensure consistent results, sample particulate matter should be removed by filtration prior to SPE analysis [43]. If measuring the degree to which the analyte is bound to contaminants in the solution or, conversely, the degree to which the analyte is unassociated, or in true solution is important, the sample should be filtered prior to analysis by SPE or LLE. Glass-fiber filters, which have no organic binders, should be inert toward the analyte of interest while trapping particulate matter [43]. Particles with a diameter of 1 μm or greater tend to settle out of solution by gravity. Nominal filter sizes of 0.7, 0.45, or 0.22 μm are commonly reported in literature in conjunction with preparation of a sample for SPE. An appropriate level of filtration should be determined for the particular sample matrix being analyzed and used consistently prior to SPE analysis. The material retained on the filter may be analyzed separately to determine the level of bound analyte. The analyst must carefully assess whether rinsing the filter with water or an organic solvent and recombining the rinsings with the filtered sample meet the objectives sought and are appropriate for the given analysis.

Prefiltering samples prior to SPE in a standardized manner using glass-fiber filters having no organic binders and testing the analytes of interest to establish that they are not adsorbed on the filter selected is recommended [43]. Alternatively, Simpson and Wynne [76] present the counter viewpoint

that sample filtration is not always appropriate when the analyte adheres to biological or environmental particulates. They suggest that SPE devices more tolerent to the buildup of matrix solids, such as in-line filters, high-flow frits, or large-particle-size beds, should be tested. The analyst must be knowledgeable about the particulate/colloidal matter present in the sample matrix in order to consider these technical decisions about sample processing.

2.4.1. Sorbents in SPE

Appropriate SPE sorbent selection is critical to obtaining efficient SPE recovery of semivolatile organics from liquids. Henry [58] notes that an SPE sorbent "must be able to sorb rapidly and reproducibly, defined quantities of sample components of interest." Fritz [73] states that "successful SPE has two major requirements: (1) a high, reproducible percentage of the analytical solutes must be taken up by the solid extractant; and (2) the solutes must then be easily and completely eluted from the solid particles." The sorption process must be reversible. In addition to reversible sorption, SPE sorbents should be porous with large surface areas, be free of leachable impurities, exhibit stability toward the sample matrix and the elution solvents, and have good surface contact with the sample solution [68,73].

Obviously, knowledge of the chemistry and character of commonly used SPE sorbents is important to achieving successful extractions. Liska [60] describes developments from the late 1960s until the early 1980s as the "age of searching" for a universal SPE sorbent that culminated in the introduction of polymeric materials and bonded silicas. These sorbents have proven useful for a wide variety of applications. However, the realization that no single optimal sorbent for all purposes exists prompts current efforts to optimize a sorbent for a particular application [60], that is, for a specific analyte in a specific matrix. Poole et al. [77] categorize the SPE sorbents available today as either general purpose, class specific, or compound specific. This discussion covers polar, polymeric, bonded silica, and graphitized carbon sorbents of general applicability as well as functionalized polymeric resins, ion-exchange sorbents, controlled-access sorbents, immunoaffinity sorbents, and molecularly imprinted polymers designed for more specific purposes.

Polar Sorbents

The earliest applications of *chromatography*, a term coined by Tswett in 1906, used polar sorbents to separate analytes dissolved in nonpolar solvents. Using light petroleum as the nonpolar mobile phase, Tswett separated

a colored extract from leaves using column chromatography on a polar calcium carbonate column [78,79]. The alternate system, in which the sorbent is nonpolar while a polar solvent is used, was not used in chromatography until the late 1940s to early 1950s [80–83]. Howard and Martin [83] introduced the term *reversed-phase* to describe separation of fatty acids using solid-supported liquid paraffin or *n*-octane as nonpolar stationary phases that were eluted with polar aqueous solvents. At that time, these systems appeared to be ''reversed'' to the ''normal'' arrangement of polar stationary phases used with less polar eluents. Although reversed-phase applications outnumber normal-phase chromatographic applications today, the nomenclature still applies.

The most common polar sorbents used for normal-phase SPE are silica $(SiO_2)_x$, alumina (Al_2O_3), magnesium silicate ($MgSiO_3$ or Florisil), and the bonded silica sorbents in which silica is reacted with highly polar functional groups to produce aminopropyl $[(SiO_2)_x{-}(CH_2)_3NH_2]$-, cyanopropyl $[(SiO_2)_x{-}(CH_2)_3CN]$-, and diol $[(SiO_2)_x{-}(CH_2)_3OCH_2CH(OH)CH_2(OH)]$-modified silica sorbents (Figure 2.21). Polar SPE sorbents are often used to

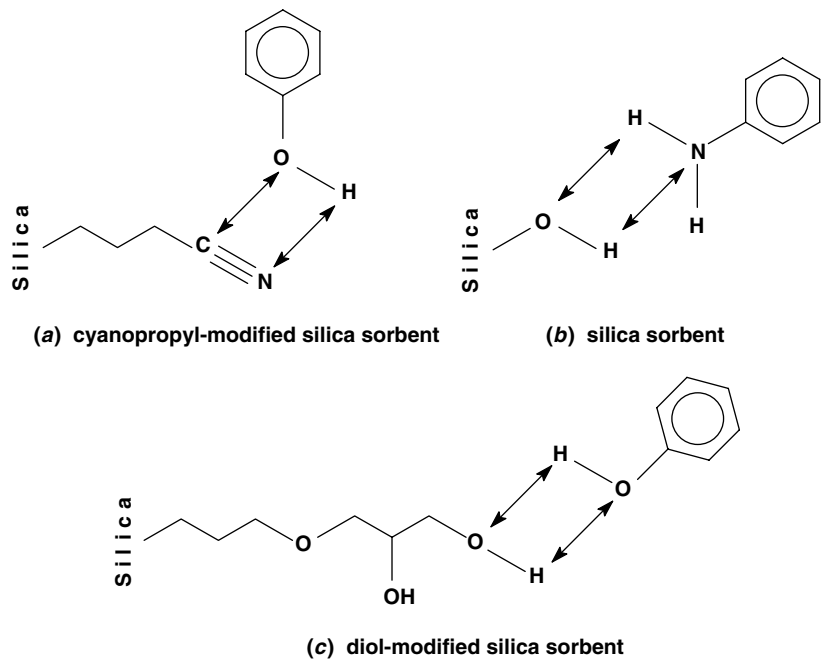


Figure 2.21. Interactions between analytes and polar sorbents via dipolar attraction or hydrogen bonding.

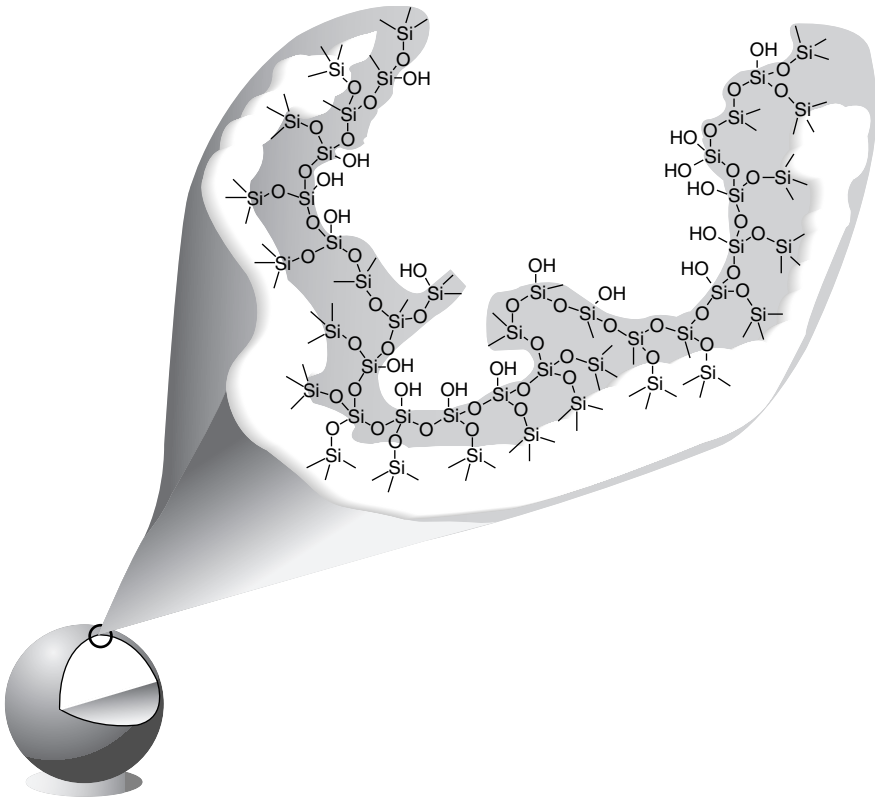


Figure 2.22. Representation of an unbonded silica particle. (Reprinted with permission from Ref. 84. Copyright © 2002 Waters Corporation.)

remove matrix interferences from organic extracts of plant and animal tissue [73]. The hydrophilic matrix components are retained by the polar sorbent while the analyte of interest is eluted from the sorbent. The interactions between solute and sorbent are controlled by strong polar forces including hydrogen bonding, dipole–dipole interactions, π–π interactions, and induced dipole–dipole interactions [75].

Porous silica (Figure 2.22) is an inorganic polymer $(SiO_2)_x$ used directly as a sorbent itself and for the preparation of an important family of sorbents known as chemically bonded silicas that are discussed later. Silica consists of siloxane backbone bridges, –Si–O–Si–, and silanol groups, –Si–OH. Colin and Guiochon [85] proposed that there are five main types of silanol group sites on the surface of a silica particle, depending on the method of preparation and pretreatment of the silica, including free silanol, silanol with

physically adsorbed water, dehydrated oxide, geminal silanol, and bound and reactive silanol. Porous silica consists of a directly accessible external surface and internal pores accessible only to molecules approximately less than 12,000 Da [86]. Pesek and Matyska [87] have reviewed the chemical and physical properties of silica.

Silica particles used for SPE sorbents are typically irregularly shaped, 40 to 60 μm in diameter. Silica particles used for sorbents in high-performance liquid chromatographic (HPLC) columns are generally spherical and 3 to 5 μm in diameter. Due to the differences in size and shape, SPE sorbents are less expensive than HPLC sorbents. Much greater pressures are required to pump solvents through the smaller particle sizes used in HPLC.

Apolar Polymeric Resins

Synthetic styrene–divinylbenzene and other polymers, particularly the trademarked XAD resins developed by Rohm & Haas, were used for SPE in the late 1960s and early 1970s. However, the particle size of the XAD resins is too large for efficient SPE applications, and therefore the resins require additional grinding and sizing. Also, intensive purification procedures are needed for XAD resins [73,75].

In the latter half of the 1990s, porous, highly cross-linked polystyrene–divinylbenzene (PS-DVB) resins with smaller, spherical particle sizes more suitable for SPE uses became available (Figure 2.23). The new generation of apolar polymeric resins is produced in more purified form, reducing the level of impurities extracted from the sorbent. Polymeric resins are discussed in more detail by Huck and Bonn [69], Fritz [73], Thurman and Mills [75], and Pesek and Matyska [87].

The enhanced performance of PS-DVB resins is due to their highly hydrophobic character and greater surface area as compared to the bonded silica sorbents, which are discussed in the following section. The strong sorption properties of PS-DVB resins may arise from the aromatic, poly-

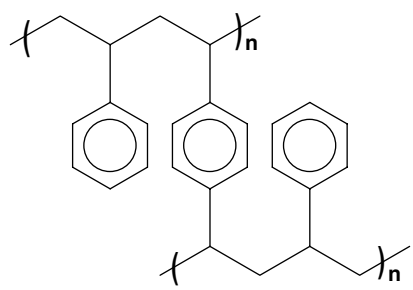


Figure 2.23. Cross-linked styrene–divinylbenzene copolymer.

meric structure that can interact with aromatic analytes via π–π interactions. However, because PS-DVB sorbents are highly hydrophobic, they are less selective. Also, PS-DVB sorbents exhibit low retention of polar analytes.

Polymeric organic sorbents can reportedly be used at virtually any pH, 2 to 12 [75] or 0 to 14 [73,88], increasing the potential to analyze simultaneously multiresidue samples containing acidic, basic, and neutral compounds. Polymeric sorbents contain no silanol groups and thereby avoid the problems caused by residual silanol groups when bonded silica sorbents are used [73,75].

The PS-DVB sorbents can be more retentive than the bonded silica sorbents. Polymeric sorbents have been shown to be capable of retaining chemicals in their ionized form even at neutral pH. Pichon et al. [88] reported SPE recovery of selected acidic herbicides using a styrene–divinylbenzene sorbent so retentive that no adjustment of the pH of the solution was necessary to achieve retention from water samples at pH 7. At pH 7 the analytes were ionized and thereby retained in their ionic form. To effect retention of acidic compounds in their nonionized form using bonded silica sorbents, it is necessary to lower the pH of the sample to approximately 2. Analysis at neutral pH can be preferable to reduced pH because at lower pHs undesirable matrix contaminants, such as humic substances in environmental samples, can be coextracted and coeluted with the analytes of interest and subsequently may interfere with chromatographic analyses.

Bonded Silica Sorbents

The first class of sorbents used for modern-era SPE were bonded-phase silicas. In the early 1970s, bonded silica sorbents found popularity as a stationary phase for HPLC. HPLC was not commonly used until the early 1970s, nor SPE until the late 1970s, until the application of silanized, or bonded silica sorbents, was realized. May et al. [89] and Little and Fallick [90] are credited with the first reports of applying bonded phases to accumulate organic compunds from water [60]. The first article about SPE on commercially available bonded-phase silica (an octadecyl, C_{18}, phase) was published by Subden et al. [91] and described the cleanup of histamines from wines.

Chemically bonded silica sorbents are currently the most commonly used solid phase for SPE. Bonded stationary phases are prepared by "grafting" organic nonpolar, polar, or ionic ligands (denoted R) to a silica particle via covalent reaction with the silanol groups on its surface. The importance of this advancement to chromatography in general and particularly to solid-phase extraction was the ability to produce highly hydrophobic phases that were more attractive to organic solutes in aqueous solution than any other

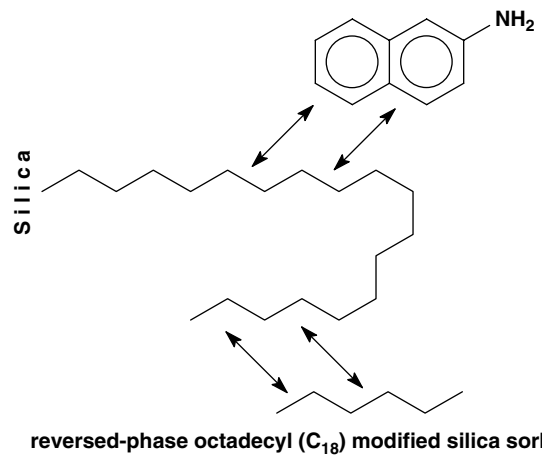


Figure 2.24. Interactions between analytes and nonpolar bonded silica sorbents via van der Waals forces.

sorbents available at the time. Reversed-phase bonded silica sorbents having alkyl groups covalently bonded to the silica gel backbone interact primarily with analytes via van der Waals forces (Figure 2.24).

Bonded-phase sorbents are stable to aqueous solvents over a pH range of 1 to 8.5, above which the silica backbone itself begins to dissolve and below which the Si–C bond is attacked. Manufacturers have continued to extend these ranges through improved products, and researchers have stretched the limits of these restrictions. The development of bonded silica sorbents led to a proliferation of pharmaceutical and environmental applications for extracting semivolatile organics from aqueous solution.

The bonded phases produced by manufacturers vary according to the nature of the silica used to prepare the bonded phase and in the reactants and reaction conditions used. The variations are closely guarded, proprietary manufacturing processes. However, it is generally known that the most common commercially manufactured bonded-phase sorbents are based on chemical reaction between silica and organosilanes via the silanol groups on the silica surface to produce chemically stable Si–O–Si–C covalent linkages to the silica backbone [75,87]. Nonpolar, polar, or ionic bonded phases can be prepared by varying the nature of the organic moiety bonded to the silica surface.

Bonded phases can be obtained as monomeric or polymeric coverage of an organic ligand group, R, on the silica surface depending on whether a monofunctional (R_3SiX) or a trifunctional ($RSiX_3$) reactant is used, respec-

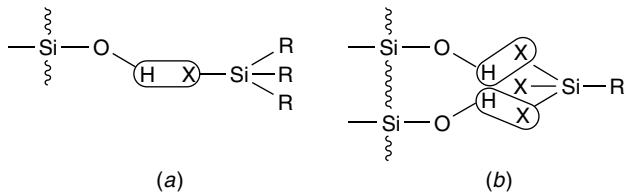


Figure 2.25. Reaction of a (*a*) monofunctional or (*b*) trifunctional organosilane with silanol groups on the silica surface.

tively (Figure 2.25). The organosilane contains a reactive group, X, that will interact chemically with the silanol groups on the silica surface. Typically, the reactant is an organochloro- or organoalkoxysilane in which the moiety, X, is chloro, methoxy, or ethoxy.

One or two Si–X groups can remain unreacted per bonded functional group because of the stoichiometry observed when trifunctional reactant modifiers are used. Hydrolysis of the Si–X group occurs in the workup procedure and results in the re-formation of new silanol groups (Figure 2.26), thereby reducing the hydrophobic character of the sorbent surface. The reactions result in the formation of a cross-linked polymeric network and/or a multilayer adsorbent. The monomeric types of bonded sorbents are obtained by using monofunctional organosilanes such as alkyldimethylmonochlorosilane to preclude the possibility of re-forming unreacted silanol groups.

A polymeric surface structure can result in slower mass transfer of the analyte in the polymer coating compared with the more ''brush- or bristle-like'' bonding of monomeric phases and thereby lead to higher efficiencies with monomeric phases. However, Thurman and Mills [75] note that the trifunctional reagent yields a phase that is more stable to acid because the

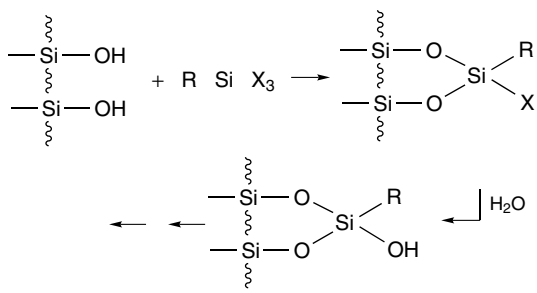


Figure 2.26. Reformation of additional silanol groups during processing when trifunctional modifiers are used.

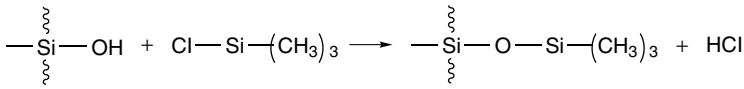


Figure 2.27. Accessible silanol groups are endcapped by reaction with trimethylchlorosilane.

organosilane is attached to the silica surface by multiple linkages to the silica backbone.

Silanol groups can be left unreacted on the silica surface, due to reaction conditions or steric inhibition, or generated during subsequent processing of polymeric bonded phases. In either case, they can have an effect on the sorption of the target analyte. Hennion [92] notes that silanol groups are uncharged at pH 2 and become increasingly dissociated above pH 2. Experimentally observable effects due to negatively charged silanols are evident above pH 4. The presence of unmasked silanol groups may have a positive, negative, or little effect, depending on the specific analyte of interest [93]. A positively charged competing base, such as triethylamine or tetrabutylammonium hydrogen sulfate, can be added to the sample to mask residual silanols.

To reduce the number of accessible silanol groups remaining on the sorbent, a technique known as *capping* or *endcapping* is sometimes used. With this technique, a small silane molecule such as trimethylchlorosilane is allowed to react with the bonded silica (Figure 2.27) to produce a more hydrophobic surface.

When using bonded silica SPE sorbents (or HPLC columns), a monomeric or polymeric phase may be best for a given analyte–matrix situation. Similarly, an endcapped or unendcapped product may be best. The preceding discussion should be helpful to analysts when consulting with manufacturers regarding the nature of the bonded surface of the sorbents produced. Hennion [92] recently published a table listing characteristics of some common, commercially available bonded silicas, including data on porosity, mean particle diameter, functionality of the silane used for bonding (i.e., mono- or trifunctional), endcapping, and percent carbon content.

Bonded silica sorbents are commercially available with many variations in the organic ligand group, R. Common bonded phases produced for reversed-phase applications include hydrophobic, aliphatic alkyl groups, such as octadecyl (C_{18}), octyl (C_8), ethyl (C_2), or cyclohexyl, covalently bonded to the silica gel backbone. Aromatic phenyl groups can also be attached. The R ligand can contain cyanopropyl or diol hydrophilic functional groups that result in polar sorbents used in normal-phase applications. Ionic functional groups, including carboxylic acid, sulfonic acid, aminopropyl, or qua-

ternary amines, can also be bonded to the silica sorbent to produce ion-exchange sorbents.

The primary disadvantages of the bonded silica sorbents are their limited pH stability and the ubiquitous presence of residual silanol groups. Despite these difficulties, the bonded silicas have been the workhorse sorbents of SPE applications for the last two decades and are still the most commonly used SPE sorbents.

Graphitized Carbon Sorbents

Graphitized carbon sorbents are earning a reputation for the successful extraction of very polar, extremely water soluble organic compounds from aqueous samples. The retention behavior of the graphitized carbon sorbents is different than that of the apolar polymeric resins or the hydrophobic bonded silica sorbents. Two types of graphitized carbon sorbents, graphitized carbon blacks (GCBs) and porous graphitic carbons (PGCs), are commercially available for SPE applications.

GCBs do not have micropores and are composed of a nearly homogeneous surface array of graphitelike carbon atoms. Polar adsorption sites on GCBs arise from surface oxygen complexes that are few in number but interact strongly with polar compounds. Therefore, GCBs behave both as a nonspecific sorbent via van der Waals interactions and as an anion-exchange sorbent via electrostatic interactions [92,94,95]. GCBs have the potential for simultaneous extraction of neutral, basic, and acidic compounds. In some cases no pH adjustment of the sample is necessary. Desorption can be difficult because GCB is very retentive.

PGC sorbents have even more highly homogeneous hydrophobic surfaces than GCB sorbents. PGCs are macroporous materials composed of flat, two-dimensional layers of carbon atoms arranged in graphitic structure. The flat, homogeneous surface of PGC arranged in layers of carbons with delocalized π electrons makes it uniquely capable of selective fractionation between planar and nonplanar analytes such as the polychlorinated biphenyls [92,94,95].

Functionalized Polymeric Resins

Adding polar functional groups to cross-linked, apolar polymeric resins by covalent chemical modification has developed particularly for generation of SPE sorbents suitable for recovery of polar compounds. Hydrophilic functional groups such as acetyl, benzoyl, *o*-carboxybenzoyl, 2-carboxy-3/4-nitrobenzoyl, 2,4-dicarboxybenzoyl, hydroxymethyl, sulfonate, trimethylammonium, and tetrakis(*p*-carboxyphenyl)porphyrin have been chemically

introduced into the structural backbone of PS-DVB copolymers [96]. Generation of a macroporous copolymer consisting of two monomer components, divinylbenzene (lipophilic) and *N*-vinylpyrrolidone (hydrophilic), produced a hydrophilically–lipophilically balanced SPE sorbent [69]. Chemically modifying apolar polymeric sorbents in this way improves wettability, surface contact between the aqueous sample and the sorbent surface, and mass transfer by making the surface of the sorbent less hydrophobic (i.e., more hydrophilic [73,75,96,97]). The sulfonate and trimethylammonium derivatives are used as ion-exchange sorbents, a type of sorbent that is considered in a later section.

Higher breakthrough volumes (i.e., indicating greater attraction of the sorbent for the analyte) for selected polar analytes have been observed when the hydrophilic functionalized polymeric resins are used as compared to classical hydrophobic bonded silicas or nonfunctionalized, apolar polymeric resins. In addition to having a greater capacity for polar compounds, functionalized polymeric resins provide better surface contact with aqueous samples. The bonded silica sorbents and the polymeric resins (discussed in earlier sections) have hydrophobic surfaces and require pretreatment, or conditioning, with a hydrophilic solvent to activate the surface to sorb analytes. Using covalent bonding to incorporate hydrophilic character permanently in the sorbent ensures that it will not be leached from the sorbent as are the common hydrophilic solvents (e.g., methanol, acetonitrile, or acetone) used to condition bonded silica sorbents or polymeric resins [69,73,96].

Ion-Exchange Sorbents

SPE sorbents for ion exchange are available based on either apolar polymeric resins or bonded silica sorbents. Ion-exchange sorbents contain ionized functional groups such as quaternary amines or sulfonic acids, or ionizable functional groups such as primary/secondary amines or carboxylic acids. The charged functional group on the sorbent associates with the oppositely charged counterion through an electrostatic, or ionic, bond (Figure 2.28).

The functional group on the sorbent can be positively or negatively charged. When the sorbent contains a positively charged functional group and the exchangeable counterion on the analyte in the liquid sample matrix is negatively charged, the accumulation process is called *anion exchange*. Conversely, if the functional group on the sorbent surface is negatively charged and the exchangeable counterion on the analyte in the liquid sample matrix is positively charged, the accumulation process is called *cation exchange*.

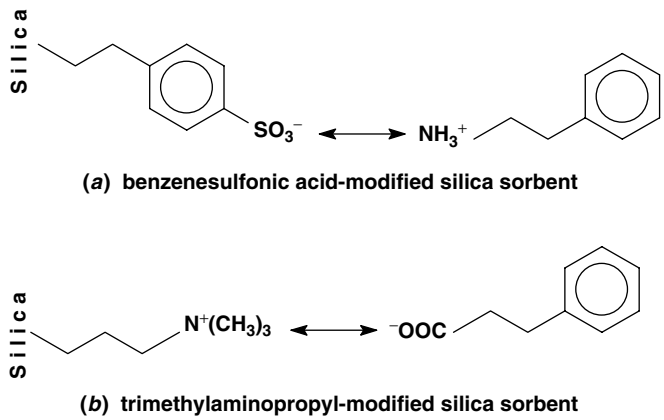


Figure 2.28. Interactions between analytes and ion-exchange sorbents: (*a*) strong cation-exchange sorbent and (*b*) strong anion-exchange sorbent.

The theoretical principles of acid–base equilibria discussed earlier in this chapter apply to the sorbent, the analyte, and the sample in ion-exchange processes. The pH of the sample matrix must be adjusted in consideration of the pK_a of the sorbent (Table 2.5) and the pK_a of the analyte such that the sorbent and the analyte are oppositely charged under sample loading conditions.

Anion-exchange sorbents for SPE contain weakly basic functional groups such as primary or secondary amines which are charged under low-pH conditions or strongly basic quaternary ammonium groups which are charged at all pHs. Cation-exchange sorbents for SPE contain weakly acidic functional

Table 2.5. Ionization Constants of Ion-Exchange Sorbents

Ion-Exchange Sorbents	Sorbent pK_a
Cation exchange	
$-CH_2CH_2COOH$	4.8
$-CH_2CH_2CH_2SO_3H$	<1.0
$-CH_2CH_2\phi SO_3H$	$\ll 1.0$
Anion exchange	
$-CH_2CH_2CH_2NHCH_2CH_2NH_2$	10.1 and 10.9
$-CH_2CH_2CH_2N(CH_2CH_3)_2$	10.7
$-CH_2CH_2CH_2N^+(CH_3)_3Cl^-$	Always charged

Source: Data from Ref. 98.

groups such as carboxylic acids, which are charged under high-pH conditions, or strongly acidic aromatic or aliphatic sulfonic acid groups, which are charged at all pH levels. ''Weakly'' acidic or basic ion-exchange sorbents are pH dependent because they dissociate incompletely, while ''strongly'' acidic or basic ion-exchange sorbents are pH independent because they dissociate completely.

In SPE, the ionic interaction between an ion-exchange sorbent and an analyte is a stronger attraction than the hydrophobic interactions achievable with apolar polymeric resins or with aliphatic/aromatic bonded silica sorbents. In ion exchange, the distribution coefficient, K_D [equation (2.2)], generally increases with the charge and bulkiness of the exchanging ion [73]. The kinetics of the ion-exchange process is slower than with nonpolar or polar interaction mechanisms. Simpson [99] discusses the kinetic effects on SPE by ion-exchange extraction.

The counterion associated with the sorbent when it is manufactured is replaced by another ion of like charge existing on the analyte to achieve retention. However, analyte retention is affected by the ionic strength of the sample matrix because other ions present will compete with the analyte of interest for retention by ion-exchange mechanisms [75].

Controlled-Access Sorbents

Controlled-access sorbents are intended to be either ''inclusive'' or ''exclusive'' of large molecules and macromolecules. Wide-pore, or large-pore, sorbents are designed intentionally to allow accessibility of macromolecules to the internal pore structure of the sorbent such that they will be retained. Conventional SPE sorbents commonly have pores of 60 Å, whereas wide-pore SPE sorbents have pores of 275 to 300 Å [75].

Conversely, restricted access materials or restricted access media (RAM) retain small molecules while excluding macromolecules such as biological proteins in their presence (Figure 2.29). Small molecules are retained by sorption processes in the pores of the sorbent while the large molecules are excluded and elute at the interstitial volume of the sorbent. This separation leads to size-selective disposal of interfering macromolecular matrix constituents.

Unlike conventional steric exclusion sorbents, RAM sorbents exhibit *bifunctional* or *dual-zone* character, in that the inner and outer surfaces are different. The outer surface is designed to exclude macromolecules physically and is rendered chemically hydrophilic to discourage retention of biomolecules. Small molecules penetrate to an inner surface, where they are retained by any of the various other sorptive surface chemistries already discussed [92].

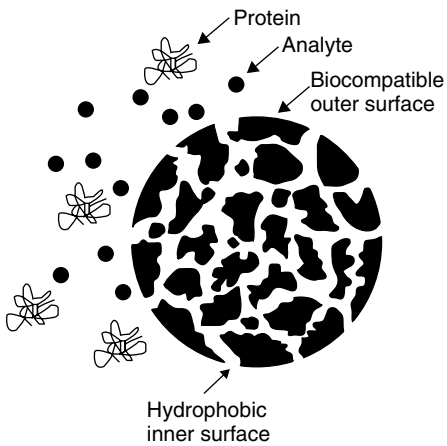


Figure 2.29. Schematic representation of a sorbent particle for restricted-access media chromatography. This medium allows proteins and macromolecules to be excluded and elute in the solvent front, while small analyte molecules enter the pores and are retained. (Reprinted with permission from Ref. 100. Copyright © 2000 Elsevier Science.)

Immunoaffinity or Immunosorbents

The driving force behind development of more selective sorbents is minimizing the problem of coextracting matrix interferences that are usually present in much greater concentration than the trace levels of the analyte of interest. More selective sorbents also permit extraction of larger sample volumes, thereby reducing the level of detection of the analyte of interest.

A recent approach to producing highly selective sorbents for SPE is based on molecular recognition technology and utilizes antibodies immobilized by covalent reaction onto solid supports such as silica (Figure 2.30). Preparation of immunoaffinity sorbents for SPE was reviewed by Stevenson [101] and Stevenson et al. [102]. Using immunosorbents, efficient cleanup is achieved from complex biological and environmental samples.

Antibodies can cross-react with closely related analytes within a chemical family. This disadvantage has been used to advantage in SPE. Therefore, immunosorbents have been designed for a single analyte, a single analyte and its metabolites, or a class of structurally related analytes [92]. The approach is therefore useful for chemical class-specific screening of compounds, such as triazines, phenylureas, or polyaromatic hydrocarbons. The specificity of the antibody is used for extraction by chemical class. Following SPE, analytical chromatographic techniques such as HPLC and GC separate structurally similar analytes for quantification.

Molecularly Imprinted Polymeric Sorbents

Another approach to selective SPE based on molecular recognition is the development of molecularly imprinted polymers (MIPs), which are said to

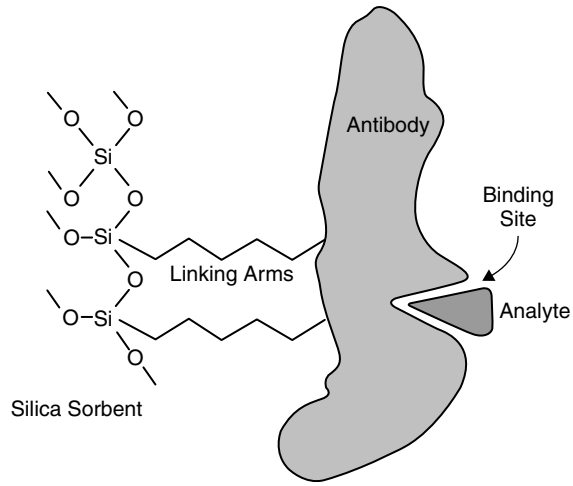


Figure 2.30. Diagrammatic representation of an immunoaffinity SPE binding an analyte. (Reprinted with permission from Ref. 75. Copyright © 1998 John Wiley & Sons, Inc.)

be an attempt to synthesize antibody mimics [92,101]. Produced by chemical synthesis, MIPs are less expensive and more easily and reproducibly prepared than immunosorbents that are prepared from biologically derived antibodies [102].

SPE sorbents that are very selective for a specific analyte are produced by preparing (MIPs) in which the target analyte is present as a molecular template when the polymer is formed. Sellergren [103] is credited with first reporting of the use of MIP sorbents for SPE. Subsequently, MIP-SPE has been applied to several biological and environmental samples [92,104–106].

MIP sorbents are prepared by combining the template molecule with a monomer and a cross-linking agent that causes a rigid polymer to form around the template (Figure 2.31). When the template is removed, the polymer has *cavities* or *imprints* designed to retain the analyte selectively. Retention of the analyte on these sorbents is due to shape recognition, but other physicochemical properties, including hydrogen bonding, ionic interactions, and hydrophobic interactions, are important to retention as well [92,104,107].

MIP-SPE sorbents are stable in both aqueous and organic solvents and are very selective for the analyte of interest. Increased selectivity relative to other sorbents produces increased sensitivity because larger sample volumes can be extracted. Also, increased selectivity results in efficient sample cleanup of the analyte in the presence of complex biological or environmental matrix

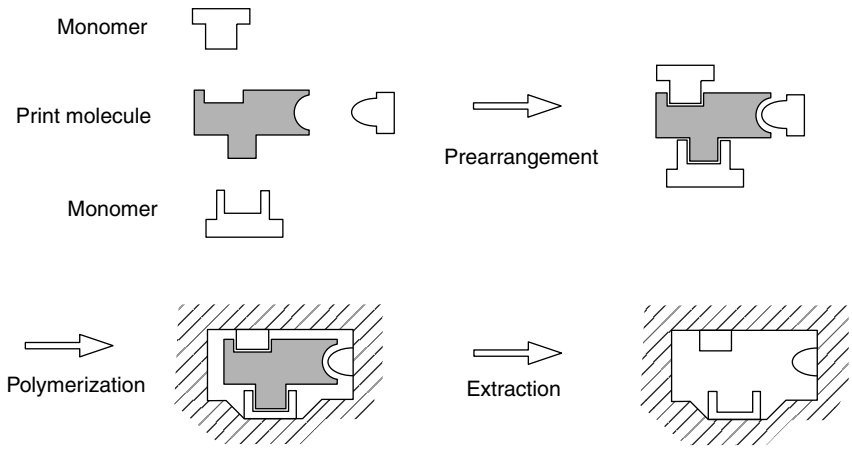


Figure 2.31. Schematic depiction of the preparation of molecular imprints. (Reprinted with permission from Ref. 105. Copyright © 2000 Elsevier Science.)

interferences. However, desorption is usually more difficult if any sorbent has increased affinity for the analyte.

One problem noted in MIP-SPE is incomplete removal of the template molecule from the polymer, resulting in leaching of the analyte during subsequent trace analyses. Stringent cleaning of the sorbent and analytical confirmation of the lack of interfering compound can reduce this problem. Alternatively, another approach has been to use a structural analog of the target analyte as the template used to create the MIP sorbent [105,106]. This approach is successful if the structural analog creates an imprint that is selective for the target analyte and if the structural analog and the target analyte can be separated chromatographically for quantitation after extraction.

Mixed-Mode Sorbents and Multiple-Mode Approaches

Each of the types of SPE sorbents discussed retains analytes through a primary mechanism, such as by van der Waals interactions, polar dipole–dipole forces, hydrogen bonding, or electrostatic forces. However, sorbents often exhibit retention by a secondary mechanism as well. Bonded silica ion-exchange sorbents primarily exhibit electrostatic interactions, but the analyte also experiences nonpolar interaction with the bonded ligand. Nonpolar bonded silicas primarily retain analytes by hydrophobic interactions but exhibit a dual-retention mechanism, due to the silica backbone and the presence of unreacted surface silanol groups [72]. Recognition that a dual-

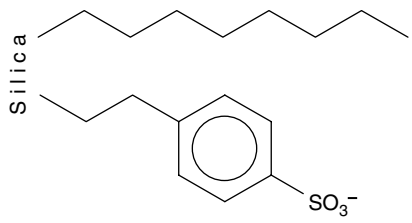


Figure 2.32. Example of a mixed-mode sorbent consisting of silica modified with octyl (C_8) alkyl chains and strong cation-exchange sites bonded on the same sorbent particle.

retention mechanism is not always detrimental to an analysis [93] has led to the production of mixed-mode sorbents by design. The development of mixed-mode sorbents and multiple-mode approaches to capitalize on multiple retention mechanisms has evolved as a logical extension of the observation of secondary interactions [108].

A mixed-mode sorbent is designed chemically to have multiple retentive sites on an individual particle (Figure 2.32). These sites exploit different retention mechanisms by chemically incorporating different ligands on the same sorbent. For example, sorbents have been manufactured that contain hydrophobic alkyl chains and cation-exchange sites on the same sorbent particle [92]. Mixed-mode sorbents exploit interaction with different functional groups on a single analyte or different functional groups on multiple analytes. Mixed-mode SPE sorbents are particularly useful for the extraction of analytes from bodily fluids [68].

Alternatively, there are several different mechanical approaches to achieving multiple-mode retention (Figure 2.33). Sorbent particles of different types (i.e., a hydrophobic sorbent and an ion-exchange sorbent) that exhibit separate mechanisms of retention can be homogeneously *admixed*, or *blended*, in the same column, or they can be *layered* into the same column by packing one phase over another [97]. Additionally, multiple phases can be *stacked* by arranging in tandem series sorbents of different retention mechanisms contained in separate columns. The technique of stacking or sequencing sorbents in tandem columns, termed *chromatographic mode sequencing* (CMS), can produce very selective isolation of analytes [109].

2.4.2. Sorbent Selection

Thurman and Mills [75] point out that knowing the analyte structure is the clue to effective isolation by SPE. A sorbent selection chart (Figure 2.34) is a useful guide for matching the analyte with the appropriate sorbent. Most manufacturers of SPE sorbents provide such guidelines either in printed product literature or on the Internet. To use a sorbent selection scheme, the analyst must be prepared to answer the following questions:

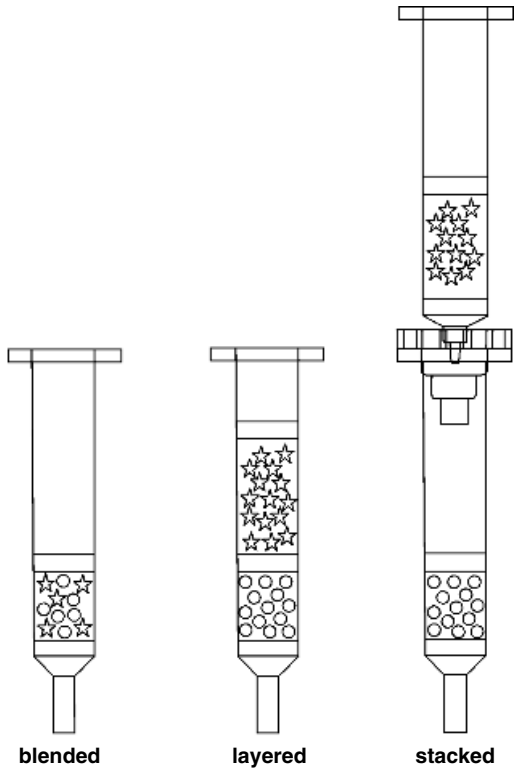


Figure 2.33. SPE multiple-mode approaches.

- Is the sample matrix miscible primarily with water or organic solvents?
- If the sample matrix is water soluble, is the analyte ionized or nonionized?
- If ionized, is the analyte permanently ionized (pH independent) or ionizable (pH dependent); is the analyte anionic or cationic?
- If the analyte is nonionized or ionization can be controlled (by pH suppression or ion pairing), is it nonpolar (hydrophobic), moderately polar, or polar (hydrophilic)?
- If the sample matrix is organic solvent miscible, is it miscible only in nonpolar organic solvents such as hexane, or is it also miscible in polar organic solvents such as methanol?
- Is the analyte nonpolar (hydrophobic), moderately polar, or polar (hydrophilic)?

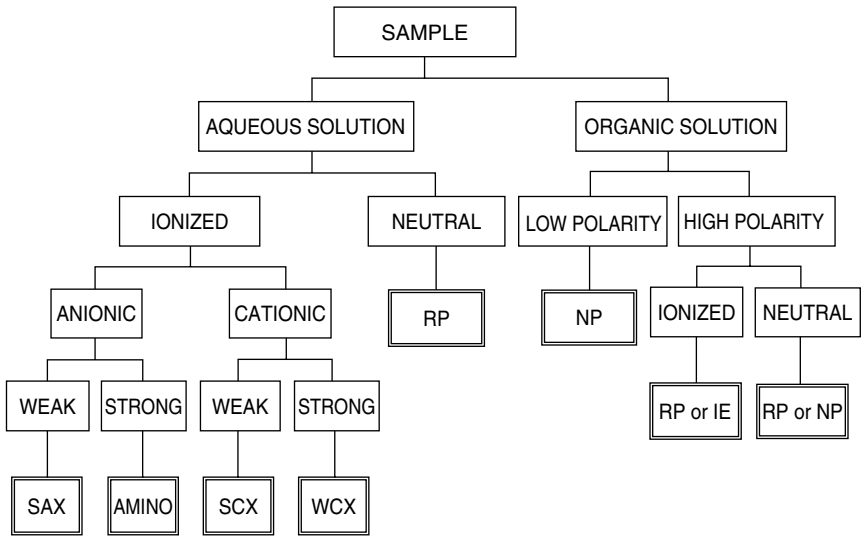


Figure 2.34. Method selection guide for the isolation of organic compounds from solution. SAX, strong anion exchanger; SCX, strong cation exchanger; WCX, weak cation exchanger; RP, reversed-phase sampling conditions; NP, normal-phase sampling conditions; IE, ion-exchange sampling conditions. (Reprinted with permission from Ref. 77. Copyright © 2000 Elsevier Science.)

Various types of sorbents used for SPE can be grouped (Table 2.6) according to the primary mechanism by which the sorbent and the analyte interact [32,72]. Reversed-phase bonded silica sorbents having alkyl groups such as octadecyl (C_{18}, C18), octyl (C_8, C8), or ethyl (C_2, C2) covalently bonded to the silica gel backbone or cyclohexyl (CH) or phenyl groups and sorbents composed of polymeric resins such as polystyrene–divinylbenzene

Table 2.6. SPE Sorbent–Analyte Interaction Mechanisms

Primary Interaction Mechanism	Sorbents	Energy of Interaction[a] (kcal/mol)
Van der Waals	Octadecyl, octyl, ethyl, phenyl, cyclohexyl, styrene–divinylbenzene, graphitized carbon	1–10
Polar/dipole–dipole	Cyano, silica, alumina Florisil	1–10
Hydrogen bonding	Amino, diol	5–10
Electrostatic	Cation exchange, anion exchange	50–200

[a]Data from Ref. 97.

interact primarily with analytes via van der Waals forces. Nonionic water-soluble compounds can be retained by reversed-phase sorbents but may not be as well retained as analytes that are soluble in methanol or methanol–water miscible mixtures. Normal-phase polar sorbents, such as silica, alumina, and Florisil, and cyano (CN) bonded phases interact by polar-dipole/dipole forces between polar functional groups in the analyte and the polar surface of the sorbent. Amino (NH_2) and diol sorbents interact with analytes by hydrogen bonding. Hexane-soluble analytes are best retained by normal-phase sorbents such as silica or Florisil or polar functionally substituted bonded phases such as amino or diol. Strong cation-exchange (SCX) and strong anion-exchange (SAX) sorbents interact primarily through electrostatic attractions between the sorbent and the analyte. Graphitized carbon sorbents exhibit both nonspecific van der Waals interactions and anion-exchange, or electrostatic, attraction for analytes.

2.4.3. Recovery

Recovery from spiked samples is calculated by measuring the amount of analyte eluted from the sorbent and comparing the original concentration to the concentration remaining after SPE. Retention and elution are two separate phases of the SPE method. However, the value measured is the overall recovery, which depends on both the sorption and elution efficiencies. Therefore, protocol development is confounded by the interdependence of sorption and desorption processes:

$$\text{recovery} = \text{sorption efficiency} \times \text{desorption efficiency} \qquad (2.33)$$

If sorption is 50% efficient but desorption is 100% efficient, the recovery measured is 50% and it is impossible to know whether sorption or desorption was inefficient or if reduced recovery was produced by a combination of both. Therefore, method development requires either optimizing sorption while controlling desorption, or vice versa using an iterative approach [67,72]. Alternatively, a statistical factorial design can be used to determine and optimize quickly variables important to SPE [110]. Using either approach, it is important to consider the major factors influencing retention, including sample pH, sample volume, and sorbent mass.

Dependence of Sorption on Sample pH

If a compound is ionizable, the extraction will be pH dependent. Data collected by Suzuki et al. [111] are graphically represented for selected data in Figure 2.35 to illustrate the influence of pH on SPE recovery. The effects

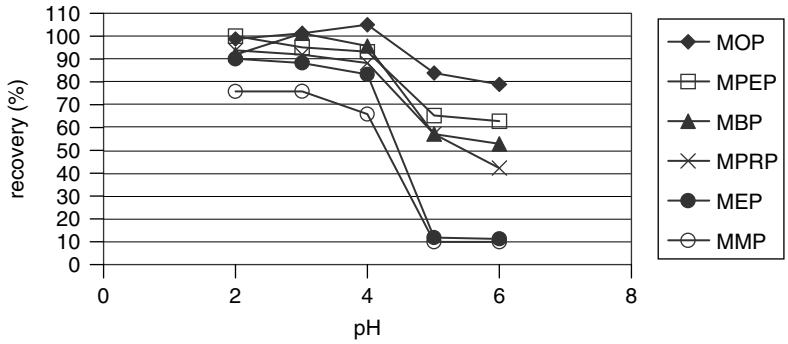


Figure 2.35. Dependence of SPE sorption on sample pH. Graphic based on selected data from Ref. 111.

of sample pH on SPE recovery of phthalic acid monoesters were evaluated using a styrene–divinylbenzene apolar polymeric phase. The effect of pH on the recovery of the free acid form of the monomethyl (MMP), monoethyl (MEP), mono-*n*-propyl (MPRP), mono-*n*-butyl (MBP), mono-*n*-pentyl (MPEP), and mono-*n*-octyl (MOP) phthalates was determined. The data clearly illustrate the principles discussed in Section 2.1.4.

Phathalic acid monoesters are weakly acidic compounds, due to the presence of a carboxyl group. At pH 2, the SPE recovery ranges from 76% for monomethyl phthalate to 99% for mono-*n*-octyl phthalate and 100% for mono-*n*-pentyl phthalate. As the pH increases, recovery gradually decreases but declines rapidly between pH 3 and 5. Recovery levels off between pH 5 and 6. The appearance of the data leads to the conclusion that the pK_a of the phthalic acid monoesters is between 3 and 5. The pK_a of this family of compounds appears to be approximately the same for each member of the series; that is, the electronic character of the carboxylic acid group is relatively unaffected by changes in the chain length of the alkyl group. At pH 2, these compounds are therefore nonionized, and at pH 6 they exist substantially in the ionized state. However, even at pH 6, recovery ranges from 10% for monomethyl phthalate to 79% for mono-*n*-octyl phthalate. This illustrates two principles discussed earlier in the chapter. First, even in the ionized state, these compounds retain a substantial degree of hydrophobicity. Second, the styrene–divinylbenzene sorbent is highly retentive, as illustrated by the degree of retention of the phthalic acid monoesters in the ionized state.

The order of recovery in the data at pH 2 and 6 is correlated approximately with the increase in the number of carbons in the alkyl chain, which in turn is roughly correlated with an increase in hydrophobicity. This exam-

ple is a good illustration of the difficulty in recovering all analytes effectively from a single extraction when they range from hydrophilic to hydrophobic extremes [43]. Potential ways to increase the recovery of the least hydrophobic compound in this series, that is, the monomethyl phthalate, might include increasing the mass of the sorbent, decreasing the volume of the sample, or adding salt to the sample for a salting-out effect. However, using these approaches to improve recovery of the monomethyl phthalate may indeed reduce recovery of the most hydrophobic components in this family of compounds.

If, in this example, the best recovery were observed for the monomethyl phthalate and the least recovery observed for the mono-*n*-octyl phthalate (i.e., the order in recovery at pH 2 were reversed), an inadequate volume or eluotropic strength of the elution solvent might be the cause of reduced recovery for the more hydrophobic analytes.

Dependence of Sorption on Sample Volume

Breakthrough volume is the maximum sample volume from which 100% recovery can be achieved [112]. Since that value is somewhat difficult to predict or derive experimentally (as are peaks in the stock market), it is helpful to use Poole and Poole's [113] definition, which arbitrarily defines breakthrough volume as the point at which 1% of the sample concentration at the entrance of the sorbent bed is detected at the outlet of the sorbent bed. The type and quantity of sorbent, hydrophobicity and ionizability of the analytes, and sample volume and pH interactively determine the breakthrough volume. The breakthrough volume for a specific mass of sorbent can be established by either loading variable-volume samples of constant concentration or variable-volume samples of variable concentration, in which case the latter comprises a constant molar amount loaded [112]. Alternatively, methods exist for predicting the breakthrough volume [113].

Selected data published by Patsias and Papadopoulou-Mourkidou [114] illustrate sorption's dependence on sample volume (Figure 2.36). Their research pursues development of an automated online SPE-HPLC methodology for analysis of substituted anilines and phenols. Recovery (%) was measured for numerous compounds on various polymeric sorbents, but the only data presented here are those in which a styrene–divinylbenzene polymeric sorbent was used for analysis of aniline, phenol, 4-nitroaniline, and 4-nitrophenol. Aqueous sample volumes of 5, 10, 25, 50, 75, 100, 125, and 150 mL were acidified to pH 3 before SPE.

Recovery for 4-nitroaniline and 4-nitrophenol begins to decrease when the analytes break through from the sorbent between the sample volumes of 10 and 25 mL. Breakthrough volumes for phenol and aniline are less

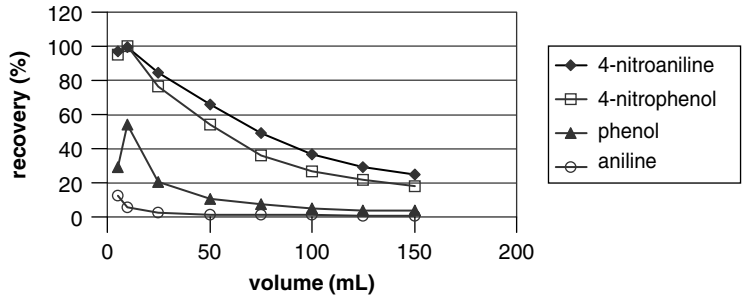


Figure 2.36. Dependence of SPE sorption on sample volume. Graphic based on selected data from Ref. 114.

than 5 mL under these conditions. The difference in the dependence of sorption upon sample loading volume between the parent molecules aniline and phenol and the nitro-substituted derivative compounds is a function of the characteristic hydrophobicity of the analytes involved as influenced by the acid dissociation constant of the analyte and the pH of the solution. The hydrophobic substituent parameter values, π_x [equation (2.8)], for para-substituted nitroaniline relative to aniline and for para-substituted nitrophenol relative to phenol (see Table 2.1) are positive, indicating that the nitro-substituted compounds are more hydrophobic than the parent compounds. The relative differences in hydrophobicity are reflected in the degree of recovery illustrated for these compounds in Figure 2.36. At each sample volume tested, the recovery is greater for the nitro-substituted compounds than for phenol and aniline.

Using a styrene–divinylbenzene sorbent, as in this example, the primary interaction mechanism is via van der Waals forces; therefore, the more hydrophobic the compound, the larger the breakthrough volume will be and the larger the sample size from which quantitative recovery can be expected. This observation can be generalized to other sorbents by stating that regardless of the primary interaction mechanism between the analyte and the sorbent (see Table 2.6), it holds true that the stronger the interaction, the larger the breakthrough volume will be.

Dependence of Sorption on Sorbent Mass

Increasing the amount of sorbent will increase the sample volume that can be passed through the sorbent before breakthrough. The dependence of sorption on sorbent mass is illustrated (Figure 2.37) for SPE recovery from a 50-mL sample volume (72 ppb) in which two C_8 columns, each contain-

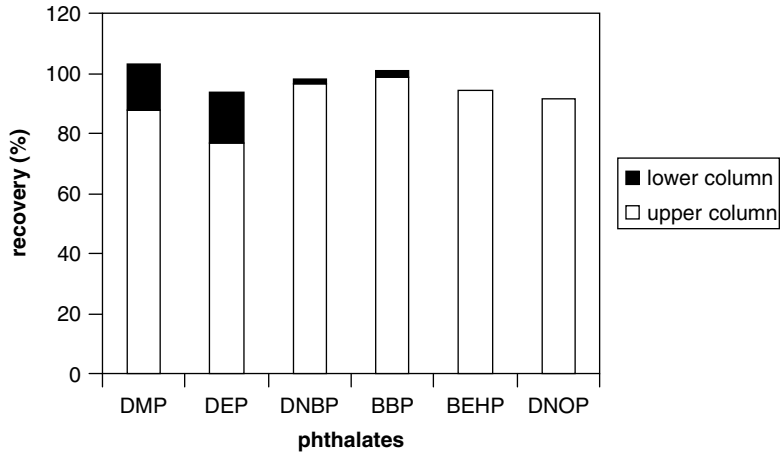


Figure 2.37. Dependence of SPE sorption on sorbent mass. Graphic based on data from Ref. 115.

ing 1.0 g of sorbent, were arranged in tandem [115]. Selected phthalates, including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DNBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), and di-*n*-octyl phthalate (DNOP), were monitored. After sample loading was complete, the two columns were separated and eluted separately with 10 mL of hexane to establish the recovery for each separate mass of sorbent. The analytes in Figure 2.37 are arranged on the *x*-axis from left to right in order of increasing hydrophobicity. The results demonstrate that 1.0 g of C_8 sorbent (the upper column in the two-column tandem arrangement) is enough to sorb BEHP and DNOP but is not enough to sorb DMP, DEP, DNBP, and BBP completely. The latter compounds are less hydrophobic than the former, and the breakthrough volumes are therefore smaller. Approximately 16% recovery for DMP and DEP was detected in the bottom column of the tandem stack. A small amount of DNBP and BBP (about 2%) was also recovered from the bottom column. BEHP and DNOP were retained completely on the upper column. BEHP and DNOP are highly hydrophobic, and the breakthrough volumes are larger. BEHP and DNOP require a smaller amount of sorbent to achieve optimized recoveries. For DMP, DEP, DNBP, and BBP, the van der Waals interactions with the sorbent are less, so more sorbent mass is needed for sorption. For BEHP and DNOP, van der Waals forces are strong, so less sorbent mass is required for sorption. Optimum recovery of all six compounds from this sample volume requires 2.0 g of C_8 sorbent.

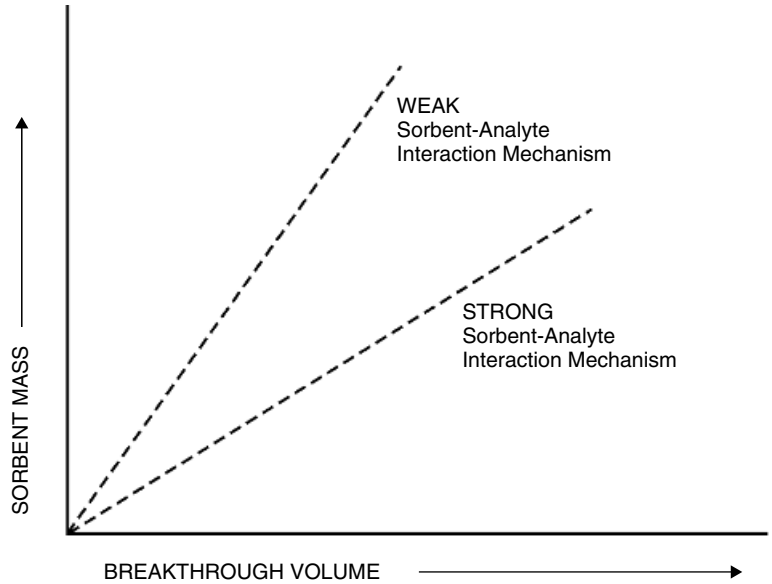


Figure 2.38. SPE interaction between sorbent mass and breakthrough volume.

Analyte sorption is dependent on both sample volume and sorbent mass (Figure 2.38). For a given amount of sorbent, the breakthrough volume is smaller for an analyte that interacts less strongly with the sorbent. For any given sample volume up to and including the breakthrough volume, the analyte that interacts more strongly with the sorbent will require a smaller amount of sorbent to achieve quantitative recovery.

Dependence of Sorption on Sample Concentration

Concentration-dependent recovery is an analytical chemist's nightmare. If an SPE method is to be useful, the analyst must demonstrate that sorption is not dependent on sample concentration in the expected concentration range of samples to be analyzed.

Dependence of Desorption on Eluting Solvent Strength

Relative elution solvent strength (or eluotropic strength) is depicted in solvent polarity charts (Figure 2.39). The relative elution strength for a solvent on a polar, normal-phase sorbent such as silica or alumina increases in reverse order to that measured on a nonpolar, reversed-phase sorbent. Ac-

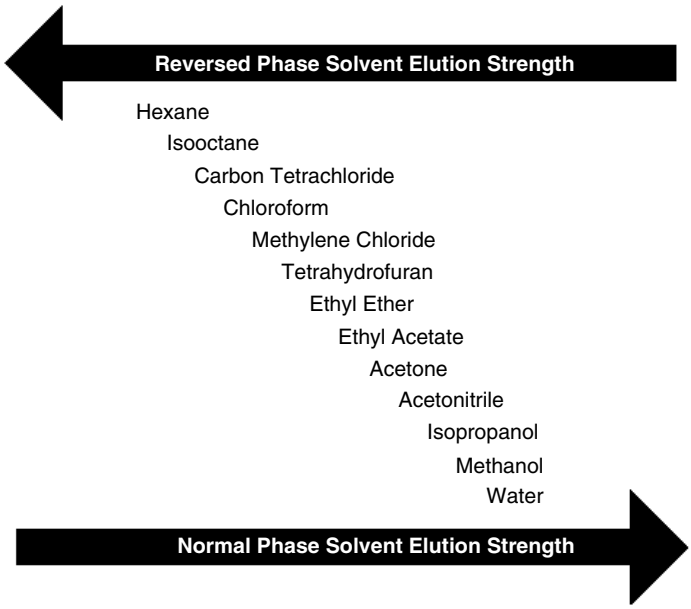


Figure 2.39. Solvent polarity chart indicates relative elution strength. (Reprinted with permission from Ref. 116. Copyright © 2002 Alltech Associates.)

cording to this chart, water is considered to be a weak solvent and hexane a strong solvent on reversed-phase sorbents. The eluting power increases as the solvent polarity decreases. Mixtures of miscible solvents can provide elution solvents of intermediate eluotropic strength.

When selecting a desorption solvent, the effect of the solvent on recovery of sample matrix contaminants should be considered. If available, a control sample matrix should be screened against potential elution solvents to assess which solvents can be used to maximize recovery of the analyte of interest and minimize the elution of sample contaminants.

Suzuki et al. [111] screened three solvents—methylene chloride, diethyl ether, and benzene—to determine their ability to produce optimum elution of phthalic acid monoesters sorbed on a styrene–divinylbenzene polymer (Figure 2.40). The effect of elution solvent strength on the recovery of the free acid form of the monomethyl (MMP), ethyl (MEP), *n*-propyl (MPRP), *n*-butyl (MBP), *n*-pentyl (MPEP), and *n*-octyl (MOP) phthalates is compared. The phthalic acid monoesters are arranged in Figure 2.40 in the order of increasing number of carbons in the alkyl chain, which in turn is roughly correlated with an increase in hydrophobicity.

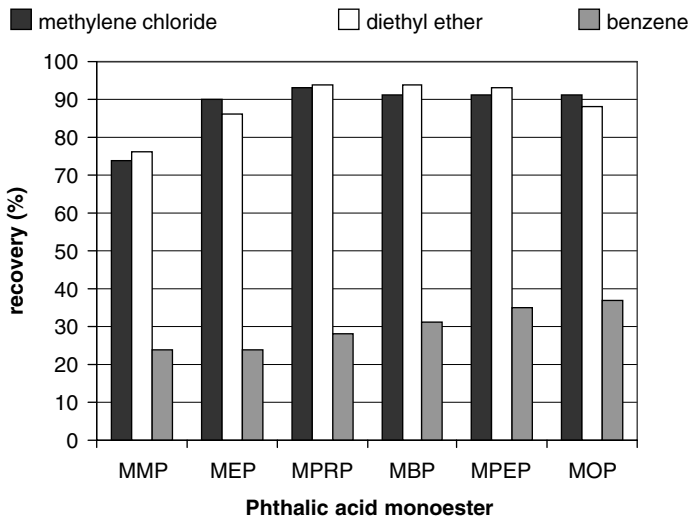


Figure 2.40. Dependence of SPE desorption on elution solvent eluotropic strength. Graphic based on selected data from Ref. 111.

When using benzene, recovery of the analytes upon elution increased with increasing hydrophobicity of the analyte but ranged from a low of 24% for the monomethyl phthalate to a high of 37% for the mono-*n*-octyl phthalate. Although benzene is expected to be a strong eluent on an apolar polymeric sorbent, it was not in this instance. Benzene may be incapable of wetting the sorbent in the presence of absorbed/adsorbed water because of its nonpolar nature. The layer of sorbed water on a sorbent phase is difficult to remove completely, even after drying with vacuum, and may be the cause of the inadequate recovery observed in this data when benzene is used. Similar results have been observed in other instances when hexane was used as an eluting solvent [112,117].

Recovery using methylene chloride or diethyl ether as eluting solvents was 86% or more for the monoesters depicted in Figure 2.40, except for the monomethyl phthalate. Relative to benzene, the polar character of methylene chloride and diethyl ether improves the wetability of the apolar sorbent having polar water molecules sorbed to the surface. The reduced recovery of mono-methyl-phthalate using methylene chloride or diethyl ether is probably due to incomplete sorption (i.e., the breakthrough volume may have been exceeded) rather than to incomplete desorption, because the more hydrophobic components were more completely desorbed.

Dependence of Desorption on Eluting Solvent Volume

Using SPE, the initial sample volume (V_i) divided by the final, or eluting, solvent volume (V_f) indicates the degree of concentration expected on 100% recovery (e.g., an optimized method for a 1000-mL sample loading volume recovered with a 10-mL eluting solvent volume is expected to produce a 100-fold increase in concentration). Therefore, the smallest amount of solvent that produces efficient recovery is generally used to produce the greatest degree of sample concentration. However, desorbing the sample using a larger volume of a solvent of lower eluting strength rather than a smaller volume of a solvent of stronger eluting strength can leave strongly retained contaminants on the sorbent as the analyte of interest is recovered.

Selected phthalates were extracted from a 50-mL sample volume (25 ppb) by SPE using 1.0 g of C_8 sorbent [115]. Extraction of dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DNBP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), and di-*n*-octyl phthalate (DNOP) illustrates (Figure 2.41) the dependence of elution, and therefore recovery, upon solvent volume. The recovery of all analytes in this example increased with increasing elution volume from 5 mL to 10 mL of hexane. In this graph, the analytes are arranged within each elution volume and compared in order of increasing hydrophobicity. The least hydrophobic members, DMP and DEP, of this group are probably retained incompletely by 1.0 g of C_8 sorbent. Among all the members of this group of analytes, the

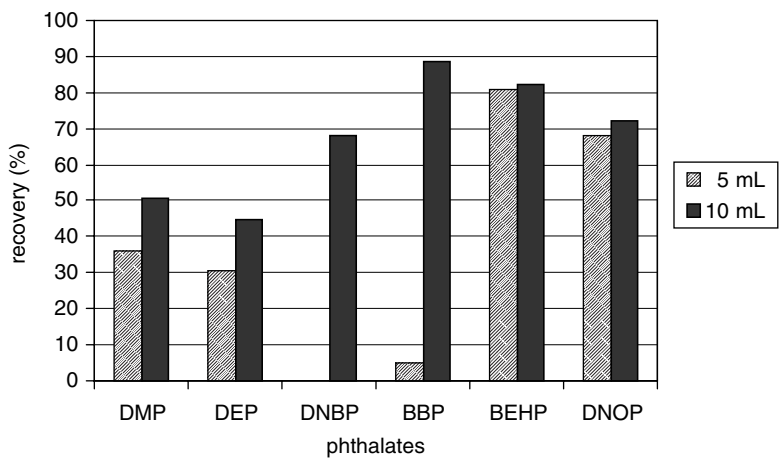


Figure 2.41. Dependence of SPE desorption on eluting solvent volume. Graphic based on data from Ref. 115.

extremely hydrophobic BEHP and DNOP compounds are eluted best when using a 5-mL elution volume of hexane. Perhaps the extreme hydrophobicity of BEHP and DNOP or their extended chain length relative to the other compounds makes it possible for hexane to better interact with these analytes than those with shorter chain lengths that are more intimately associated with the layer of water sorbed on the sorbent surface. Figure 2.41 clearly illustrates the importance of examining the dependence of desorption on sample volume.

2.4.4. Methodology

Generally, SPE consists of four steps (Figure 2.42): column preparation, or prewash, sample loading (retention or sorption), column postwash, and sample desorption (elution or desorption), although some of the recent advances in sorbent technology reduce or eliminate column preparation procedures. The prewash step is used to condition the stationary phase if necessary, and the optional column postwash is used to remove undesirable contaminants. Usually, the compounds of interest are retained on the sorbent while interferences are washed away. Analytes are recovered via an elution solvent.

SPE is not a single type of chromatography. SPE is a nonequilibrium procedure combining nonlinear modes of chromatography (Figure 2.43): the sample loading, or retention step, involves frontal chromatography and the sample desorption, or elution, step involves stepwise, or gradient, desorption, or displacement development [43,119]. In contrast, HPLC is a form of linear, or elution, chromatography that leads to dilution of the analyte as opposed to concentration of the analyte that is achieved with SPE.

In HPLC, the sample is introduced via elution development (Figure 2.44*a*) in which "the mixture is applied as a small quantity at the head of the column ... and the individual components are separated by being transported along the stationary phase by the continuous addition and movement of the mobile phase" [120]. Sample introduction in SPE is conducted as frontal chromatography (Figure 2.44*b*) in which there is "the continuous addition of the dissolved mixture to the column, with the result that the least sorbed compound is obtained in a pure state" [120]. Linear chromatography is distinguished from nonlinear chromatography by the different way in which the sample is fed into the sorbent. Therefore, SPE results in greater concentration of the analyte in the final elution volume than in the original sample, while HPLC, for example, dilutes the sample in the effluent relative to the original sample.

SPE sorbents are commercially available in three formats: contained within cartridges, in columns fashioned like syringe barrels, or in disks

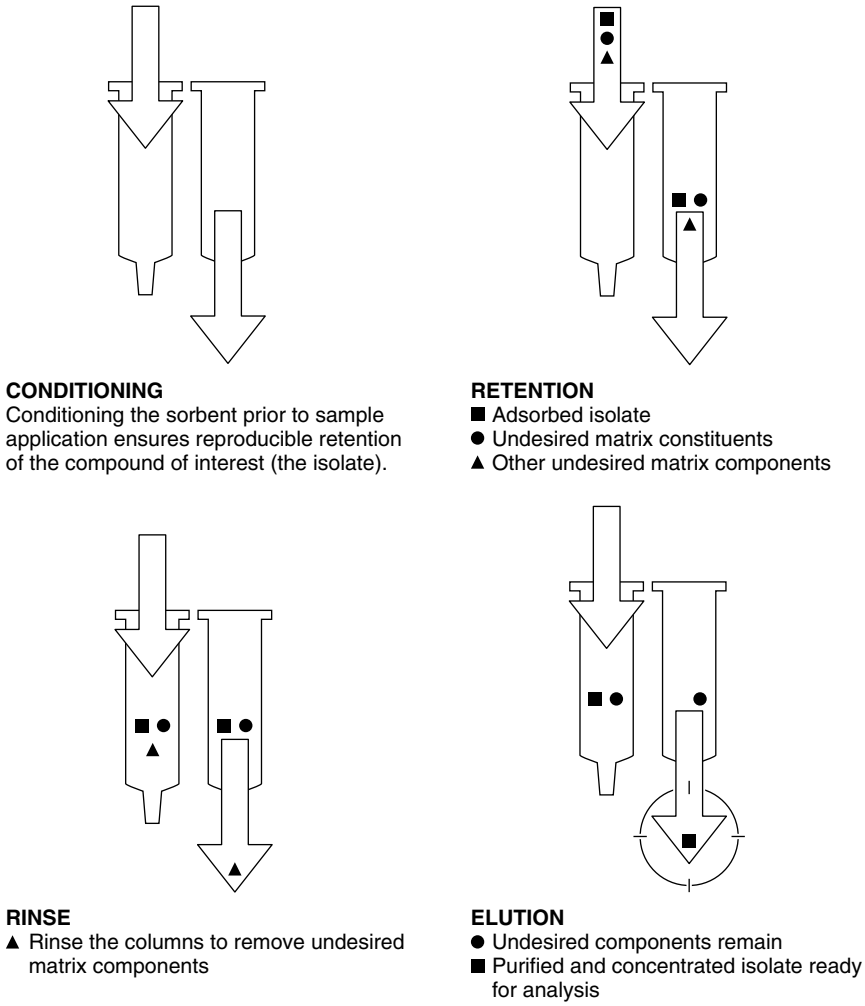


Figure 2.42. Four basic steps for solid-phase extraction. (Reprinted with permission from Ref. 118. Copyright © 2002 Varian, Inc.)

(Figure 2.45). Bulk sorbent phases can also be purchased. Typical column housings are manufactured of polypropylene or glass, and the sorbent is contained in the column by using porous frits made of polyethylene, stainless steel, or Teflon. Pesek and Matyska [87] describe three types of disk construction: (1) the sorbent is contained between porous disks, which are inert with respect to the solvent extraction process; (2) the sorbent is en-

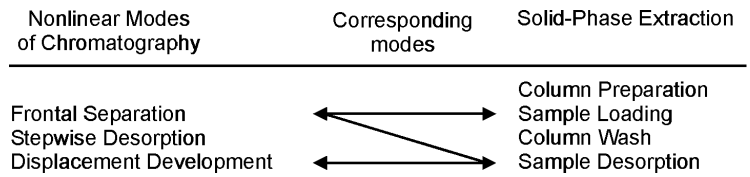


Figure 2.43. Nonlinear modes of chromatography. (Reprinted with permission from Ref. 43. Copyright © 2000 Marcel Dekker, Inc.)

meshed into a web of Teflon or other inert polymer; and (3) the sorbent is trapped in a glass fiber or paper filter. The commercial availability of SPE sorbents in 96-well formats (i.e., 96 individual columns contained in a single molded block) has made parallel processing with robotic automated workstations possible. Solvents can be passed through SPE sorbents by positive pressure, or hand pumping, or can be pulled through by vacuum.

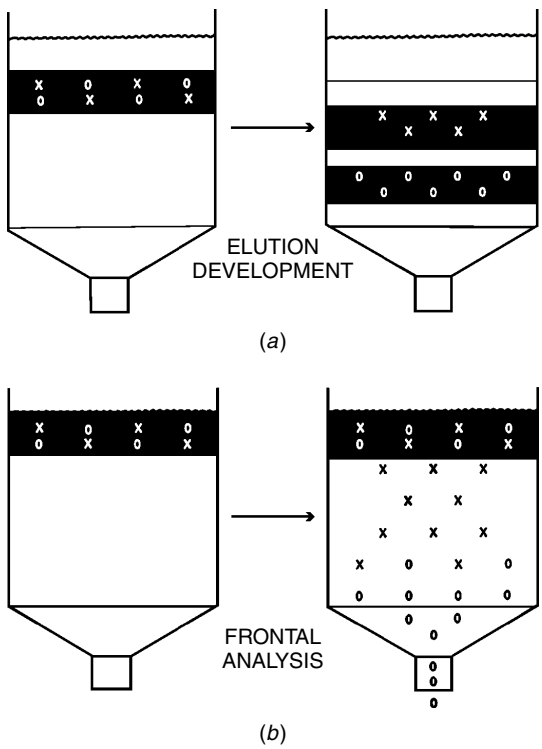


Figure 2.44. Comparison of (*a*) elution development and (*b*) frontal chromatography.

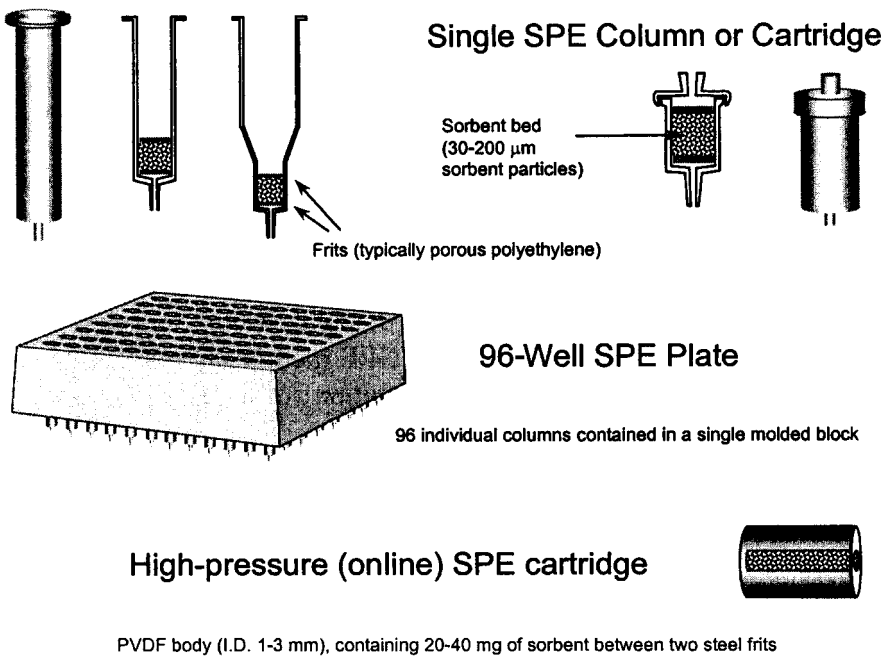


Figure 2.45. SPE formats. (Reprinted with permission from Ref. 87. Copyright © 2000 Marcel Dekker, Inc.)

2.4.5. Procedures

Ionized Analytes

Ionic water-soluble compounds can be retained by ion-exchange sorbents or by reversed-phase (RP) sorbents if ionization is controlled by ion suppression (i.e., by pH control that produces the nonionized form). In ion-exchange SPE, retention occurs at a sample pH at which the analyte is in its ionic form, whereas the analyte is desorbed in its neutral form; if the analytes are ionic over the entire pH range, desorption occurs by using a solution of appropriate ionic strength [92].

Alternatively, ionic compounds can be recovered from solution on hydrophobic sorbents using ion-pair SPE (IP-SPE). Carson [121] notes that advantages of IP-SPE over ion-suppression RP-SPE or ion-exchange SPE include selectivity, compatibility with aqueous samples and rapid evaporative concentration of eluents, and potential application to multiclass multiresidue analysis. IP reagents (e.g., 1-dodecanesulfonic acid for pairing with basic analytes or tetrabutylammonium hydrogen sulfate for pairing with

acidic analytes) are molecules typically composed of a long-chain aliphatic hydrocarbon and a polar acidic or basic functional group. IP reagents improve the sorption of analytes on hydrophobic sorbents in two ways: (1) the reagent and analyte form a neutral complex pair, and (2) the IP reagent usually contains a hydrophobic and/or bulky portion of the molecule that increases the overall hydrophobicity of the complex relative to the unpaired analyte.

Multistage SPE

Basic SPE procedures consist of four steps, as illustrated earlier (Figure 2.42). However, using multiple processing steps such as selective sorption, selective desorption and multiple mode processes such as chromatographic mode sequencing (Figure 2.33) are possible and can lead to increased selectivity [66,70,71]. The number of theoretical plates of an SPE column is roughly two orders of magnitude less than for HPLC columns. However, SPE columns have considerable capacity for chemical class separations and can be used to isolate compounds selectively from multicomponent samples. Multistage procedures exploit differences in analyte hydrophobicity, polarity, and ionogenicity. Multistage processes lead to multiple extracts or fractions that separate components and lead to improvement in the subsequent analytical results. Selective sorption in SPE can be accomplished by controlling the sample matrix or the sorbent. Selective desorption is accomplished by utilizing differences in the eluotropic strength, ionic strength, pH, or volume of the eluting solvent to produce multistep serial elution of the sorbent. Chromatographic mode sequencing (CMS) is the serial use of different chromatographic sorbents for SPE [109].

Automation

During the past decade, SPE process automation has become a reality. High-throughput 96-well workstations and extraction plates are commercially available and allow numerous samples to be processed simultaneously [122]. Among the advantages of automated SPE, Rossi and Zhang [100] list timesaving; high throughput with serial sample processing (25 to 50 samples per hour) and even greater throughput using parallel processing systems (up to 400 samples per hour); improved precision and accuracy; reduced analyst exposure to pathogenic or hazardous samples; reduced tedium; and the possibility of automated method development. The advantages of automated systems outweigh the limitations, but the disadvantages should be considered and include the potential for carryover, systematic errors that can occur undetected and decrease precision, and sample stability issues.

2.4.6. Recent Advances in SPE

Microfluidics and miniaturization hold great promise in terms of sample throughput advantages [100]. Miniaturization of analytical processes into microchip platforms designed for micro total analytical systems (μ-TASs) is a new and rapidly developing field. For SPE, Yu et al. [123] developed a microfabricated analytical microchip device that uses a porous monolith sorbent with two different surface chemistries. The monolithic porous polymer was prepared by in situ photoinitiated polymerization within the channels of the microfluidic device and used for on-chip SPE. The sorbent was prepared to have both hydrophobic and ionizable surface chemistries. Use of the device for sorption and desorption of various analytes was demonstrated [123].

As analytical capabilities improve, multiple procedures are linked together in series to effect analyses. Procedures combined in this manner are called *hyphenated techniques*. Ferrer and Furlong [124] combined multiple techniques—accelerated solvent extraction (ASE) followed by online SPE coupled to ion trap HPLC/MS/MS—to determine benzalkonium chlorides in sediment samples. Online SPE, especially coupled to HPLC, is being used more routinely. This approach allowed online cleanup of the ASE extract prior to introduction to the analytical column.

2.5. SOLID-PHASE MICROEXTRACTION

Solid-phase microextraction (SPME) was introduced by Arthur and Pawliszyn [125]. The original concept of miniaturizing extractions (*microextraction*) using solid-phase sorbents has evolved (Figure 2.46) into a family of different approaches that strain the ability of the term *SPME* to adequately describe all techniques. According to Lord and Pawliszyn [51], one problem in the terminology applied today is that the extracting phases are not always solids. However, changing the term to *stationary-phase microextraction* or *supported-phase microextraction* in reference to the extraction phase being stationary during extraction or supported on a solid framework would not be all-inclusive either; although usually true (Figure 2.46*a*,*b*,*c*,*e*,*f*), it is not always true that the sorbent phase is stationary or supported (Figure 2.46*d*). For this discussion, all of the configurations depicted in Figure 2.46 will be considered as variations on the basic SPME theme. Most SPME applications published to date use sorption via exposure of the sample to a thin layer of sorbent coated on the outer surface of fibers (Figure 2.46*a*) or on the internal surface of a capillary tube (Figure 2.46*b*). One application of in-tube, suspended-particle SPME (which appears to this author to be a

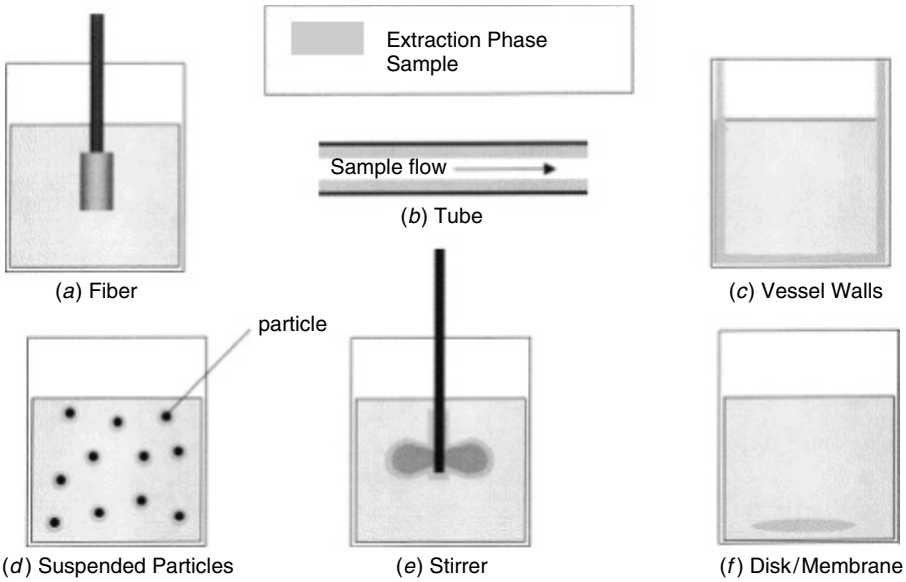


Figure 2.46. Configurations of solid-phase microextraction: (*a*) fiber, (*b*) tube, (*c*) vessel walls, (*d*) suspended particles, (*e*) stirrer, and (*f*) disk/membrane. (Reprinted with permission from Ref. 51. Copyright © 2000 Elsevier Science.)

miniaturized version of classical batch LSE and a hybrid of Figure 2.46*b* and *d*) has been published [126] and is discussed further in Section 2.5.4. The "stirrer" variation of SPME (Figure 2.46*e*) is rapidly evolving into a term and acronym in its own right [i.e., stir bar sorptive extraction (SBSE)] and is discussed later in this chapter.

Understanding analytical nomenclature is important, but it is more important to understand the underlying common extraction mechanism that leads to grouping all the approaches depicted in Figure 2.46. Exhaustive extraction of analyte from the sample matrix is not achieved by SPME, nor is it meant to occur (although SBSE techniques approach exhaustive extraction and therefore probably do deserve their own acronym). By SPME, samples are analyzed after equilibrium is reached or at a specified time prior to achieving equilibrium. Therefore, SPME operationally encompasses nonexhaustive, equilibrium and preequilibrium, batch and flow-through microextraction techniques. Thus defined, SPME is distinctly different from SPE because SPE techniques, including semimicro SPE (SM-SPE) and miniaturized SPE (M-SPE) [73], are exhaustive extraction procedures.

The distribution constant, K_{fs}, between the coated fiber SPME sorbent and the aqueous sample matrix is given by

$$K_D = \frac{[\mathrm{X}]_\mathrm{B}}{[\mathrm{X}]_\mathrm{A}} = K_\mathrm{fs} = \frac{C_f}{C_s} \tag{2.34}$$

where C_f is the concentration of analyte in the fiber sorbent and C_s is the concentration of analyte in the aqueous sample phase. As with the other extraction techniques discussed, if the value of K_fs is larger, the degree of concentration of the target analytes in the sorbent phase is greater, and the analytical procedure is more sensitive [127].

When equilibrium conditions are reached, the number of moles, n, of analyte extracted by the fiber coating is independent of increases in extraction time, such that

$$n = \frac{K_\mathrm{fs} V_f V_s C_0}{K_\mathrm{fs} V_f + V_s} \tag{2.35}$$

where V_f is the fiber coating volume, V_s the sample volume, and C_0 the initial concentration of a given analyte in the sample [51,128–130]. K_fs values are influenced by temperature, salt, pH, and organic solvents [130].

Examination of equation (2.35) leads to the conclusion that when the sample volume is very large (i.e., $K_\mathrm{fs} V_f \ll V_s$), the amount of extracted analyte is independent of the volume of the sample, such that

$$n = K_\mathrm{fs} V_f C_0 \tag{2.36}$$

If the amount of extracted analyte is independent of sample volume, the concentration extracted will correspond directly to the matrix concentration [51,128]. Therefore, SPME is directly applicable for field applications in air and water sampling.

However, it is not necessary to continue an extraction by SPME until equilibrium is reached. A quantitative result may be achieved by careful control of time and temperature. Ulrich [130] notes that important kinetic considerations of the relationship between analyte concentration and time by SPME include:

- The time of extraction is independent of the concentration of analyte in the sample.
- The relative number of molecules extracted at a distinct time is independent of analyte concentration.
- The absolute number of molecules extracted at a distinct time is linearly proportional to the concentration of analyte.

One of the major advantages of SPME is that it is a solventless sample preparation procedure, so solvent disposal is eliminated [68,131]. SPME is a relatively simple, straightforward procedure involving only sorption and desorption [132]. SPME is compatible with chromatographic analytical systems, and the process is easily automated [131,133]. SPME sampling devices are portable, thereby enabling their use in field monitoring.

SPME has the advantages of high concentrating ability and selectivity. Conventional SPE exhaustively extracts most of the analyte (>90%) from a sample, but only 1 to 2% of the sample is injected into the analytical instrument. SPME nonexhaustively extracts only a small portion of the analyte (2 to 20%), whereas all of the sample is injected [68,73,75]. Furthermore, SPME facilitates unique investigations, such as extraction from very small samples (i.e., single cells). SPME has the potential for analyses in living systems with minimal disturbance of chemical equilibria because it is a nonexhaustive extraction system [51].

Despite the advantages of an equilibrium, nonexhaustive extraction procedure, there are also disadvantages. Matrix effects can be a major disadvantage of a sample preparation method that is based on equilibration rather than exhaustive extraction [134]. Changes in the sample matrix may affect quantitative results, due to alteration of the value of the distribution constant relative to that obtained in a pure aqueous sample [68,134].

SPME can be used to extract semivolatile organics from environmental waters and biological matrices as long as the sample is relatively clean. Extraction of semivolatile organic compounds by SPME from dirty matrices is more difficult [134]. One strategy for analyzing semivolatiles from dirty matrices is to heat the sample to drive the compound into the sample headspace for SPME sampling; another approach is to rinse the fiber to remove nonvolatile compounds before analysis [134].

2.5.1. Sorbents

For structural integrity, SPME sorbents are most commonly immobilized by coating onto the outside of fused silica fibers or on the internal surface of a capillary tube. The phases are not bonded to the silica fiber core except when the polydimethylsiloxane coating is 7 μm thick. Other coatings are cross-linked to improve stability in organic solvents [135]. De Fatima Alpendurada [136] has reviewed SPME sorbents.

Apolar, Single-Component Absorbent Phase

Polydimethylsiloxane (PDMS) is a single-component, nonpolar liquid absorbent phase coated on fused silica commercially available in film thick-

nesses of 7, 30, and 100 μm [137]. The PDMS phases can be used in conjunction with analysis by GC or HPLC. The thickest coating, 100 μm, used for volatile compounds by headspace procedures is not discussed in this chapter. The intermediate coating level, 30 μm, is appropriate for use with nonpolar semivolatile organic compounds, while the smallest-diameter coating, 7 μm, is used when analyzing nonpolar, high-molecular-weight compounds. The use of PDMS fibers is restricted to a sample pH between 4 and 10 [136].

Polar, Single-Component Absorbent Phase

Polyacrylate (PA) is a single-component polar absorbent coating commercially available in a film thickness of 85 μm [137]. The sorbent is used with GC or HPLC analyses and is suitable for the extraction of polar semivolatile compounds.

Porous, Adsorbent, Blended Particle Phases

Multiple-component phases were developed to exploit adsorbent processes for SPME. Adsorbent blended phases commercially available for SPME contain either divinylbenzene (DVB) and/or Carboxen particles suspended in either PDMS, a nonpolar phase, or Carbowax (CW), a moderately polar phase [55]. The solid particle is suspended in a liquid phase to coat it onto the fiber.

PDMS-DVB is a multiple-component bipolar sorbent coating. PDMS-DVB is commercially available in a film thickness of 65 μm for SPME of volatile, amine, or nitroaromatic analytes for GC analyses or in a film thickness of 60 μm for SPME of amines and polar compounds for final determination by HPLC [137]. DVB is suspended in the PDMS phase [135].

CW-DVB is a multiple-component, polar sorbent manufactured in 65- or 70-μm film thicknesses for GC analyses. SPME using CW-DVB is appropriate for the extraction of alcohols and polar compounds [137]. DVB is suspended in the Carbowax phase [135].

Carboxen/PDMS is a multiple-component bipolar sorbent (75 or 85 μm thickness) used for SPME of gases and low-molecular-weight compounds with GC analyses [137]. Carboxen is suspended in the PDMS phase [135]. Carboxen is a trademark for porous synthetic carbons; Carboxen 1006 used in SPME has an even distribution of micro-, meso-, and macropores. Carboxens uniquely have pores that travel through the entire length of the particle, thus promoting rapid desorption [135]. Among the SPME fibers currently available, the 85-μm Carboxen/PDMS sorbent is the best choice for extracting analytes having molecular weights of less than 90, regardless of

functional groups present with the exception of isopropylamine [138]. The Carboxen particles extract analytes by adsorption.

DVB/Carboxen-PDMS is a multiple-component bipolar phase that contains a combination of DVB-PDMS (50 μm) layered over Carboxen-PDMS (30 μm) [55,137]. This arrangement expands the analyte molecular weight range, because larger analytes are retained in the meso- and macropores of the outer DVB layer, while the micropores in the inner layer of Carboxen retain smaller analytes [55]. The dual-layered phase is used for extraction of odor compounds and volatile and semivolatile flavor compounds with GC analysis. DVB sorbents have a high affinity for small amines; consequently, the combination coating of DVB over Carboxen is the best sorbent choice for extracting isopropylamine [138].

CW/templated resin (TPR), 50 μm, is used for analysis of surfactants by HPLC. The templated resin in CW/TPR is a hollow, spherical DVB formed by coating DVB over a silica template. When the silica is dissolved, the hollow, spherical DVB particle formed has no micro- or mesopores [135].

2.5.2. Sorbent Selection

Analyte size, concentration levels, and detection limits must all be taken into consideration when selecting SPME sorbents [55]. Physical characteristics, including molecular weight, boiling point, vapor pressure, polarity, and presence of functional groups, of the analytes of interest must be considered [135]. Analyte size is important because it is related to the diffusion coefficient of the analyte in the sample matrix and in the sorbent.

When selecting an SPME sorbent (Table 2.7), the polarity of the sorbent coating should match the polarity of the analyte of interest, and the coating should be resistant to high-temperature conditions and extremes in pH, salts, and other additives [130]. In addition to selecting sorbents having a high affinity for the analyte of interest, it is important to select sorbents with a lack of affinity for interfering compounds [134].

Recovery

Extraction recovery can be optimized by changing sample conditions such as pH, salt concentration, sample volume, temperature, and extraction time [130,132,133,136]. Currently, all commercially available SPME sorbents are neutral, such that the sample pH should be adjusted to ensure that the analyte of interest is also neutral [131].

The detection limits for SPME headspace sampling are equivalent to SPME liquid sampling for volatile compounds. However, semivolatile organic compounds diffuse slowly into the headspace so that SPME headspace sampling is not appropriate for semivolatile compounds [134].

Table 2.7. SPME Fiber Selection Guide

Analyte Class	Fiber Type	Linear Range
Acids (C2–C8)	Carboxen-PDMS	10 ppb–1 ppm
Acids (C2–C15)	CW-DVB	50 ppb–50 ppm
Alcohols (C1–C8)	Carboxen-PDMS	10 ppb–1 ppm
Alcohols (C1–C18)	CW-DVB	50 ppb–75 ppm
	Polyacrylate	100 ppb–100 ppm
Aldehydes (C2–C8)	Carboxen-PDMS	1 ppb–500 ppb
Aldehydes (C3–C14)	100 μm PDMS	50 ppb–50 ppm
Amines	PDMS-DVB	50 ppb–50 ppm
Amphetamines	100 μm PDMS	100 ppb–100 ppm
	PDMS-DVB	50 ppb–50 ppm
Aromatic amines	PDMS-DVB	5 ppb–1 ppm
Barbiturates	PDMS-DVB	500 ppb–100 ppm
Benzidines	CW-DVB	5 ppb–500 ppb
Benzodiazepines	PDMS-DVB	100 ppb–50 ppm
Esters (C3–C15)	100 μm PDMS	5 ppb–10 ppm
Esters (C6–C18)	30 μm PDMS	5 ppb–1 ppm
Esters (C12–C30)	7 μm PDMS	5 ppb–1 ppm
Ethers (C4–C12)	Carboxen-PDMS	1 ppb–500 ppb
Explosives (nitroaromatics)	PDMS-DVB	1 ppb–1 ppm
Hydrocarbons (C2–C10)	Carboxen-PDMS	10 ppb–10 ppm
Hydrocarbons (C5–C20)	100 μm PDMS	500 ppt–1 ppb
Hydrocarbons (C10–C30)	30 μm PDMS	100 ppt–500 ppb
Hydrocarbons (C20–C40+)	7 μm PDMS	5 ppb–500 ppb
Ketones (C3–C9)	Carboxen-PDMS	5 ppb–1 ppm
Ketones (C5–C12)	100 μm PDMS	5 ppb–10 ppm
Nitrosamines	PDMS-DVB	1 ppb–200 ppb
PAHs	100 μm PDMS	500 ppt–1 ppm
	30 μm PDMS	100 ppt–500 ppb
	7 μm PDMS	500 ppt–500 ppb

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Thicker phase coatings extract a greater mass of analyte, but the extraction time is longer than for a thinner coating [135]. Because the coated fiber sorbents are reused multiple times, ease and completeness of desorption of the fiber is an issue in order to reduce sample carryover [134].

2.5.3. Methodology

Although various ways to implement SPME are proposed and are being developed (Figure 2.46), there are two primary approaches to conducting SPME (Figure 2.47): with the sorbent coated on the outer surface of fibers

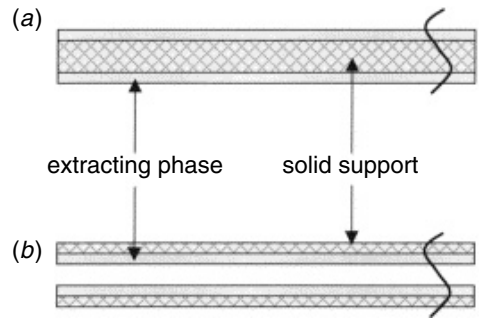

Figure 2.47. Two different implementations of the SPME technique: (*a*) polymer coated on outer surface of fiber; (*b*) polymer coated on internal surface of capillary tube. (Reprinted with permission from Ref. 51. Copyright © 2000 Elsevier Science.)

or with the sorbent coated on the internal surface of a capillary tube [51]. The fiber design can be interfaced with either GC or HPLC. However, the in-tube design has developed as an easier approach for interfacing SPME with HPLC.

In the fiber design, a fused silica core fiber is coated with a thin film (7 to 100 μm) of liquid polymer or a solid sorbent in combination with a liquid polymer (Figure 2.47*a*). Fiber lengths are generally 1 cm, although different-sized fibers can be prepared. In addition to standard fused silica fibers, silica fibers coated in a thin layer of plastic are also available. The plastic coating makes the fiber more flexible, and the sorbent phase coating bonds to the plastic layer better than the bare fused silica [55]. The in-tube design for SPME uses 0.25-mm-ID capillary tubes with about 0.1 μL of coating of the sorbent on the internal surface of the tube [51].

The theoretical calculations of the phase volume of the sorbent are facilitated by considering the fiber to be a right cylinder. The dimensions of the fused silica fiber are accurately known so that the volume of the fused silica core can be subtracted from the total volume of the fiber to yield the phase volume of the sorbent.

SPME (Figure 2.48) can be conducted as a direct extraction in which the coated fiber is immersed in the aqueous sample; in a headspace configuration for sampling air or the volatiles from the headspace above an aqueous sample in a vial (headspace SPME analyses are discussed elsewhere); or by a membrane protection approach, which protects the fiber coating, for analyses of analytes in very polluted samples [136]. The SPME process consists of two steps (Figure 2.49): (a) the sorbent, either an externally coated fiber or an internally coated tube, is exposed to the sample for a specified period of time; (b) the sorbent is transferred to a device that interfaces with an ana-

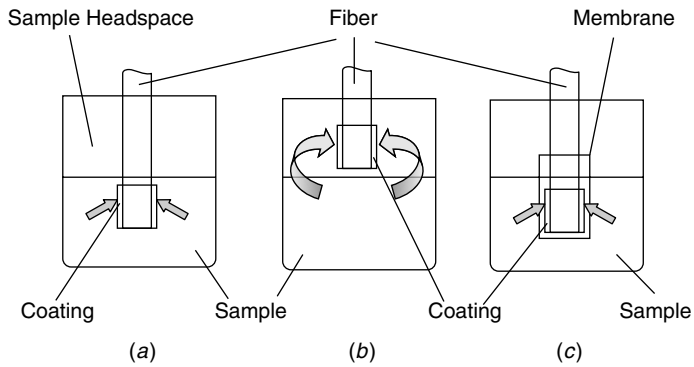

Figure 2.48. Modes of SPME operation: (*a*) direct extraction; (*b*) headspace SPME; (*c*) membrane-protected SPME. (Reprinted with permission from Ref. 51. Copyright © 2000 Elsevier Science.)

lytical instrument for thermal desorption using GC or for solvent desorption when using HPLC.

In the fiber mode, the sorbent coated fiber is housed in a microsyringe-like protective holder. With the fiber retracted inside the syringe needle, the needle is used to pierce the septum of the sample vial. The plunger is depressed to expose the sorbent-coated fiber to the sample. After equilibrium is reached or at a specified time prior to reaching equilibrium, the fiber is retracted into the protection of the microsyringe needle and the needle is withdrawn from the sample. The sorbent is then interfaced with an analytical instrument where the analyte is desorbed thermally for GC or by solvents for HPLC or capillary electrophoresis. For the in-tube mode, a sample aliquot is repeatedly aspirated and dispensed into an internally coated capillary. An organic solvent desorbs the analyte and sweeps it into the injector [68,130,133]. An SPME autosampler has been introduced by Varian, Inc., that automates the entire process for GC analyses.

Procedures

Determination of the optimum time for which the SPME sorbent will be in direct contact with the sample is made by constructing an extraction-time profile of each analyte(s) of interest. The sorption and desorption times are greater for semivolatile compounds than for volatile compounds. To prepare the extraction-time profile, samples composed of a pure matrix spiked with the analyte(s) of interest are extracted for progressively longer times. Constant temperature and sample convection must be controlled. Stirring the

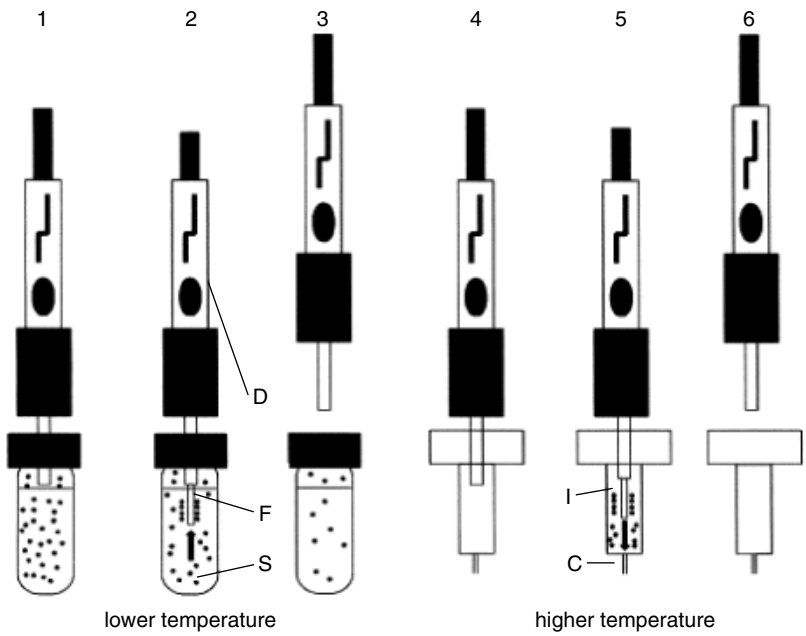

Figure 2.49. Principle of SPME: 1, introduction of syringe needle of the SPME device (D) into the sample vial and close to the sample (S), 2, moving the fiber (F) into the position outside the syringe and into the sample (extraction), 3, moving the fiber back into the syringe needle and subsequent transfer of the device to the GC injector port (1) and capillary head (C), 4, penetration of the septum with syringe needle, 5, moving the fiber into the position outside the syringe (desorption), 6, moving the fiber back into the syringe needle and withdrawing the needle. (Reprinted with permission from Ref. 130. Copyright © 2000 Elsevier Science.)

sample during sorption is necessary to reduce the diffusion layer at the sample matrix/sorbent interface and reach equilibrium faster [132]. A graph is prepared of time plotted on the x-axis and the detector response, or amount of analyte extracted, plotted on the y-axis (Figure 2.50). The extraction-time profile enables the analyst to select a reasonable extraction time while taking into consideration the detection limit of the analyte [134,136].

The SPME extraction-time profile prepared in this manner is typically composed of three distinct stages: the initial period of greatest amount of analyte extracted per time in which the graph rises sharply and has the greatest slope (however, small errors in the time measurement can lead to large errors in estimating the amount of analyte extracted); second, the profile enters an intermediate stage in which the slope of the plot is positive but smaller in magnitude relative to the initial stages of the plot; and finally, under ideal conditions equilibrium is reached such that the plot is a plateau

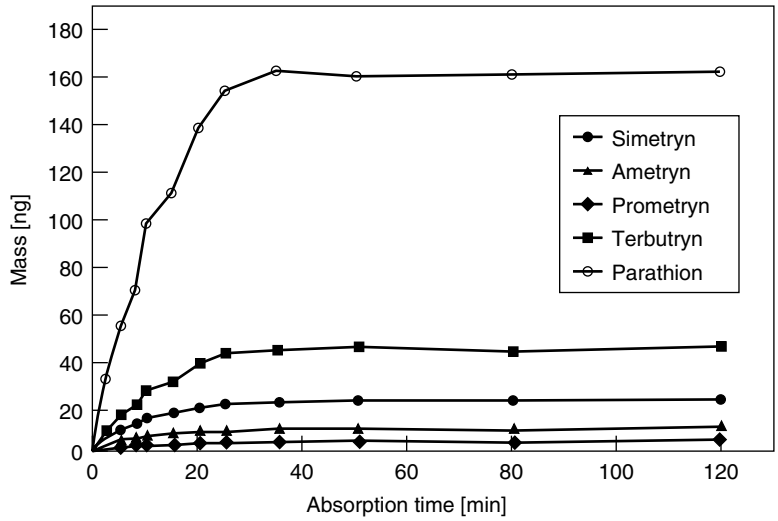

Figure 2.50. SPME absorption–time profile for four *s*-triazines and parathion using magnetic stirring. (Reprinted with permission from Ref. 139. Copyright © 1997 Elsevier Science.)

where the slope is equal to zero and there is no further increase in analyte extracted regardless of increases in contact time (Figure 2.51). Under equilibrium conditions, small errors in the time measurement produce small errors in estimating the amount of analyte extracted. Essentially, it is appropriate to conduct SPME under either the intermediate or equilibrium conditions in order to minimize the standard deviation of the analytical

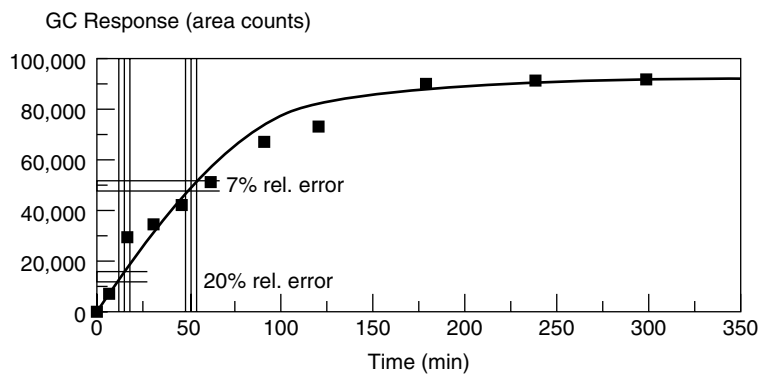

Figure 2.51. Selection of the extraction time based on extraction time profile of *p,p′*-DDT. (Reprinted with permission from Ref. 128. Copyright © 1997 John Wiley & Sons, Inc.)

measurements. In the first stage of the extraction-time profile, contact times are short, which shortens the overall analytical time, but the degree of error in the measurement is large. To reach true equilibrium, contact times may be long, but the degree of error in the measurement is small. Choosing a contact time within the intermediate region of the extraction-time profile strikes a balance between the contact time required for measurement and the anticipated degree of error. When intermediate contact times are used that do not reach equilibrium, the longest reasonable extraction time should be selected for quantitation in order to maximize the limit of detection and minimize the relative error of determination.

Quantitation of extraction under nonequilibrium conditions is based on the proportional relationship between the sorbed analyte and initial concentration [68]. Calibration of the SPME technique can be based on internal calibration using isotopically labeled standards or standard addition if recovery is matrix dependent. External calibration can be used if the standard matrix and the sample matrix are closely similar or identical [128,132,134].

2.5.4. Recent Advances in Techniques

Mullett et al. [126] recently published an automated application of a variation on the in-tube SPME approach for the analysis of propranolol and other β-blocker class drugs. The analytes were extracted from serum samples using a molecularly imprinted polymeric (MIP) adsorbent phase. MIP phases were discussed earlier as an emerging type of sorbent being used for SPE analyses. MIP phases are polymeric sorbents prepared in the presence of a target analyte that performs as a molecular template. When the template is removed, cavities that are selective recognition sites for the target analyte remain in the sorbent. In this approach, the MIP sorbent based on propranolol was passed through a 50-μm sieve and the fines removed by sedimentation in methanol. A slurry of the sorbent in methanol was placed into an 80-mm length of polyether ether ketone (PEEK) tubing of 0.76 mm ID such that the particles were not packed but suspended in the tube to allow easy flow through of the sample (Figure 2.46*d*). The MIP SPME capillary column was placed between the injection loop and the injection needle of an HPLC autosampler. The extraction process utilized the autosampler to aspirate and dispense the sample repeatedly across the extraction sorbent in the capillary column. In this technique, the sorbent is a "solid-phase" and the procedure is a "microscale extraction." The technique is not SPE because the particles are loosely packed and the sample passes back and forth through the column. However, the surface contact area between the sorbent and the sample is much greater than in the coated fiber or coated inner surface tubing SPME procedures described earlier. To this author, the

extraction phase of the SPME procedural variation reported in this paper is more closely related to classical batch LSE, with a miniaturization of scale, than it is to classical SPME. Regardless of terminology, the approach taken in this paper is analytically elegant, and along with other examples discussed in this chapter, well illustrates the fact that the lines between strict definitions of LLE and LSE procedures and among LSE procedures are becoming blurred as analysts derive new procedures. The techniques available represent a continuum array of extraction approaches for today's analyst.

Koster et al. [140] conducted on-fiber derivatization for SPME to increase the detectability and extractability of drugs in biological samples. Amphetamine was used as a model compound. The extraction was performed by direct immersion of a 100-μm polydimethylsiloxane-coated fiber into buffered human urine. On-fiber derivatization was performed with pentafluorobenzoyl chloride either after or simultaneously with extraction.

2.6. STIR BAR SORPTIVE EXTRACTION

Stir bar sorptive extraction (SBSE), an approach theoretically similar to SPME, was recently introduced [141] for the trace enrichment of organic compounds from aqueous food, biological, and environmental samples. A stir bar is coated with a sorbent and immersed in the sample to extract the analyte from solution. To date, reported SBSE procedures were not usually operated as exhaustive extraction procedures; however, SBSE has a greater capacity for quantitative extraction than SPME. The sample is typically stirred with the coated stir bar for a specified time, usually for less than 60 minutes, depending on the sample volume and the stirring speed, to approach equilibrium. SBSE improves on the low concentration capability of in-sample solid-phase microextraction (IS-SPME).

The stir bar technique has been applied to headspace sorptive extraction (HSSE) [142–144]. However, headspace techniques are discussed elsewhere, as they are more applicable to volatile organic compounds than to the semivolatile organic compounds that comprise the focus of this chapter.

2.6.1. Sorbent and Analyte Recovery

To date, the only sorbent used reportedly for coating the stir bar is polydimethylsiloxane (PDMS), although the use of stir bars coated with polar sorbents is predicted for the future [141]. Using this sorbent, the primary mechanism of interaction with organic solutes is via absorption or partitioning into the PDMS coating such that the distribution constant [equation (2.37)] between PDMS and water ($K_{PDMS/W}$) is proposed to be proportional

to the octanol–water partition coefficient (K_{OW}) [141]:

$$K_D = \frac{[X]_B}{[X]_A} = K_{PDMS/W} \approx K_{OW} \tag{2.37}$$

According to the theoretical development for this technique given in Baltussen et al. [141],

$$K_{OW} \approx K_{PDMS/W} = \frac{[X]_{PDMS}}{[X]_W} = \frac{m_{PDMS}}{m_W} \times \frac{V_W}{V_{PDMS}} \tag{2.38}$$

where $[X]_{PDMS}$ and $[X]_W$, and m_{PDMS} and m_W, are the analyte concentration and the analyte mass in the PDMS and water phase, respectively, while V_{PDMS} and V_W represent the volume of the PDMS sorbent and water phase, respectively. Therefore, the parameters determining the mass of an analyte recovered by SBSE using the PDMS sorbent are the partition coefficient of the analyte (K_{OW}) and the phase ratio (V_W/V_{PDMS}) of the volume of the water phase to the volume of the PDMS coating on the stir bar.

Baltussen et al. [141] theoretically compared recovery by SBSE using a stir bar assumed to be coated with a 100-μL volume of PDMS to recovery by IS-SPME having an assumed coating volume of 0.5 μL of PDMS. For the extraction of a 10-mL sample of water, it was demonstrated (Figure 2.52) that with SBSE, a more favorable extraction of analytes having lower K_{OW} values should be possible than with SPME. The small volume of the PDMS sorbent used in SPME results in a large phase ratio that implies [equation (2.38)] that a high octanol–water partition coefficient is required for efficient extraction. For SPME using PDMS, the analyte K_{OW} value is estimated (Figure 2.52) to be 20,000 ($\log K_{OW} = 4.3$) or greater for high recovery efficiency from a 10-mL sample volume [141,145], whereas, using SBSE with PDMS, analytes with a K_{OW} value of 500 ($\log K_{OW} = 2.7$) or greater can be extracted more quantitatively [141] due to the higher volume of PDMS coating for SBSE devices relative to SPME fibers. However, since larger volumes of PDMS are used in SBSE than in SPME, more time is required to reach equilibrium because more analyte mass will be transferred to the PDMS sorbent phase [145].

In comparing the same compounds while using PDMS sorbent, recovery from aqueous solution by SBSE was demonstrated [141] to be greater than recovery by SPME. Tredoux et al. [146] noted enrichment factors for benzoic acid in beverages to be approximately 100 times higher for SBSE relative to SPME, and Hoffmann et al. [147] reported sensitivities 100 to 1000 times higher by SBSE than by SPME for the extraction of analytes in orange juice and wine.

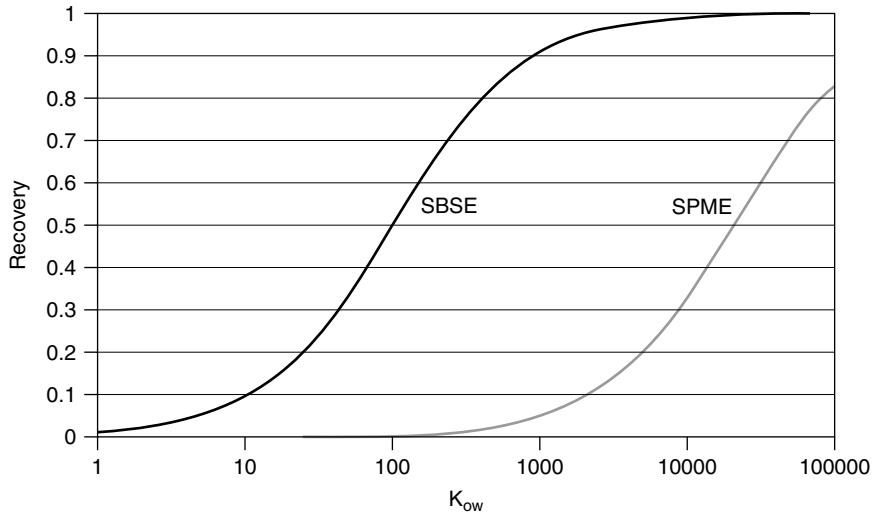

Figure 2.52. Theoretical recovery of analytes in SBSE and SPME from a 10-mL water sample as a function of their octanol–water partitioning constant. Volume of PDMS on SPME fiber: 0.5 μL; volume of PDMS on SBSE stir bar: 100 μL. (Reprinted with permission from Ref. 141. Copyright © 1999 John Wiley & Sons, Inc.)

2.6.2. Methodology

The stir bar consists of a stainless steel rod encased in a glass sheath (Figure 2.53). The glass is coated with PDMS sorbent. The length of the stir bar is typically 10 to 40 mm. The PDMS coating varies from 0.3 to 1 mm, resulting in PDMS phase volumes of 55 to 220 μL [145]. With a larger stir bar, more PDMS coating is deposited, and consequently, a larger sample volume can be extracted.

A thermodesorption unit that will accept the PDMS-coated stir bar is used to transfer the analytes into a gas chromatograph (Figure 2.54). The analyte is desorbed from the stir bar and cryofocused on a precolumn. Subsequent flash heating transfers analytes into the gas chromatograph. After desorption, the stir bar can be reused.

Procedures

Extraction of aqueous samples occurs during stirring at a specified speed for a predefined time. After a given stirring time, the bar is removed from the sample and is usually thermally desorbed into a gas chromatograph.

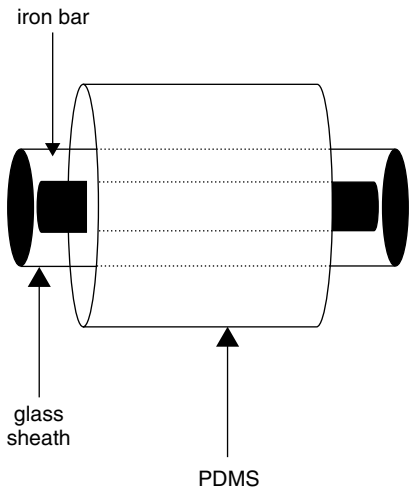

Figure 2.53. Schematic representation of a stir bar applied for SBSE. (Reprinted with permission from Ref. 145. Copyright © 2001 American Chemical Society.)

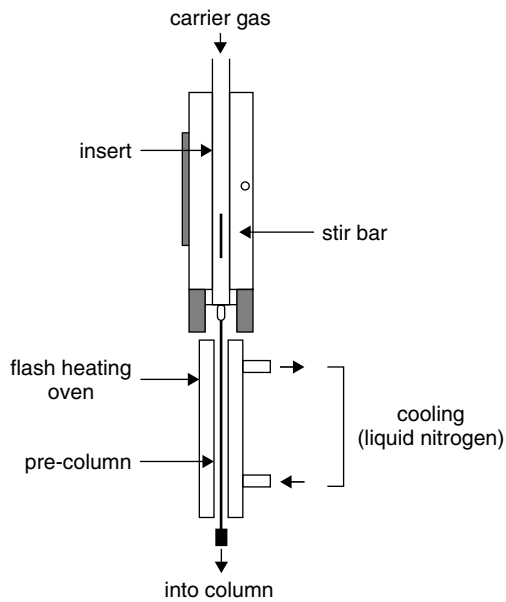

Figure 2.54. Schematic representation of the desorption unit. (Reprinted with permission from Ref. 145. Copyright © 2001 American Chemical Society.)

However, Popp et al. [148] desorbed extracted polycyclic aromatic hydrocarbons by ultrasonic treatment of the stir bar in acetonitrile or acetonitrile–water mixtures in order to perform liquid chromatographic analyses of the extract.

Although the development of this technique is still in its infancy, SBSE should have many useful analytical applications. Extraction remains a balancing act between sorbent mass and sample volume, and it appears that the primary advantage of SBSE using the PDMS sorbent (i.e., greater concentration capability than SPME) will also be its greatest disadvantage. The nonselective sorptive capability of the PDMS sorbent co-concentrates undesirable matrix components from solution. SBSE produces analyte accumulation in the sorbent but not sample cleanup. Sandra et al. [149] reported that for SBSE of fungicides in wine, standard addition methods were necessary for quantification due to matrix effects of the wine on recovery, and Ochiai et al. [150] added surrogate internal standards to compensate for sample matrix effects and coextracted analytes. Benijts et al. [151] also reported matrix suppression when SBSE on PDMS was applied to the enrichment of polychlorinated biphenyls (PCBs) from human sperm. The lipophilic medium lowered recoveries from the sperm matrix proportionally with PCB polarity.

Nevertheless, SBSE is attractive because it is a solventless enrichment technique. That coupled with the rapidity and ease of use of this procedure will make it a desirable approach for analysts. The introduction of more selective sorbents will overcome problems with matrix effects.

2.6.3. Recent Advances in Techniques

SBSE appears to be particularly useful for the extraction of a variety of components from beverages and sauces. Applications have included coffee [144], soft drinks [150], orange juice [147], lemon-flavored beverages [146], wine [147,149,150], balsamic vinegar [150], and soy sauce [150].

SBSE was recently applied [152] to the analysis of off-flavor compounds, including 2-methylisoborneol (2-MIB) and geosmin, in drinking water. These organic compounds cause taste and odor problems at very low concentrations and are notoriously difficult to extract. Detection limits by SBSE ranged from 0.022 to 0.16 ng/L. The recoveries ranged from 89 to 109% with relative standard deviations of 0.80 to 3.7%.

Vercauteren et al. [145] used SBSE to determine traces of organotin compounds in environmental samples at part per quadrillion (ppq) levels. The limits of detection reported using SBSE are the lowest ever determined for these compounds.

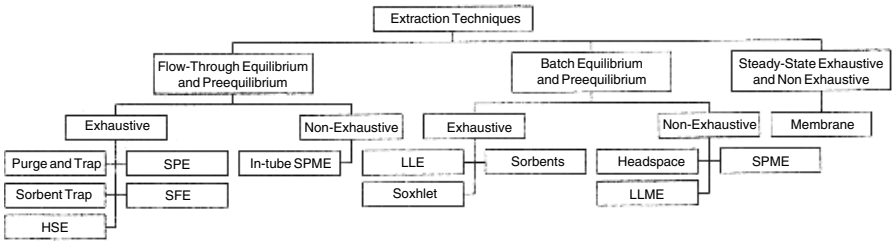

Figure 2.55. Classification of sample preparation techniques. (Reprinted with permission from Ref. 155. Copyright © 2001 NRC Research Press.)

2.7. METHOD COMPARISON

LLE, SPE, SPME, and SBSE applications for the extraction of semivolatile organics from liquids were discussed. Others [134,153,154] have compared sample preparation techniques. When examined collectively for perspective, the sample processing techniques can be perceived as variations on a single theme as practiced by today's analysts (Figure 2.55).

Two fundamentals drive extraction procedures: (1) determining the value of K_D for a given analyte–sample matrix–sorbent combination, which will indicate if the process is an equilibrium procedure (in nonequilibrium procedures, K_D approaches infinity during sorption), and (2) determining if the majority of the analyte (>90%) is recovered from the sample (Table 2.8), which will indicate if the process used is exhaustive. K_D is the continuum that relates the procedures discussed here and those to be developed in the future. As commonly implemented, K_D values for the studied procedures decrease in the order $K_{D(\mathrm{SPE})} > K_{D(\mathrm{LLE})} \simeq K_{D(\mathrm{SBSE})} > K_{D(\mathrm{SPME})}$. As commonly practiced, SPE and SPME exist at opposite ends of the continuum in method fundamentals. LLE is an equilibrium procedure, but through application of repeated extractions, nearly quantitative, or exhaustive, recovery of analytes can be achieved. SBSE is a recently emerging procedure that appears to lie on the extraction continuum between LLE and SPME. The capacity of SBSE for exhaustive extraction is greater than SPME but less

Table 2.8. Extraction Method Fundamentals

SPE	Nonequilibrium	Exhaustive
LLE	Equilibrium	Exhaustive
SBSE	Equilibrium	Nonexhaustive
SPME	Equilibrium	Nonexhaustive

than LLE. The capacity for quantitative, or exhaustive, transfer is related to the K_D value and the total mass of sorbent utilized. More sorbent mass is typically present in SBSE than in SPME; therefore, more analyte is transferred to the sorbent in SBSE.

Compared to nonequilibrium methods, equilibrium methods tend to be simpler, less expensive, more selective, therefore require less cleanup, require determination of preequilibrium/equilibrium status, are time, temperature, and matrix dependent, and require internal standards for calibration [43,75,128,156].

Extraction approaches differ, but the choice of methodology depends on the analyst's objectives and resources and the client's expectations. In practice, an analyst may prefer equilibrium or nonequilibrium procedures. However, no stigma should be placed on whether an extraction method is exhaustive or nonexhaustive or equilibrium or nonequilibrium.

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CHAPTER

3

EXTRACTION OF SEMIVOLATILE ORGANIC COMPOUNDS FROM SOLID MATRICES

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3.1. INTRODUCTION

This chapter covers techniques for the extraction of semivolatile organics from solid matrices. The focus is on commonly used and commercially available techniques, which include Soxhlet extraction, automated Soxhlet extraction, ultrasonic extraction, supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE). The underlying principles, instrumentation, operational procedures, and selected applications of these techniques are described. In a given application, probably all the methods mentioned above will work, so it often boils down to identifying the most suitable one. Consequently, an effort is made to compare these methodologies.

The U.S. Environmental Protection Agency (EPA) has approved several methods for the extraction of pollutants from environmental samples. These standard methods are listed under EPA publication SW-846, *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods* [1]. Many of them were approved only in the last decade. Automated Soxhlet was promulgated in 1994, SFE and ASE in 1996, and MAE in 2000. The Association of Official Analytical Chemists (AOAC) has published its own standard extraction methods for the food, animal feed, drug, and cosmetics industries [2]. Some extraction methods have also been approved by the American Society for Testing and Materials (ASTM) [3]. Table 3.1 summarizes the standard methods from various sources.

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Table 3.1. Methods Accepted as Standards for the Extraction of Semivolatile Organics from Solid Matrices

Technique	Analytes	Standard Method
Soxhlet extraction	Semivolatile and nonvolatile organics	EPA 3540C
	Fat in cacao products	AOAC 963.15
Automated Soxhlet extraction	Semivolatile and nonvolatile organics	EPA 3541
Pressurized fluid extraction (PFE)	Semivolatile and nonvolatile organics	EPA 3545A
Microwave-assisted extraction (MAE)	Semivolatile and nonvolatile organics	EPA 3546
	Total petroleum hydrocarbons, organic compounds	ASTM D-5765 ASTM D-6010
	Fat in meat and poultry products	AOAC 991.36
Ultrasonic extraction	Semivolatile and nonvolatile organics	EPA 3550C
Supercritical fluid extraction (SFE)	Semivolatile petroleum hydrocarbons, PAHs, PCBs, and organochlorine pesticides	EPA 3560 EPA 3561 EPA 3562

3.1.1. Extraction Mechanism

Extraction of organics from solids is a process in which solutes desorb from the sample matrix and then dissolve into the solvent. Extraction efficiency is influenced by three interrelated factors: solubility, mass transfer, and matrix effects. Much of the discussion in Chapter 2 on solvents and solubility is also relevant to solid matrices. The solubility of an analyte depends largely on the type of the solvent, and for a selected solvent, its solubility is affected by temperature and pressure. *Mass transfer* refers to analyte transport from the interior of the matrix to the solvent. It involves solvent penetration into the matrix and removal of solutes from the adsorbed sites. Mass transfer is dependent on the diffusion coefficient as well as on the particle size and structure of the matrix. High temperature and pressure, low solvent viscosity, small particle size, and agitation facilitate mass transfer [4]. It is a more important issue than solubility when the analyte concentration in the extraction solvent is below its equilibrium solubility (i.e., when the analyte is readily soluble in the solvent). Matrix effects are the least understood of the three factors. A highly soluble compound can be ''unextractable'' because it is locked in the matrix pores, or is strongly bound to its surface. For example, analytes in aged soil bind more strongly than in a clean soil when spiked with the same analyte. Desorption is more difficult and may take longer. Some extraction techniques, such as SFE, are found to be matrix dependent

[5]. Different extraction parameters are employed for different groups of analytes in different matrices.

Solvent selection depends largely on the nature of the analytes and the matrix. Although the discussions in Chapter 2 can be used as a guideline to account for the solvent–analyte interactions, the matrix effects are often unpredictable. There is no single solvent that works universally for all analytes and all matrices. Sometimes, a mixture of water-miscible solvents (such as acetone) with nonmiscible ones (such as hexane or methylene chloride) are used. The water-miscible solvents can penetrate the layer of moisture on the surface of the solid particles, facilitating the extraction of hydrophilic organics. The hydrophobic solvents then extract organic compounds of like polarity. For instance, hexane is efficient in the extraction of nonpolar analytes, and methylene chloride extracts the polar ones.

As temperature and pressure play important roles in extraction kinetics, extraction techniques can be classified based on these parameters. Classical methods include Soxhlet extraction, automated Soxhlet extraction, and ultrasonic extraction. They are operated under atmospheric pressure, with heating or ultrasonic irradiation. These methods consume relatively large volumes of organic solvents, and the extraction may take a long time. The other group consists of SFE, ASE, and MAE, which are performed under elevated pressure and/or temperature. The extraction is faster, more efficient, and sample throughput is high. With relatively less consumption of organic solvents, these methods are more environmentally friendly. Moreover, the costs of solvent purchase and waste disposal are reduced. Despite the high initial equipment cost, these methods may be more economical in the long run, especially for the routine analysis of a large number of samples.

3.1.2. Preextraction Procedures

Most extraction methods perform best on dry samples with small particle size. If possible, samples may be air-dried and ground to a fine powder before extraction. However, this procedure is not recommended if the sample contains volatile analytes and/or worker exposure is a concern. Instead, the sample can be dried by mixing with anhydrous sodium sulfate or palletized diatomaceous earth. In certain applications such as in MAE, water can be used as a part of the solvent mixture [6,7]. Instead of drying, water is added into the sample to maintain a certain moisture level.

3.1.3. Postextraction Procedures

Some extraction techniques generate large volumes of solvent extract. The extract needs to be concentrated to meet the detection limit of the analytical method. Moreover, in most cases, extracts of soil, sludge, and waste samples

require some degree of cleanup prior to analysis. The purpose of cleanup is to remove interfering compounds and high-boiling materials that may cause error in quantification, equipment contamination, and deterioration of chromatographic resolution. The details of postextraction techniques have been discussed in Chapter 1.

3.2. SOXHLET AND AUTOMATED SOXHLET

Soxhlet extraction and automated Soxhlet extraction are described in this section. Soxhlet extraction was named after Baron Von Soxhlet, who introduced this method in the mid-nineteenth century. It had been the most widely used method until modern extraction techniques were developed in the 1980s. Today, Soxhlet is still a benchmark method for the extraction of semivolatile organics from solid samples. Automated Soxhlet extraction (Soxtec being its commercial name) offers a faster alternative to Soxhlet, with comparable extraction efficiency and lower solvent consumption.

3.2.1. Soxhlet Extraction

A schematic diagram of a typical Soxhlet apparatus is shown in Figure 3.1. The system has three components. The top part is a solvent vapor reflux condenser. In the middle are a thimble holder with a siphon device and a side tube. The thimble holder connects to a round-bottomed flask at the bottom. The sample is loaded into a porous cellulous sample thimble and placed into the thimble holder. Typically, 300 mL of solvent(s) (for a 10-g sample) is added to the flask. A couple of boiling chips are also added, and the flask is gently heated on a heating mantle. Solvent vapor passes through the side tube and goes to the reflux condenser, where it condenses and drips back to the thimble chamber. When the analyte-laden solvent reaches the top of the thimble holder, it is drained back into the bottom flask through the siphon device. This cycle repeats many times for a predetermined time period. Since the extracted analytes have higher boiling points than the extraction solvent, they accumulate in the flask while the solvent recirculates. Consequently, the sample is always extracted with fresh solvents in each cycle.

Because the sample is extracted with cooled, condensed solvents, Soxhlet is slow and can take between 6 to 48 hours. The extract volume is relatively large, so a solvent evaporation step is usually needed to concentrate the analytes prior to extract cleanup and analysis. The sample size is usually 10 g or more. Multiple samples can be extracted on separate Soxhlet units, and the extraction can be run unattended. Soxhlet is a rugged, well-established

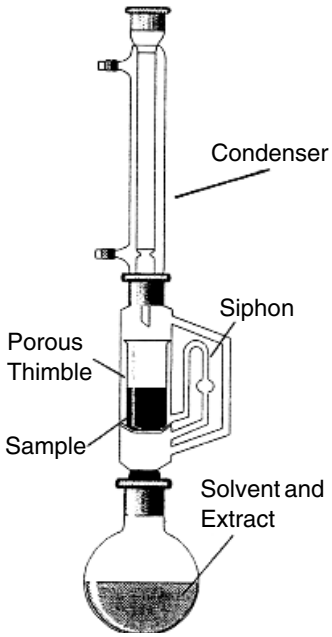

Figure 3.1. Schematic diagram of a Soxhlet apparatus. (Reproduced from Ref. 93, with permission from Nelson Thornes Ltd.)

technique that is often used as the benchmark for comparing other methods. Few parameters can affect the extraction. The main drawbacks are the long extraction time and relatively large solvent consumption. The routine use of Soxhelt is decreasing as faster extraction techniques are finding their way into the analytical arena.

3.2.2. Automated Soxhlet Extraction

In 1994, automated Soxhlet extraction (Soxtec, commercially) was approved by EPA as a standard method. A shematic diagram of Soxtec is shown in Figure 3.2. The extraction is carried out in three stages: boiling, rinsing, and solvent recovery. In the first stage, a thimble containing the sample is immersed in the boiling solvent for about 60 minutes. Extraction here is faster than Soxhlet, because the contact between the solvent and the sample is more vigorous, and the mass transfer in a high-temperature boiling solvent is more rapid. In the second stage, the sample thimble is lifted above the boiling solvent. The condensed solvent drips into the sample, extracts the organics, and falls back into the solvent reservoir. This rinse–extract process is similar to Soxhlet and is usually set for 60 minutes. The third stage is a concentration step for 10 to 20 minutes. The solvent is evaporated to 1 to

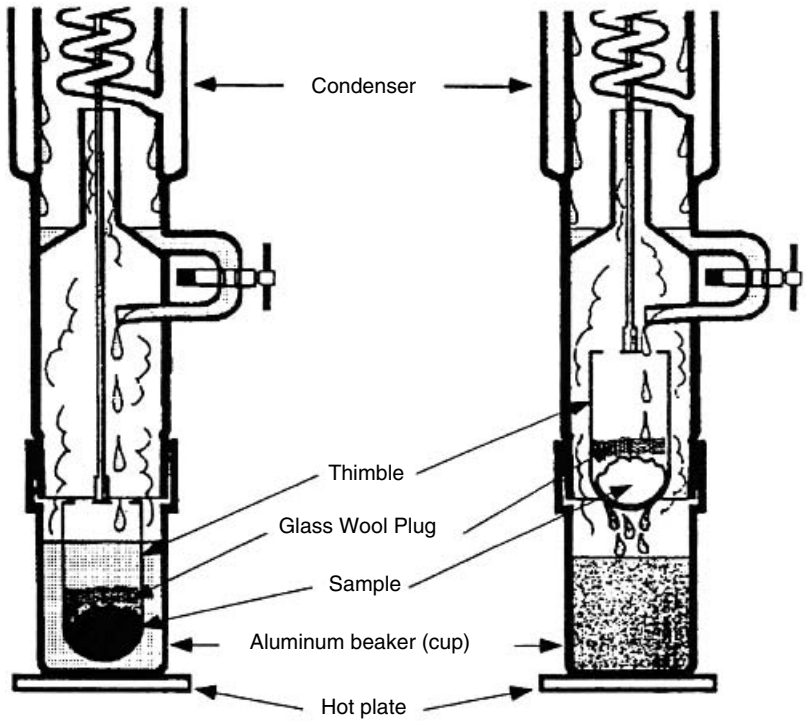

Figure 3.2. Schematic diagram of an automatic Soxhlet extraction device (Soxtec).

2 mL, as would occur in a Kuderna–Danish concentrator. Since the concentration step is integrated in Soxtec, the extract is ready for cleanup and analysis.

Lopez-Avila et al. [8] published a study in 1993 that evaluated the Soxtec extraction of 29 target compounds (seven nitroaromatic compounds, three haloethers, seven chlorinated hydrocarbons, and 12 organochlorine pesticides) from spiked sandy clay loam and clay loam. Among the five factors investigated (matrix type, spike level, anhydrous sodium sulfate addition, total extraction time, and immersion/extraction time ratio), matrix type, spike level, and total extraction time had the most pronounced effects on method performance at the 5% significance level for 16 of the 29 target compounds. The two solvent mixtures, hexane–acetone (1 : 1) and methylene chloride–acetone (1 : 1), performed equally well. Four compounds were not recovered at all, and apparently were lost from the spike matrix. Limited experimental work was performed with 64 base–neutral–acidic compounds spiked onto clay loam, and with three standard reference materials certified

for polycyclic aromatic hydrocarbons (PAHs). For the 64 compounds spiked onto clay loam at 6 mg/kg, 20 had recoveries more than 75%, 22 between 50 and 74%, 12 between 25 and 49%, and 10 less than 25%.

3.2.3. Comparison between Soxtec and Soxhlet

Soxhlet can be applied universally to almost any sample. It is not uncommon to use Soxhlet as the benchmark method for validating other extraction techniques. Soxtec reduces the extraction time to 2 to 3 hours as compared to 6 to 48 hours in Soxhlet. It also decreases solvent use from 250 to 500 mL per extraction to 40 to 50 mL per extraction. Two to six samples can be extracted simultaneously with a single Soxtec apparatus.

Recent studies comparing Soxtec with Soxhlet show comparable or even better results for Soxtec. Brown et al. [9] compared the efficiency of the standard Soxhlet method against three different protocols using the Soxtec extractor (Tecator, Inc. Silver Spring, MD). Organic mutagens were extracted from municipal sewage sludge using MeOH and CH_2Cl_2 as solvents. Both the Soxtec (with 5 minutes of boiling time and 55 minutes of rinsing time), and Soxhlet procedures yielded reproducible mutagenic responses within the variability of the bioassay. The data indicate that the Soxtec extraction, which was faster and required less solvent, provided adequate extraction of organic mutagens from sewage sludge.

Foster and Gonzales [10] reported a collaborative study by 11 laboratories of Soxtec and Soxhlet methods for the determination of total fat in meat and meat products. Each lab analyzed six samples: canned ham, ground beef, frankfurters, fresh pork sausage, hard salami, and beef patties with added soy. In general, results for the Soxtec system showed improved performance. The method was first adopted by AOAC International for the extraction of fat from meat. Membrado et al. [11] tested Soxtec against Soxhlet extraction for the extraction of coal and coal-derived products. Optimization of Soxtec operating conditions reduced the total extraction time to 10% of what was needed by Soxhlet extraction. The recovery and precision by the two methods were comparable.

3.3. ULTRASONIC EXTRACTION

Ultrasonic extraction, also known as *sonication*, uses ultrasonic vibration to ensure intimate contact between the sample and the solvent. Sonication is relatively fast, but the extraction efficiency is not as high as some of the other techniques. Also, it has been reported that ultrasonic irradiation may lead to the decomposition of some organophosphorus compounds [12].

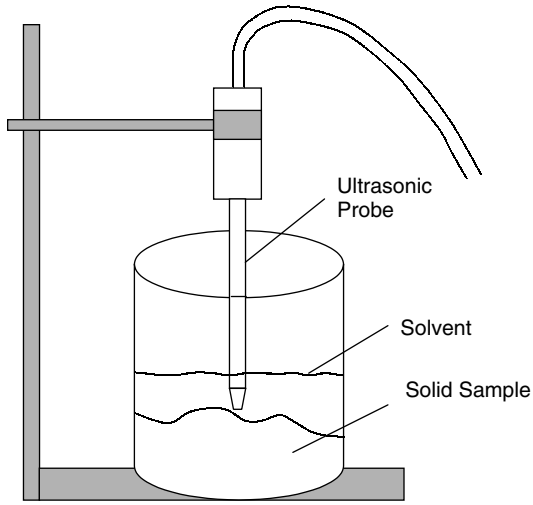

Figure 3.3. Schematic diagram of an ultrasonic extraction device.

Thus, the selected solvent system and the operating conditions must demonstrate adequate performance for the target analytes in reference samples before it is implemented for real samples. This is particularly important for low-concentration [parts per billion (ppb) level] samples.

Figure 3.3 shows a schematic diagram of a sonication device. It is a horn-type ultrasonic disruptor equipped with a titanium tip. There are two types of disruptors. A $\frac{3}{4}$-in. horn is typically used for low-concentration samples and a $\frac{1}{8}$-in. tapered microtip attached to a $\frac{1}{2}$-in. horn for medium/high-concentration samples. The sample is usually dried with anhydrous sodium sulfate so that it is free flowing. For trace analysis, the sample size is typically 30 g. Then a certain volume (typically, 100 mL) of selected solvents are mixed with the sample. The most common solvent system is acetone–hexane (1:1 v/v) or acetone–methylene chloride (1:1 v/v). For nonpolar analytes such as polychlorinated biphenyls (PCBs), hexane can also be used. The extraction is performed in the pulsed mode, with ultrasonic energy being on and off rather than continuous. The disruptor horn tip is positioned just below the surface of the solvent, yet above the sample. Very active mixing between the sample and the solvent should be observed. Extraction can be carried out in duration as short as 3 minutes. Since it is a fast procedure, it is important that one strictly follow the specific operating conditions. For low-concentration samples, the sample needs to be extracted two or more times, each time with the same amount of fresh solvents. Then the extracts from the different extractions are combined. For high-concentration

(over 20 ppm) samples, approximately 2 g of sample is needed, and a single extraction with 10 mL of solvents may be adequate. After extraction, the extract is filtrated or centrifuged, and some form of cleanup is generally needed prior to analysis.

3.3.1. Selected Applications and Comparison with Soxhlet

Like Soxhlet, sonication is also recognized as an established conventional method, although it is not as widely used. Limited research has focused on sonication per se or its comparison with Soxhlet. Qu et al. [13] developed a method using sonication with methanol for the extraction of linear alkylbenzene sulfonate (LAS) in plant tissues (rice stems and leaves). Both efficiency and accuracy were found to be high. The mean recovery was 89% (84 to 93% for LAS concentration of 1 to 100 mg/kg), and the relative standard deviation (RSD) was 3% for six replicate analyses. Its advantages over Soxhlet extraction were speed (1 hour), less solvent consumption, and smaller sample requirement (2 to 3 g).

Marvin et al. [14] compared sonication with Soxhlet for the extraction of PAHs from sediments, and from an urban dust standard reference material (SRM 1649). The sonication method required less than 5 g of sample. The amount of organic materials extracted by sonication with two solvents was $2.53 \pm 0.10\%$ of the sediment samples (w/w), while $2.41 \pm 0.14\%$ was extracted by Soxhlet. Sequential sonicaion with two solvents was much faster (45 minutes) than Soxhlet (2 days), with practically the same extraction efficiency. The variation of PAH extracted by sonication from the urban dust SRM was within 15%.

Haider and Karlsson [15] developed a simple procedure for the determination of aromatic antioxidants and ultraviolet stabilizers in polyethylene using ultrasonic extraction. Chloroform was used for the isolation of Chimassorb 944 from 150-μm-thick commerical low-density polyethylene and Irganox 1010 and Irgafos 168 from 25-μm medium-density polyethylene film. The recovery of the additives increased remarkably at higher temperatures and longer extraction times. At 60°C, quantitative recovery was achieved in 15, 45, and 60 minutes for Irgafos 168, Irganox 1010, and Chimassorb 944, respectively.

Eiceman et al. [16] reported the ultrasonic extraction of polychlorinated dibenzo-*p*-dioxins (PCDDs) and other organic compounds from fly ash from municipal waste incinerators. Ten to 20 grams of sample was extracted with 200 mL of benzene for 1 hour. Results from five replicate analyses yielded averages and RSDs (ng/g) for the tetra- to octachlorinated dibenzo-*p*-dioxins of 8.6 ± 2.2, 15.0 ± 4.0, 13.0 ± 3.4, 3.2 ± 1.0, and 0.4 ± 0.1, respectively.

Golden and Sawicki [17] studied ultrasonic extraction of almost all of the polar compounds from airborne particulate material collected on Hi-Vol filters. Full recovery of PAH and good reproducibility were achieved. Total analysis time was approximately 1.5 hours. The same research group also reported a sonication procedure for the extraction of total particulate aromatic hydrocarbon (TpAH) from airborne particles collected on glass fiber filters [18]. Significantly higher recovery of TpAH and PAH were achieved by 40 minutes of sonication than by 6 to 8 hours of Soxhlet extraction.

3.4. SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction (SFE) utilizes the unique properties of supercritical fluids to facilitate the extraction of organics from solid samples. Analytical scale SFE can be configured to operate on- or off-line. In the online configuration, SFE is coupled directly to an analytical instrument, such as a gas chromatograph, SFC, or high-performance liquid chromatograph. This offers the potential for automation, but the extract is limited to analysis by the dedicated instrument. Off-line SFE, as its name implies, is a stand-alone extraction method independent of the analytical technique to be used. Off-line SFE is more flexible and easier to perform than the online methods. It allows the analyst to focus on the extraction per se, and the extract is available for analysis by different methods. This chapter focuses on off-line SFE.

The discovery of supercritical fluids by Baron Cagniard de la Tour dates back to 1822 [19]. In 1879, Hannay and Hogarth demonstrated the solvating power of supercritical ethanol [20]. Between 1964 and 1976, Zosel filed several patents on decaffeination of coffee, which signified a major development in SFE. In 1978, a decaffeination plant was opened by the Maxwell House Coffee Division. Since then, SFE has found many industrial applications. The use of supercritical fluids for analytical purposes started with capillary supercritical fluid chromatography (SFC), which was introduced by Novotny et al. in 1981 [21]. Analytical scale SFE became commercially available in the mid-1980s. In 1996, EPA approved two SFE methods, one for the extraction of total petroleum hydrocarbons (TPHs) and the other for PAHs. Another SFE method was promulgated by EPA in 1998 for the extraction of PCBs and organochlorine pesticides (OCPs).

3.4.1. Theoretical Considerations

A supercritical fluid is a substance above its critical temperature and pressure. Figure 3.4 shows a phase diagram of a pure substance, where curve

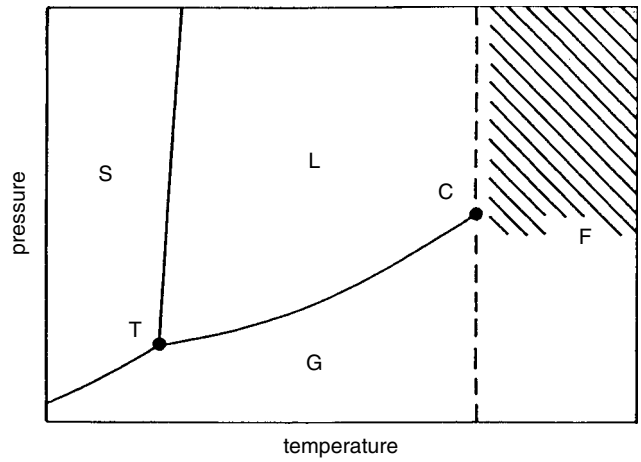

Figure 3.4. Phase diagram of a pure substance. (Reproduced from Ref. 24, with permission from Kluwer Academic Publishers.)

T–C is the interface between gas and liquid. Each point on the line corresponds to a certain temperature and the pressure needed to liquefy the gas at this temperature. Point *C* is the critical point. Beyond the critical temperature, a gas does not liquefy under increasing pressure. Instead, it is compressed into a supercritical fluid. The critical point is substance-specific. Table 3.2 shows the supercritical conditions of some selected solvents.

Table 3.2. Critical Parameters of Select Substances

Substance	Critical Temperature (°C)	Critical Pressure (atm)	Critical Density (10^3 kg/m^3)
CO_2	31.3	72.9	0.47
N_2O	36.5	72.5	0.45
SF_6	45.5	37.1	0.74
NH_3	132.5	112.5	0.24
H_2O	374	227	0.34
n-C_4H_{10}	152	37.5	0.23
n-C_5H_{12}	197	33.3	0.23
Xe	16.6	58.4	1.10
CCl_2F_2	112	40.7	0.56
CHF_3	25.9	46.9	0.52

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Table 3.3. Physical Properties of Gases, Supercritical Fluids, and Liquids

State	Conditions[a]	Density (10^3 kg/m^3)	Viscosity (mPa·s)	Self-Diffusion Coefficient (10^4 m^2/s)
Gas	30°C, 1 atm	$0.6–2 \times 10^3$	$1–3 \times 10^2$	0.1–0.4
Supercritical fluid	Near T_c, p_c	0.2–0.5	$1–3 \times 10^{-2}$	0.7×10^{-3}
	Near T_c, $4p_c$	0.4–0.9	$3–9 \times 10^{-2}$	0.2×10^{-3}
Liquid	30°C, 1 atm	0.6–1.6	0.2–3	$0.2–2 \times 10^{-5}$

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[a] T_c, critical temperature; p_c, critical pressure.

Table 3.3 presents the approximate physical properties of gases, supercritical fluids, and liquids. It shows that the densities of supercritical fluids are close to that of a liquid, whereas their viscosities are gaslike. The diffusion coefficients are in between. Due to these unique properties, supercritical fluids have good solvating power (like liquid), high diffusivity (better than liquid), low viscosity, and minimal surface tension (like gas). With rapid mass transfer in the supercritical phase and with better ability to penetrate the pores in a matrix, extraction is fast in SFE, along with high extraction efficiency.

The solubility of a supercritical fluid is influenced by its temperature, pressure, and density. Solubility correlates better to density than to pressure. An empirical equation can be used to predict solubility [22]:

$$\ln(s) = aD + bT + c \tag{3.1}$$

where s is the solubility in mole or weight percent, D the density in g/mL, T the temperature in kelvin, and a, b, and c are constants. Figure 3.5 depicts the change in analyte solubility in supercritical fluids as a function of temperature and pressure. The predicted solubility using equation (3.1) shows good agreement with the experimental data.

Carbon dioxide (CO_2) has a low supercritical temperature (31°C) and pressure (73 atm). It is nontoxic and nonflammable and is available at high purity. Therefore, CO_2 has become the solvent of choice for most SFE applications. Being nonpolar and without permanent dipole moment, supercritical CO_2 is a good solvent for the extraction of nonpolar and moderately polar compounds. However, its solvating power for polar solutes is rather poor. Moreover, when the solutes bind strongly to the matrix, the solvent strength of CO_2 is often inadequate to break the solute–matrix bond.

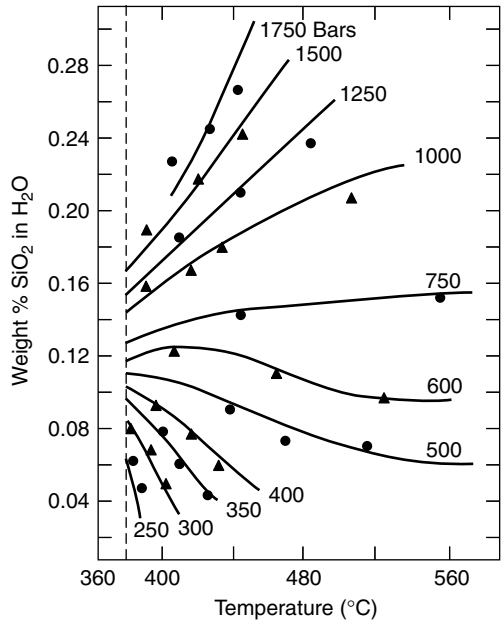

Figure 3.5. Solubility of SiO_2 in supercritical H_2O. (Reproduced from Ref. 22, with permission from Preston Publications.)

This is true even if it is capable of dissolving the solutes. Supercritical solvents such as N_2O and $CHClF_2$ are more efficient in extracting polar compounds, but their routine use is uncommon due to environmental concerns. The extraction efficiency of polar compounds by CO_2 can be improved by the addition of small quantities (1 to 10%) of polar organic solvents, referred to as *modifiers*. This is a common practice in SFE. Table 3.4 lists some common modifiers for supercritical CO_2.

Table 3.4. Commonly Used Modifiers for Supercritical CO_2

Oxygen containing	Methanol, ethanol, isopropyl alcohol, acetone, tetrahydrofuran
Nitrogen containing	Acetonitrile
Sulfur containing	Carbon disulfide, sulfur dioxide, sulfur hexafluoride
Hydrocarbons and halogenated organics	Hexane, toluene, methylene chloride, chloroform, carbon tetrachloride, trichlorofluoromethane
Acids	Formic acid

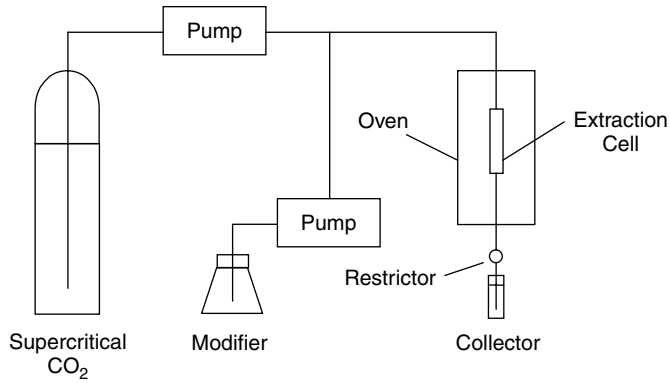

Figure 3.6. Schematic diagram of an off-line SFE system.

3.4.2. Instrumentation

The schematic diagram of an SFE system is shown in Figure 3.6. The basic components include a tank of CO_2, a high-pressure pump, an extraction cell, a heating oven, a flow restrictor, and an extract collector. A source of organic modifier and a pump for its delivery may also be needed. High-purity CO_2 is generally supplied in a cylinder with a dip tube (or eductor tube). The function of the dip tube is to allow only liquefied CO_2 to be drawn into the pump, as the liquid stays at the bottom of the vertically placed cylinder while the gaseous CO_2 is at the top. Aluminum cylinders are generally preferred over steel cylinders. Impurities in CO_2 may cause interference during analysis. The extraction cells, frits, restrictors, and multiport valves may also carry-over analytes from high-concentration samples. It has been found that contamination is more likely to be caused by SFE instrumentation and associated plumbing than by the CO_2 itself [23]. All connections in the SFE system should be metal to metal, and the use of lubricants should be avoided. The extraction system should also be cleaned after each extraction.

The basic requirement for a SFE pump is the ability to deliver constant flow (at least 2 mL/min) in the pressure range 3500 to 1000 psi. Reciprocating and syringe pumps are most common. To maintain CO_2 in a liquid state, the pump head is cooled by using a recirculating bath. There are several ways to add a modifier to the CO_2. One is to add it directly to the extraction cell, but the modifier is exhausted with the flow of extraction fluid. Another approach is to add the modifier to the CO_2 tank (i.e., it is premixed with CO_2). However, it has been reported that the ratio of modifier to CO_2 in the mixture changes with time [24]. Moreover, the modifier may contaminate

the CO_2 pump. A better alternative is to use a second pump for modifier delivery. The modifier and the CO_2 are mixed at a point after the pump but before the extraction cell. This way, the type of the modifier and its concentration can easily be controlled, and the CO_2 pump is free of modifier contamination.

The extraction cell is usually made of stainless steel, PEEK (polyether ether ketone), or any other suitable material that can withstand high pressure (up to 10,000 psi). It is fitted with fingertight frits, which eliminate use of a wrench and reduces the wear and tear that can result from overtightening. Research indicates that the shape of the cell has little impact on the extraction efficiency [24]. Short squat cells are preferred because they are easier to fill than the long thin ones. The extraction cell is placed in an oven that can heat up to 200°C.

The pressure of the supercritical fluid is controlled by the restrictor. Restrictors can be broadly classified into two types: fixed and variable. *Fixed* (diameter) *restrictors* are typically made of fused silica or metal tubing. They are inexpensive and easy to replace, but are subject to plugging problems. A common cause of plugging is water freezing at the restrictor tip because of the rapid expansion of the released supercritical fluid. Plugging can also happen when the matrix has high concentrations of extractable materials such as elemental sulfur, bulk hydrocarbons, or fats. *Variable restrictors* have an orifice or nozzle that can be adjusted electronically. They are free from plugging, and a constant flow rate can be maintained. Although variable restrictors are more expensive, they are necessary for real-world applications.

The extract is collected by depressurizing the fluid into a sorbent trap or a collection solvent. A trap may retain the analytes selectively, which may then be selectively washed off by a solvent. This can offer high selectivity, but requires an additional step. The trap can be cryogenically cooled to avoid the loss of analytes. Using a collection solvent is more straightforward. The choice of solvents often depends on the analytical instrumentation. For example, tetrachloroethene is suitable for infrared determination, while methylene chloride and isooctane are appropriate for gas chromatographic separations.

3.4.3. Operational Procedures

The sample is loaded into an extraction cell and placed into the heating oven. The temperature, pressure, flow rate, and the extraction time are set, and the extraction is started. The extract is collected either by a sorbent trap, or by a collection vial containng a solvent. Typical EPA-recommended operating conditions for the extraction of PAHs, pesticides, and PCBs are

Table 3.5. EPA-Recommended SFE Methods for Environmental Samples

	Total Recoverable Petroleum Hydrocarbons	Volatile PAHs	Less Volatile PAHs	Organochlorine Pesticides	PCBs
Extraction fluid	CO_2	CO_2	CO_2–CH_3OH–H_2O (95:1:4 v/v/v)[a]	CO_2	CO_2
Pressure (psi)	6100	1750	4900	4330	4417
Density (g/mL)	0.785	0.3	0.63	0.87	0.75
Temperature (°C)	80	80	120	50	80
Static equilibration time (min)	0	10	10	20	10
Dynamic extraction time (min)	30	10	30	30	40
Flow rate (mL/min)	1.1–1.5	2.0	4.0	1.0	2.5

[a]For HPLC determination only. CO_2–methanol–dichloromethane (95:1:4 v/v/v) should be used for GC.

presented in Table 3.5. Supercritical fluid extraction can be operated in two modes: static or dynamic. In *static extraction* the supercritical fluid is held in an extraction cell for a certain amount of time and then released to a collection device. In *dynamic extraction*, the supercritical fluid flows continuously through the extraction cell and out into a collection device.

3.4.4. Advantages/Disadvantages and Applications of SFE

SFE is fast (10 to 60 minutes) and uses minimum amount of solvents (5 to 10 mL) per sample. CO_2 is nontoxic, nonflammable, and environmentally friendly. Selective extraction of different groups of analytes can be achieved by tuning the strength of the supercritical fluids with different modifiers and by altering operating conditions. In addition, the extract from SFE does not need additional filtration, as the extraction cell has frits.

On the down side, analytical-scale SFE has limited sample size (<10 g), and the instrument is rather expensive. Furthermore, SFE has been found to be matrix dependent. Different methods have to be developed and validated

for different sample matrices and for different groups of analytes. For example, Kim et al. [25] conducted an investigation on the effect of plant matrix on the SFE recovery of five schisandrin derivatives. At 60°C and 34.0 MPa, the compounds extracted from the leaves of *Schisandra chinensis* by supercritical CO_2 were 36.9% of what were obtained by organic solvent extraction. However, under the same SFE conditions, extraction from the stem and fruits yielded more than 80% of that by organic solvents. Although the addition of 10% ethanol to CO_2 increased the yield from leaves four times, it had little effect on the extraction of stems and fruits.

SFE has a wide range of applications, which include the extraction of PAHs, PCBs, phenols, pesticides, herbicides, and hydrocarbons from environmental samples, contaminants from foods and feeds, and active gradients from cosmetics and pharmaceutical products. Table 3.6 lists some examples from the literature.

3.5. ACCELERATED SOLVENT EXTRACTION

Accelerated solvent extraction (ASE) is also known as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE). It uses conventional solvents at elevated temperatures (100 to 180°C) and pressures (1500 to 2000 psi) to enhance the extraction of organic analytes from solids. ASE was introduced by Dionex Corp. (Sunnyvale, CA) in 1995. It evolved as a consequence of many years of research on SFE [45]. SFE is matrix dependent and often requires the addition of organic modifiers. ASE was developed to overcome these limitations. It was expected that conventional solvents would be less efficient than supercritical fluids, which have higher diffusion coefficients and lower viscosity. However, the results turned out to be quite the opposite. In many cases, extraction was faster and more complete with organic solvents at elevated temperature and pressure than with SFE. Extensive research has been done on the extraction of a variety of samples with ASE. ASE was approved by EPA as a standard method in 1996.

3.5.1. Theoretical Considerations

The elevated pressure and temperature used in ASE affects the solvent, the sample, and their interactions. The solvent boiling point is increased under high pressure, so the extraction can be conducted at higher temperatures. The high pressure also allows the solvent to penetrate deeper into the sample matrix, thus facilitating the extraction of analytes trapped in matrix pores. At elevated temperatures, analyte solubility increases and the mass transfer is faster. The high temperature also weakens the solute–matrix bond due to

Table 3.6. Selected SFE Applications

Analytes	Matrix	Reference
Polycyclic aromatic hydrocarbons (PAHs)	Standard reference materials (SRMs)	5
	Wastewater sludge	26
	Soils	27
	Liver samples	28
	Toasted bread	29
Polychlorinated biphenyls (PCBs)	Wastewater sludge	26
	Chicken liver	30
Organochlorine pesticides (OCPs)	Wastewater sludge	26
	Chinese herbal medicines	31
Carbamate pesticides (carbaryl, aldicarb, and carbofuran)	Filter paper and silica gel matrixes	32
Insecticides carbosulfan and imidacloprid	Process dust waste	33
Ten triazine herbicide residues	Eggs	34
Cyanazine and its seven metabolites	Spiked silty clay loam soil	35
Aromatic acids, phenols, pesticides	Soils	27
4-Nonylphenol	Municipal sewage sludge	36
Petroleum hydrocarbons	Spiked clay–sand soil	37
Nine aliphatic hydrocarbons	Chicken liver	30
Nicarbazin (a drug used principally in poultry)	Poultry feeds, eggs, and chicken tissue	38
Fenpyroximate	Apple samples	39
Vitamins A and E	Milk powder	40
Vitamins D_2 and D_3	Pharmaceutical products	41
p-Aminobenzoate (PABA) and cinnamate, ultraviolet absorbers	Cosmetic products	42
Five of the most common sunscreen agents	Cosmetic products	43
Lanolin	Raw wool fibers	44

van der Waals forces, hydrogen bonding, and dipole attractions. In addition, the high temperature reduces the solvent viscosity and surface tension, which enhances solvent penetration into the matrix. All these factors lead to faster extraction and better analyte recovery.

3.5.2. Instrumentation

A schematic diagram of an ASE system is shown in Figure 3.7. It consists of solvent tank(s), a solvent pump, an extraction cell, a heating oven, a collec-

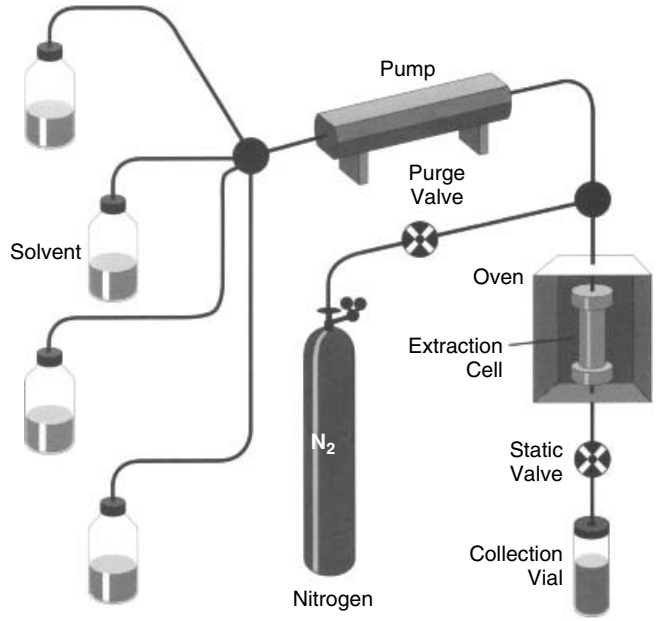

Figure 3.7. Schematic diagram of an ASE system. (Reproduced with permission from Dionex Corp.)

tion vial, and a nitrogen tank. The sample size can be anywhere between 1 and 100 mL. The extraction cells are made of stainless steel that can withstand high temperature and pressure. Each cell has two removable fingertight caps on the ends that allow easy sample loading and cleaning. The caps are fitted with compression seals for high-pressure closure. To load the cell, one end cap is screwed on to fingertightness. Then a filter is introduced into the cell, followed by the sample. The other cap is screwed on to fingertightness for complete closure. The cell is then placed in a carousel that can hold and load multiple cells.

The ASE system is fully automated. An autoseal actuator moves the cell from the carousel into the heating oven. The solvent is delivered from one or more solvent bottles into the extraction cell by a pump. The oven is heated, and the temperature and pressure in the cell rise. When the pressure reaches 200 psi above the preset value, the static valve opens to release the excessive pressure and then closes again. Then the pump delivers fresh solvent to the cell to bring the pressure back to the preset value. The addition of fresh solvent increases the concentration gradient and enhances both mass transfer and extraction efficiency. The extracts are collected in 40 or

60-mL collection vials on a removable vial tray. The vial lids have TFE-coated solvent-resistant septa. The tubing from the extraction cell to the collection vial provides enough heat loss so that additional cooling is not necessary.

An automated solvent controller is available in the latest ASE system. It allows up to four solvents to be mixed and delivered to the extraction cells. This can reduce the time for measuring and mixing solvents and decrease users' exposure to toxic solvents. The solvent controller can be programmed to change solvents between sequential extractions of multiple samples. The same sample can also be reextracted using different solvents. The ASE system has many built-in safety features, which include vapor sensors, liquid-leak detectors, vial overfill monitors, electronic and mechanical over-pressurization prevention systems, solvent flow monitors, and pneumatic source pressure monitors.

3.5.3. Operational Procedures

The steps in the ASE process are shown in Figure 3.8. The sample is loaded into the extraction cell, and then the solvent is pumped in. Then the cell is heated to the desired temperature and pressure. The heat-up time can be 5 to 9 minutes (for up to 200°C). This is referred to as the *prefill method*. Alternatively, the sample can be heated before adding the solvent, which is known as the *preheat method*. However, the preheat method is prone to the loss of volatile analytes. Therefore, the prefill approach is generally preferred [46].

After heating, the extraction can be conducted dynamically, statically, or as a combination of both. In the dynamic mode, the extraction solvent flows through the system, whereas there is no solvent flow in the static mode. Although it may have higher extraction efficiency, dynamic extraction uses more solvents and is not commonly used. Static extraction time is on the order of 5 minutes, although it can be as long as 99 minutes. After extraction, the extract is flushed into the collection vial with fresh solvents. The flush volume can be 5 to 150% of the cell volume, with 60% being the typical choice. As many as five static cycles may be chosen, although a single cycle is the most common option. The total flush volume is divided by the number of cycles, and an equal portion is used in each cycle. After the final solvent flush, the solvent is purged into the collection vial with nitrogen (typically, 1-minute purge at 150 psi). The ASE system can sequentially extract up to 24 samples in one unattended operation. The sequence of introducing and removing the cells to and from the oven can be automated. Extract filtration is not required, but concentration and/or cleanup is often necessary prior to analysis.

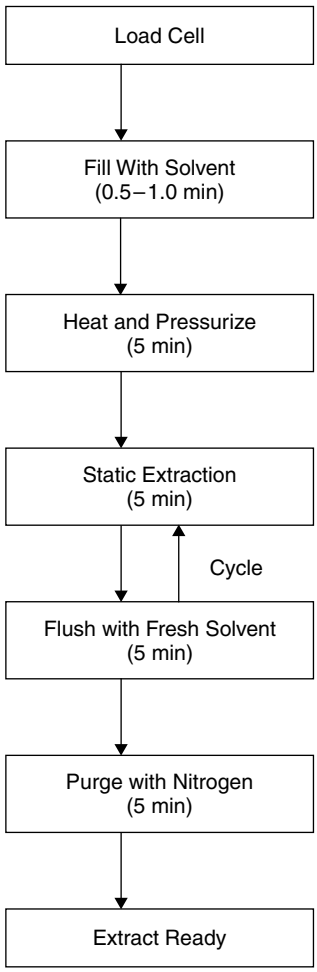

Figure 3.8. Schematic diagram of ASE procedures.

3.5.4. Process Parameters

Typical operating parameters suggested in the EPA standard method are listed in Table 3.7.

Temperature and Pressure

As mentioned before, solubility and mass transfer increase at elevated temperatures. Table 3.8 shows that both recovery and precision improved when the temperature was increased during the extraction of total petroleum

Table 3.7. Suggested System Parameters in EPA Standard Methods for the ASE of Environmental Samples

	Semivoaltiles, Organophosphorus Pesticides, Organochlorine Pesticides, Herbicides, and PCBs	Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans	Diesel Range Organics
Oven temperature (°C)	100	150–175	175
Pressure (psi)	1500–2000	1500–2000	1500–2000
Static time (min)	5 (after 5 min preheat time)	5–10 (after 7–8 min preheat time)	5–10 (after 7–8 min preheat time)
Flush volume	60% of the cell volume	60–75% of the cell volume	60–75% of the cell volume
Nitrogen purge[a]	60 s at 150 psi	60 s at 150 psi	60 s at 150 psi
Static cycles	1	2 or 3	1

[a] Purge time may be extended for larger cells.

hydrocarbons from soil [46]. Similar observations were made in other applications as well [47,48]. A certain pressure level is required to keep the solvent in its liquid state when the temperature is above its boiling point at atmospheric pressure. Pressure greater than 1500 psi has no significant influence on the recovery [45]. Typical pressures used in the extraction of environmental samples are in the range 1500 to 2000 psi.

Solvents

The general criteria for the solvent selection are high solubility of the analytes and low solubility of the sample matrix. Solvents used in conventional

Table 3.8. Effects of Temperature on the Recovery of TPHs from Soil Using ASE (1200 mg/kg Certified Value)

Temperature (°C)	Extraction Efficiency (%)	RSD (%)
27	81.2	6.0
50	93.2	5.0
75	99.2	2.0
100	102.7	1.0

Reproduced from Ref. 46, with permission from the American Chemical Society.

Table 3.9. Solvents Recommended by EPA for the ASE of Environmental Samples

Analytes	Solvents
Organochlorine pesticides, semivolatile organics	Acetone–hexane (1:1 v/v) or acetone–methylene chloride (1:1 v/v)
PCBs	Acetone–hexane (1:1 v/v) or acetone–methylene chloride (1:1 v/v) or hexane
Organophosphorus pesticides	Methylene chloride or acetone–methylene chloride (1:1 v/v)
Chlorinated herbicides	Acetone–methylene chloride–phosphoric acid solution (250:125:15 v/v/v) or acetone–methylene chloride–trifluoroacetic acid solution (250:125:15 v/v/v)
Polychlorinated dibenzodioxins and polychlorinated dibenzofurans	Toluene or toluene–acetic acid solution (5% v/v glacial acetic acid in toluene) for fly ash samples
Diesel range organics	Acetone–methylene chloride (1:1 v/v) or acetone–hexane (1:1 v/v) or acetone–heptane (1:1 v/v)

(such as Soxhlet) extraction methods can readily be applied in ASE. However, conventional solvents cannot be used in certain applications, such as the extraction of polymers. This is because the matrix itself can dissolve in the solvent at high temperature and plug the connecting tubing in the system. On the other hand, solvents that are not efficient in Soxhlet extraction may yield high recovery under ASE conditions. For example, hexane was found to be a poor solvent in the Soxhlet extraction of monomers and oligomers from nylon-6 and poly(1,4-butylene terephthalate) (PBT), but it gave satisfactory results in ASE [47]. Table 3.9 lists the solvents recommended in EPA method 3545A for the ASE of different groups of analytes from soils, clays, sediments, sludge, and waste solids.

Small sample size can reduce solvent volume, provided it meets the requirements of sensitivity and homogeneity. Ten to 30 grams of material is usually necessary. The volume of the solvent is a function of the size of the extraction cell rather than the mass of the sample. The solvent volume may vary from 0.5 to 1.4 times that of the cell [1]. Specific solvent/cell volume ratios are usually available in the instrument manufacturer's instructions.

3.5.5. Advantages and Applications of ASE

ASE has many advantages. It uses minimal amount of solvent and is fast (about 15 minutes), fully automated, and easy to use. Filtration is a built-in

step, so additional filtration is not needed. While operating at higher temperatures and pressures, ASE can employ the same solvent specified by other existing methods. Therefore, method development is simple. There are more solvents to choose from, because solvents that work poorly in conventional methods may perform well under ASE conditions. In addition, ASE provides the flexibility of changing solvents without affecting the extraction temperature and pressure. Despite high initial equipment cost, cost per sample can be relatively low.

This section is not intended to be a thorough literature survey, but it offers a general description of typical ASE applications. Table 3.10 provides

Table 3.10. Selected ASE Applications

Analytes	Matrix	Reference
PAHs	Soils	49
	Clay loam and soils	50
	Mosses and pine needles	51
	Soils, heap material, and fly ash	52
	Soil	53
PCBs	Mosses and pine needles	52
	Soil	53
Organochlorine pesticides (OCPs)	Soils, heap material, and fly ash	52
	Clay loam and soils	50
Organophosphorus pesticides	Foods	54
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans	Soils, heap material, and fly ash	52
	Chimney brick, urban dust, and fly ash	55
Hydrocarbons	Wet and dry soils	56
Chlorobenzenes, HCH isomers, and DDX	Soil	53
	Mosses and pine needles	51
Atrazine and alachlor	Soils	57
Diflufenican	Soil	58
Phenols	Spiked soil	59
Chlorophenols	Soil	60
Additive Irganox 1010	Polypropylene	61
	Polypropylene, PVC, and nylon	62
Antioxidant Irganox 1076	Linear low-density polyethylene (LLDPE)	63
Monomers and oligomers	Nylon-6 and poly(1,4-butylene terephthalate)	47
Felodipine	Medicine tablets	64
Active gradients	Medicinal plants	65
Fatty acid and lipids	Cereal, egg yolk, and chicken meat	66

a quick reference to these examples, and more detailed information can be found in some recent reviews [67,68]. In principle, ASE is a universal method that can be used in any solvent extraction. However, majority applications so far have been in the environmental area, such as the extraction of pesticides, herbicides, PAHs, PCBs, base/neutral/acid compounds, dioxins, furans, and total petroleum hydrocarbons. ASE has also been used to extract additives and plasticizers from polymers, additives, and active ingredients from pharmaceuticals, and contaminants/fat from food.

3.6. MICROWAVE-ASSISTED EXTRACTION

It should be noted that microwave-assisted extraction (MAE) discussed in this chapter is different from microwave-assisted acid digestion. The former uses organic solvents to extract organic compounds from solids, while the latter uses acids to dissolve the sample for elemental analysis with the organic contents being destroyed. Microwave-assisted digestion of metals is covered in Chapter 5.

The name *magnetron* (microwave generator) was first used in 1921 by A. W. Hall. In 1946, Percy Spencer discovered the function of microwave as a heating source. Domestic microwave ovens became available in 1967 [69]. In 1975, microwave was first applied to acid digestion for metal analysis by Abu-Samra et al. [70]. Since then much work has been done on microwave-assisted acid digestion, and it has gained widespread acceptance and approval by regulatory agencies as a standard method. Microwave-assisted organic extraction was first carried out in 1986 by Ganzler et al. [71] for the extraction of fats and antinutrients from food and pesticides from soil. In 1992, Pare [72] patented a process called MAP (microwave-assisted process) for the extraction of essential oils from biological materials. This technique was later extended to analytical as well as large-scale applications. In the year 2000, MAE was approved by the EPA as a standard method for the extraction of semivoaltile and nonvolatile compounds from solid samples.

3.6.1. Theoretical Considerations

Microwaves are electromagnetic radiation in the frequency range 0.3 to 300 GHz (corresponding to 0.1 to 100 cm wavelength). They are between the radio frequency and the infrared regions of the electromagnetic spectrum. Microwave is used extensively in radar transmission (1 to 25 cm wavelength) and telecommunications. To avoid interference with communication networks, all microwave heaters (domestic or scientific) are designed to work at

either 2.45 or 0.9 GHz. Domestic ovens operate at 2.45 GHz only. When mircowave radiation is applied to molecules in the gas phase, the molecules absorb energy to change their rotational states. The microwave spectrum of molecules shows many sharp bands in the range 3 to 60 GHz. This has been used in microwave spectroscopy to obtain fundamental physical–chemical data such as bond lengths and angels, and to identify gaseous molecules (e.g., molecular species in outer space).

In the liquid and solid states, molecules do not rotate freely in the microwave field; therefore, no microwave spectra can be observed. Molecules respond to the radiation differently, and this is where microwave heating comes in. The mechanism of microwave heating is different from that of conventional heating. In conventional heating, thermal energy is transferred from the source to the object through conduction and convection. In microwave heating, electromagnetic energy is transformed into heat through ionic conduction and dipole rotation. *Ionic conduction* refers to the movement of ions in a solution under an electromagnetic field. The friction between the solution and the ions generates heat. *Dipole rotation* is the reorientation of dipoles under microwave radiation. A polarized molecule rotates to align itself with the electromagnetic field at a rate of 4.9×10^9 times per second. The larger the dipole moment of a molecule, the more vigorous is the oscillation in the microwave field.

The ability of a material to transform electromagnetic energy into thermal energy can be defined as

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'}$$

where $\tan \delta$ is the loss tangent or tangent delta; ε'' is the dielectric loss coefficient, a measure of the efficiency of a material to transform electromagnetic energy to thermal energy; and ε' is the dielectric constant, a measure of the polarizibility of a molecule in an electric field. Table 3.11 lists the physical constants of some selected organic solvents. Polar solvents such as acetone, methanol, and methylene chloride have high $\tan \delta$ values and can be heated rapidly. Nonpolar solvents such as hexane, benzene, and toluene cannot be heated because they lack dipoles and do not absorb microwave.

3.6.2. Instrumentation

In general, organic extraction and acid digestion use different types of microwave apparatus, as these two processes require different reagents and different experimental conditions. A new commercial system, Mars X (CEM Corp., Matthews, NC) offers a duel unit that can perform both proce-

Table 3.11. Physical Constants of Organic Solvents Used in MAE[a]

Solvent	Boiling Point (°C)	Vapor Pressure		ε'	Dipole Moment (debye)	$\tan\delta \times 10^4$
		torr	kPa			
Methylene chloride	40	436	58.2	8.93	1.14	—
Acetone	56	184	24.6	20.7	2.69	—
Methanol	65	125	16.7	32.7	2.87	6400
Tetrahydrofuran	66	142	19.0	7.58	1.75	—
Hexane	69	120	16.0	1.88	<0.1	—
Ethyl acetate	77	73	9.74	6.02	1.88	—
Ethanol	78	—	—	24.3	1.69	2500
Methyl ethyl ketone	80	91	12.1	18.51	2.76	—
Acetonitrile	82	89	11.9	37.5	3.44	—
2-Propanol	82	32	4.27	19.92	1.66	6700
1-Propanol	97	14	1.87	20.33	3.09	~2400[b]
Isooctane	99	49	6.54	1.94	0	—
Water	100	760	101.4	78.3	1.87	1570
Methyl isobutyl ketone	116	20	2.67	13.11	—	—
Dimethyl formamide	153	2.7	0.36	36.71	3.86	—
Dimethyl acetamide	166	1.3	0.17	37.78	3.72	—
Dimethyl sulfoxide	189	0.6	0.08	46.68	3.1	—
Ethylene glycol	198	—	—	41.0	2.3	10,000
N-Methyl pyrrolidinone	202	4.0	0.53	32.0	4.09	—

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[a] Boiling points were determined at 101.4 kPa; vapor pressures were determined at 25°C, dielectric constants were determined at 20°C; dipole moments were determined at 25°C.

[b] Value was determined at 10°C.

dures. In this chapter only the instrumentation for organic extraction is discussed.

The basic components of a microwave system include a microwave generator (magnetron), a waveguide for transmission, a resonant cavity, and a power supply. For safety and other reasons, domestic microwave ovens are not suitable for laboratory use. There are two types of laboratory microwave units. One uses closed extraction vessels under elevated pressure; the other uses open vessels under atmospheric pressure. Table 3.12 lists the features of some commercial MAE systems.

Closed-Vessel Microwave Extraction Systems

Closed-vessel units were the first commercially available microwave ovens for laboratory use. A schematic diagram of such a system is shown in

Table 3.12. Features of Some Commercial MAE Systems

Model/Manufacturer	Power (W)	Sensors	Max. Pressure (bar)	Vessel Volume (mL)	Vessel Material[a]	Number of Vessels	Max Temp. (°C)
Multiwave/Anton Parr GmbH, Austria	1000	Pressure control and infrared temperature measurement in all vessels	70	100	TFM/ceramics	12	230
			70	100	TFM/ceramics	6	280
			130	50	TFM/ceramics	6	280
			130	50	Quartz	8	300
			130	20	Quartz	8	300
Mars-8/CEM, United States	1500	Infrared temperature measurement in all vessels	35	100	TFM	14	300
			100	100	TFM	12	300
Ethos 900/1600, Milestone, United States	1600	Pressure control and temperature measurement in all vessels	30	120	TFM/PFA	10	240
			100	120	TFM	6	280
			30	120	TFM/PFA	12	240
			100	120	TFM	10	280
Soxwave 100/3.6, Prolabo, France	250	Temperature control	Open vessel	250	Quartz	1	—
			Open vessel	100 or 260	Quartz	1	—

[a] TFM, tetrafluoromethoxyl polymer; PFA, perfluoroalkoxy.

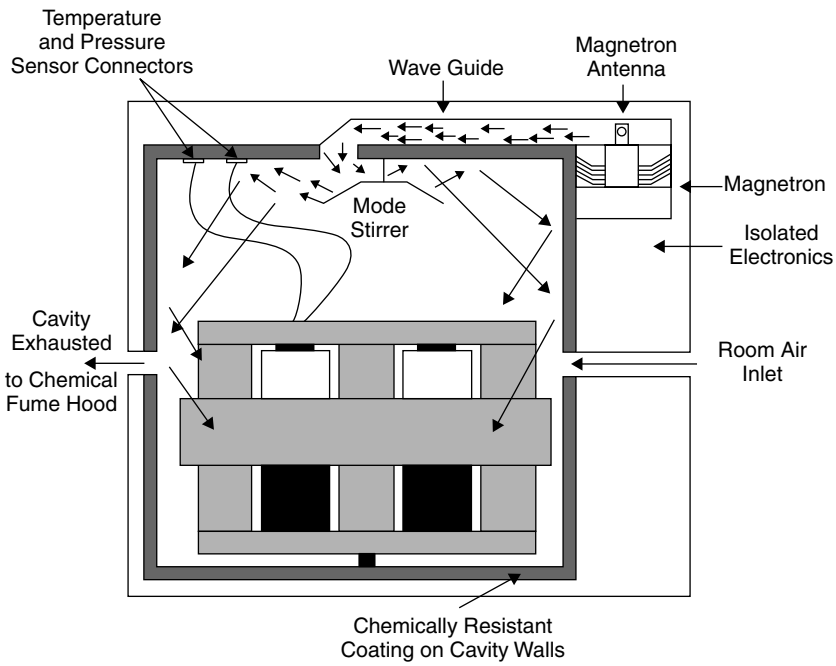

Figure 3.9. Schematic diagram of a closed-vessel cavity MAE system. (Reproduced from Ref. 85, with permission from the American Chemical Society.)

Figure 3.9. In the oven cavity is a carousel (turntable or rotor) that can hold multiple extraction vessels. The carousel rotates 360° during extraction so that multiple samples can be processed simultaneously. The vessels and the caps are constructed of chemically inert and microwave transparent materials such as TFM (tetrafluoromethoxyl polymer) or polyetherimide. The inner liners and cover are made of Teflon PFA (perfluoroalkoxy). The vessels can hold at least 200 psi of pressure. Under elevated pressures, the temperature in the vessel is higher than the solvent's boiling point (see Table 3.11), and this enhances extraction efficiency. However, the high pressure and temperature may pose safety hazards. Moreover, the vessels need to be cooled down and depressurized after extraction.

One of the extraction vessels is equipped with a temperature and pressure sensor/control unit. Figure 3.10 shows the schematic diagram of a control vessel as well as a standard vessel. A fiber-optic temperature probe is built into the cap and the cover of the control vessel. The standard EPA method requires the microwave extraction system to be capable of sensing the temperature to within ± 2.5°C and adjusting the microwave field output power

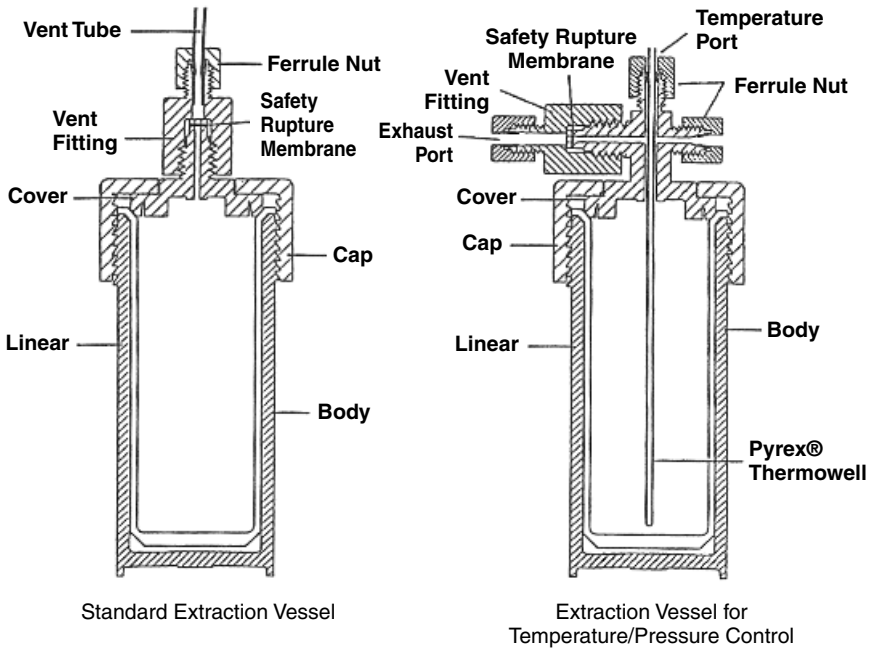

Figure 3.10. Schematic diagram of a closed vessel for MAE. (Reproduced from Ref. 85, with permission from the American Chemical Society.)

automatically within 2 seconds of sensing. The temperature sensor should be accurate to $\pm 2^{\circ}C$.

Safety features are essential to a microwave apparatus. An exhaust fan draws the air from the oven to a solvent vapor detector. Should solvent vapors be detected, the magnetron is shut off automatically while the fan keeps running. Each vessel has a rupture membrane that breaks if the pressure in the vessel exceeds the preset limit. In the case of a membrane rupture, solvent vapor escapes into an expansion chamber, which is connected to the vessels through vent tubing. To prevent excessive pressure buildup, some manufacturer use resealable vessels. A spring device allows the vessel to open and close quickly, releasing the excess pressure.

Additional features can be found in newer systems. Some have a built-in magnetic stir bar with variable speed control for simultaneous stirring in all the vessels. Stirring enhances contact between the sample and the solvents. This reportedly results in significant reduction in extraction time and improvement in analytes recoveries [68]. The stir bar is made of Weflon, a proprietary polytetrafluoroethylene (PTFE) compound that can absorb microwave. This allows the use of nonpolar solvents for extraction since

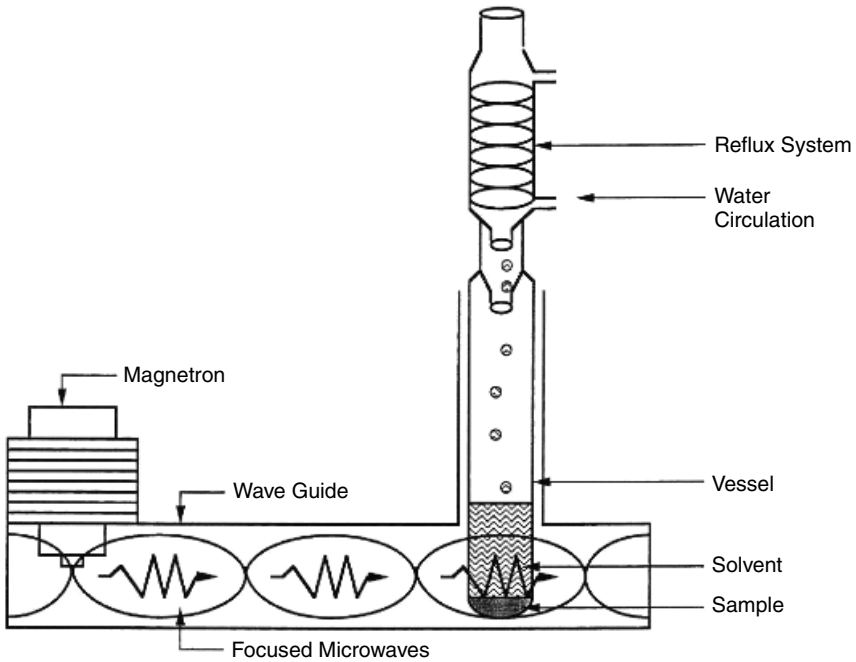

Figure 3.11. Schematic diagram of an open-vessel, waveguide-type MAE system. (Reproduced from Ref. 6, with permission from Elsevier Science.)

heating is done through the stir bar. The same solvents used in conventional methods (both polar and nonpolar) may be adopted here, thus reducing the time for method development.

Open-Vessel Microwave Extraction Systems

Open-vessel systems are also known as *atmospheric pressure microwave* or *focused microwave systems*. An example is Soxwave 100 (Prolabo Ltd., France). A schematic diagram of such a system is shown in Figure 3.11. It uses a "focused" waveguide, that directs the microwave energy into a single-vessel cavity. This provides greater homogeneity of the radiation than in closed-vessel units, where microwave is dispersed into the multivessel cavity. However, only one vessel can be heated at a time, and multiple vessels are to be processed sequentially. The vessel, typically made of glass or quartz, is connected with an air (or a water) condenser to reflux the volatile analytes and solvents. Operating somewhat like Soxhlet extraction, this type of system has been referred to as *microwave-assisted Soxhlet extraction*.

3.6.3. Procedures and Advantages/Disadvantages

In a typical application, 2 to 20 g of sample is dried, weighed, and loaded into an extraction vessel. A certain amount (less than 30 mL) of select solvents is also added. Then parameters such as temperature, pressure, and extraction time are set according to the instructions from the microwave manufacturer. A preextraction heating step (typically, 1 to 2 minutes) is needed to bring the system to the preset values. Subsequently, the samples are extracted for about 10 to 20 minutes. After the extraction, the vessels are cooled, and this normally takes less than 20 minutes. Finally, the extract is filtered, concentrated, and analyzed.

High efficiency is the major advantage of microwave extraction over conventional methods such as Soxhlet. It can achieve the same recovery in a shorter time (20 to 30 minutes) and with less solvent (30 mL). The throughput is high (up to 12 samples per hour for closed-vessel system). On the other hand, MAE has several limitations. Solvents used in Soxhlet extraction cannot readily be applied to microwave extraction because some of them do not absorb microwave. Method development is generally necessary for MAE applications. Moreover, cooling and filtration after extraction prolongs the overall process. Since MAE is quite exhaustive, normally the extract contains interfering species that require cleanup prior to analysis.

3.6.4. Process Parameters

The efficiency of MAE can be influenced by factors such as the choice of solvent, temperature, extraction time, matrix effects, and water contents. In general, some optimization of these conditions is necessary. Typical microwave conditions suggested in a standard EPA method are listed in Table 3.13.

Table 3.13. EPA Standard Procedure for MAE of Environmental Samples

Solvents	25 mL of acetone–hexane (1:1 v/v)
Temperature	100–150°C
Pressure	50–150 psi
Time at temperature	10–20 min
Cooling	To room temperature
Filtering/rinsing	With the same solvent system

Choice of Solvent

The proper choice of solvent is the key to successful extraction. In general, three types of solvent system can be used in MAE: solvent(s) of high ε'' (dielectric loss coefficient), a mixture of solvents of high and low ε'', and a microwave transparent solvent used with a sample of high ε''. Pure water was used for the extraction of triazines from soils [73], and the recovery was comparable to those using organic solvents. In the extraction of organochlorine pesticides (OCPs) from marine sediments, terahydrofuran (THF) yielded better recovery than either acetone or acetone–hexane (1:1) [74]. It was reported that dichloromethane (DCM)–methanol (9:1) was the most efficient solvent for the extraction of phenylurea herbicides (linuron and related compounds) from soils. Other solvent systems, including DCM, DCM–water (5:1), methanol–water (7:3), and methanol–water (9:1) gave poor performance [75]. For the extraction of felodipine and its degradation product H152/37 from medicine tablets [76], acetonitrile–methanol (95:5) was found to be the optimum solvent composition. Methanol was capable of dissolving the tablet's outer covering layer, while acetonitrile broke the inner matrix into small pieces. Hexane–acetone (typically 1:1) has proven to be an efficient solvent system for the extraction of PAHs, phenols, PCBs, and OCPs from environmental samples [77,78].

Temperature and Pressure

Generally, recovery increases with the increase in temperature and then levels off after a certain point. For thermally labile compounds, analyte degradation occurs at high temperatures and results in low recovery. Excessively high temperatures lead to matrix decomposition in polymer extractions and should be avoided. In general, pressure is not a critical parameter in MAE. It changes with the solvent system and the temperature used and is acceptable below a preset limit.

It was reported that the recoveries of 17 PAHs from six certified reference marine sediments and soils [77] increased from 70 to 75% when the temperature was increased from 50°C to 115°C, and remained at 75% from 115 to 145°C. In the extraction of OCPs from sediments, recovery was unchanged from 100 to 120°C [74]. In the extraction of phenylurea herbicides from soils, the recovery peaked in the range 60 to 80°C and decreased at lower or higher temperatures [75]. In the extraction of sulfonylurea herbicides from soils, recovery dropped from 70 to 80% to 1 to 30%, due to decomposition when temperature increased from 70°C to 115°C [79]. The recovery of oligomers from poly(ethyleneterephthalate) increased as temperature rose

from 70°C to 140°C [80]. However, polymer fusion occurred at temperatures above 125°C; therefore, 120°C was chosen as the optimum.

Extraction Time

Many microwave extractions can reach maximum recovery in 10 to 20 minutes. Longer extraction time is not necessary and may lead to the decomposition of thermolabile analytes. It was reported that the recovery of sulfonylurea from soil was not affected by extraction time in the range 5 to 30 minutes [79]. Similar observation was made in the extraction of PAHs from soils and sediments [6]. In the extraction of PAHs and LAHs (linear aliphatic hydrocarbons) from marine sediments, the extraction time was found to be dependent on the irradiation power and the number of samples extracted per run [81]. When the irradiation power was 500 W, the extraction time varied from 6 minutes for one sample to 18 minutes for eight samples [74]. The recovery of OCPs from spiked marine sediments increased from 30% at 5 and 10 minutes to 60% at 20 minutes and to 74 to 99% at 30 minutes [82].

Matrix Effects and Water Content

Matrix effects have been observed in MAE applications. It was reported that recoveries of OCPs from aged soils (24 hours of aging) were lower than those from freshly spiked samples [78]. Similar matrix effects were also reported in the extraction of sulfonylurea herbcides from aged soils [79]. In another study, the average recoveries of 17 PAHs from six different standard reference materials (marine sediments and soils) varied from 50 to 100% [77].

Because water is a polar substance that can be heated by microwave irradiation, it can often improve analyte recovery. In a study of focused MAE of PAHs from soil and sediments [6], sample moisture level showed significant influence on extraction efficiency, and 30% water in the sample provided the highest recovery. Similarly, the maximum recovery of phenylurea herbicides was obtained with 10% water in soils [75]. In the extraction of triazines from soil, water content in the range 10 to 15% yielded the highest recovery [7].

Microwave power output and sample weight seem to have minor effects on extraction efficiency. It was reported that the increase in oven power gave higher recovery of PAHs from atmospheric particles [82]. The reason could be that the microwave system used in that study had no temperature control.

For an extraction conducted at a controlled temperature, the oven power output may have less influence on recovery.

3.6.5. Applications of MAE

Majority MAE applications have been in the extraction of PAHs, PCBs, pesticides, phenols, and total petroleum hydrocarbons (TPHs) from environmental samples. MAE has also been used in the extraction of contaminants and nutrients from foodstuffs, active gradients from pharmaceutical products, and organic additives from polymer/plastics. Table 3.14 lists some typical applications. Readers interested in the details of MAE applications can find more information in some recent reviews [85–87].

3.7. COMPARISON OF THE VARIOUS EXTRACTION TECHNIQUES

Table 3.15 summarizes the advantages and disadvantages of various extraction techniques used in the analysis of semivolatile organic analytes in solid samples. They are compared on the basis of matrix effect, equipment cost, solvent use, extraction time, sample size, automation/unattended operation, selectivity, sample throughput, applicability, filtration requirement, and the need for evaporation/concentration. The examples that follow show the differences among these techniques in real-world applications.

Example 1

Lopez-Avila et al. [88] compared MAE, Soxhlet, sonication, and SFE in their ability to extract 95 compounds listed in the EPA method 8250. Freshly spiked soil samples and two SRMs were extracted by MAE and Soxhlet with hexane–acetone (1:1), by sonication with methylene chloride–acetone (1:1), and by SFE with supercritical carbon dioxide modified with 10% methanol. Table 3.16 shows the number of compounds in different recovery ranges obtained by the various techniques. Sonication yielded the highest recoveries, followed by MAE and Soxhlet, whose performances were similar. SFE gave the lowest recoveries. MAE demonstrated the best precision: RSDs were less than 10% for 90 of 94 compounds. Soxhlet extraction showed the worst precision; only 52 of 94 samples gave RSDs less than 10%. No technique produced acceptable recoveries for 15 polar basic compounds. The recoveries of these compounds by MAE with hexane–acetone at 115°C for 10 minutes (1000 W power) were poor. Consequently, their extraction with MAE was investigated using acetonitrile at 50 and 115°C. Ten of the 15 compounds were recovered quantitatively (>70%) at 115°C.

Table 3.14. Selected MAE Applications

Analytes	Matrix	Vessel Type	Solvents	Extraction Conditions	Recovery (%)	RSD (%)	Reference
PAHs	SRMs, spiked, and real soil samples	Open	20 mL of acetone–hexane (1:1)	1-g sample, 10 min	96–100	<7	83
	Soil and sediments	Open	30 mL of dichloromethane	0.1- to 1-g sample, 30% water, 10 min	85–90	<15	6
	Atmospheric particles	Closed	15 mL of acetone–hexane (1:1)	2.6-g sample, 20 min, 400 W	96–103 compared with Soxhlet	<5 for 12 of 16 compounds	82
Semivolatiles, PCBs, OCPs, OPPs	Freshly spiked soils	Closed	30 mL of acetone–hexane (1:1)	5-g sample, 115°C, 10 min	80–120 for 152 of 187 compounds, 7% higher than Soxhlet and sonication	1–39	78
OCPs	Spiked and natural sediments	Closed	30 mL of terahydrofuran	5-g sample, 100°C, 30 min	74–99	1–10	74

Atrazine, OPPs	Orange peel	Closed	10 mL of acetone–hexane (1:1)	1.5- to 2.5-g sample, 90°C, 9 min	93–101	1–3	84
Triazines	Aged spiked soil	Closed	30 mL of water	1-g sample, 0.5 MPa, 4 min	88–91	6–7	73
Sulfonylurea herbicides	Freshly spiked and aged sandy soils	Closed	20 mL of dichloromethane–methanol (9:1)	10-g sample, 60°C, 100 psi, 10 min	70–100	1–10	79
Phenylurea herbicides	Freshly Spiked soils (FSS) and aged soils (AS)	Closed	20 mL of dichloromethane–methanol (9:1)	5-g sample, 10% water, 690 kPa, 70°C, 10 min	80–120 for FSS, 41–113 for AS	<12 for SFS, 1–35 for AS	75
Felodipine, H152/37	Tablets	Closed	10 mL of methanol–acetonitrile (5:95)	Whole tablet, 80°C, 10 min	99–100	2–5	76
Oligomers	Poly(ethylene-terephthalate) (PET)	Closed	40 mL of dichloro-methane	8-g sample, 120°C, 150 psi, 120 min	94 compared with Soxhlet	5	80

Table 3.15. Advantages and Disadvantages of Various Extraction Techniques

Technique	Advantages	Disadvantages
Soxhlet extraction	Not matrix dependent Very inexpensive equipment Unattended operation Rugged, benchmark method Filtration not required	Slow extraction (up to 24–48 hrs) Large amount of solvent (300–500 mL) Mandatory evaporation of extract
Automated Soxhlet extraction	Not matrix dependent Inexpensive equipment Less solvent (50 mL) Evaporation integrated Filtration not required	Relatively slow extraction (2 hours)
Ultrasonic extraction	Not matrix dependent Relatively inexpensive equipment Fast extraction (10–45 min) Large amount of sample (2–30 g)	Large amount of solvent (100–300 mL) Mandatory evaporation of extract Extraction efficiency not as high Labor intensive Filtration required
Supercritical fluid extraction (SFE)	Fast extraction (30–75 min) Minimal solvent use (5–10 mL) CO_2 is nontoxic, nonflammable, environmentally friendly Controlled selectivity Filtration not required Evaporation not needed	Matrix dependent Small sample size (2–10 g) Expensive equipment Limited applicability
Accelerated solvent extraction (ASE)	Fast extraction (12–18 min) Small amount of solvent (15–40 mL) Large amount of sample (up to 100 g) Automated Easy to use Filtration not required	Expensive equipment Cleanup necessary
Microwave-assisted extraction (MAE)	Fast extraction (20–30 min) High sample throughput Small amount of solvent (30 mL) Large amount of sample (2–20 g)	Polar solvents needed Cleanup mandatory Filtration required Moderately expensive equipment Degradation and chemical reaction possible

Table 3.16. Number of Compounds in Different Recovery Ranges Obtained by Various Extraction Techniques

	Recovery			
Technique	>80%	50–79%	29–49%	<19%
Sonication	63	25	4	2
MAE	51	33	8	2
Soxhlet	50	32	8	4
SFE	37	37	12	8

Example 2

A study compared ASE and SFE to Soxhlet and sonication in the determination of long-chain trialkylamines (TAMs) in marine sediments and primary sewage sludge [89]. The recoveries of these compounds by SFE at 50°C and 30 MPa with CO_2 (modified dynamically with methanol or statically with triethylamine) were 10 to 77% higher than those by Soxhlet or sonication with dichloromethane–methanol (2:1). ASE at 150°C and 17 MPa with the same solvent mixture as Soxhlet showed the highest extraction efficiency among the extraction methods evaluated. SFE exhibited the best precision because no cleanup was needed, whereas Soxhlet, sonication, and ASE extracts required an alumina column cleanup prior to analysis. SFE and ASE used less solvent and reduced the extraction time by a factor of 3 and a factor of 20 compared to sonication and Soxhlet, respectively.

Example 3

Heemken et al. [90] compared ASE and SFE with Soxhlet, sonication, and methanolic saponificaion extraction (MSE) for the extraction of PAHs, aliphatic and chlorinated hydrocarbons from a certified marine sediment samples, and four suspended particulate matter (SPM) samples. Average PAH recovery in three different samples using SFE was between 96 and 105% of that by Soxhlet, sonication, and MSE; for ASE the recovery was between 97 and 108%. Compared to the certified values of sediment HS-6, the average recoveries of SFE and ASE were 87 and 88%; for most compounds the results were within the limits of confidence. For alkanes, SFE recovery was between 93 and 115%, and ASE recovery was between 94 and 107% of that by Soxhlet, sonication, and MSE. While the natural water content of the SPM sample (56%) led to insufficient recovery by ASE and SFE, quantitative extractions were achieved in SFE after addition of anhydrous sodium sulfate to the sample.

Example 4

Llompart et al. [91] compared SFE and MAE with the EPA sonication protocol, for the extraction of phenolic compounds (phenol, *o*-cresol, *m*-cresol, and *p*-cresol) from soil. The samples were five artificially spiked soil matrices with carbon content ranging from 2 to 10%, and a real phenol-contaminated soil with a high carbon content (18%). The extracts from SFE and MAE were analyzed directly by a gas chromatography/mass spectrometry method without cleanup or preconcentration. These two methods showed no significant difference in precision, with RSDs in the range 3 to 15%. They were more efficient than sonication, with at least twice the recovery in both spiked and real soil samples. MAE showed the best recoveries (>80%) for the five spiked matrixes, except for *o*-cresol in soils with carbon content higher than 5%. Although SFE provided satisfactory recovery from low-carbon (<5%) soils, recoveries were low in more adsorptive (high-carbon-content) soils. Extraction efficiency improved significantly when a derivatization step was combined to SFE. However, in the real soil samples, the recoveries achieved by both SFE and MAE derivatization were lower than those by SFE and MAE without derivatization.

Example 5

Vandenburg et al. [92] compared extraction of additive Irganox 1010 from freeze-ground polypropylene polymer by pressurized fluid extraction (PFE) and MAE with reflux, ultrasonic, shake-flask, and Soxhlet extraction. PFE and MAE were faster than any conventional method with comparable extraction efficiency. The times to reach 90% recovery by PFE using propan-2-ol at 150°C and acetone at 140°C were 5 and 6 minutes, respectively. Reflux with chloroform was found to be the fastest method performed under atmospheric pressure with 90% recovery in 24 minutes. Reflux with cyclohexane–propan-2-ol (1:1) required 38 minutes. Ultrasonic, shake-flask, and Soxhlet extraction required about 80 minutes (90% extraction). The total sample preparation time for PFE was 15 minutes, MAE 28 minutes, and reflux with chloroform was 45 minutes.

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CHAPTER

4

EXTRACTION OF VOLATILE ORGANIC COMPOUNDS FROM SOLIDS AND LIQUIDS

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4.1. VOLATILE ORGANICS AND THEIR ANALYSIS

From an analytical point of view, volatile organic compounds (VOCs) can be defined as organic compounds whose vapor pressures are greater than or equal to 0.1 mmHg at 20°C. For regulatory purposes, VOCs are defined by the U.S. Environmental Protection Agency (EPA) as "any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates, and ammonium carbonate, which participates in atmospheric photochemical reactions" [1]. Many VOCs are environmental pollutants. They are not only toxic but are also important ozone precursors in the formation of smog.

An important feature of VOC analysis is that in most cases the analytes are first transferred to a gas–vapor phase and then analyzed by an instrument. Gas chromatography (GC) is the instrumental method of choice for the separation and analysis of volatile compounds. GC is mature, extremely reliable, and there is a wealth of literature regarding analysis of volatile compounds by GC [2–6]. In general, the analysis of pure volatile compounds is not difficult and can be accomplished via direct injection of the analyte into a gas chromatograph [7,8]. However, the analytical task

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becomes challenging when the analytes of interest are dissolved or sorbed in a complex matrix such as soil, food, cosmetics, polymers, or pharmaceutical raw materials. The challenge is to extract the analytes from this matrix reproducibly, and to accurately determine their mass or concentration. There are several approaches to this, including static headspace extraction (SHE), dynamic headspace extraction (purge and trap), solid-phase microextraction (SPME), membrane extraction, and liquid extraction, possibly combined with large-volume GC injection for enhanced sensitivity. The choice of technique depends on the type of sample matrix, information required (quantitative or qualitative), sensitivity required, need for automation, and budget.

In this chapter, techniques for the extraction of volatile compounds from various matrices are described. Details are provided on the basic theory and applications of each technique with a focus on providing useful information to the analyst working on the analysis of volatile analytes from difficult matrices. Since the analytes are volatile, most of the techniques are geared toward preparation of samples for gas chromatography, although they are appropriate for many instrumental methods. The chapter is heavily referenced and the reader should refer to the appropriate references for more details on a particular technique or application.

4.2. STATIC HEADSPACE EXTRACTION

Static headspace extraction is also known as *equilibrium headspace extraction* or simply as *headspace*. It is one of the most common techniques for the quantitative and qualitative analysis of volatile organic compounds from a variety of matrices. This technique has been available for over 30 years [9], so the instrumentation is both mature and reliable. With the current availability of computer-controlled instrumentation, automated analysis with accurate control of all instrument parameters has become routine. The method of extraction is straightforward: A sample, either solid or liquid, is placed in a headspace autosampler (HSAS) vial, typically 10 or 20 mL, and the volatile analytes diffuse into the headspace of the vial as shown in Figure 4.1. Once the concentration of the analyte in the headspace of the vial reaches equilibrium with the concentration in the sample matrix, a portion of the headspace is swept into a gas chromatograph for analysis. This can be done by either manual injection as shown in Figure 4.1 or by use of an autosampler.

Figure 4.2 shows a typical schematic diagram for a headspace gas chromatographic (HSGC) instrumental setup. Typically, the analyte is

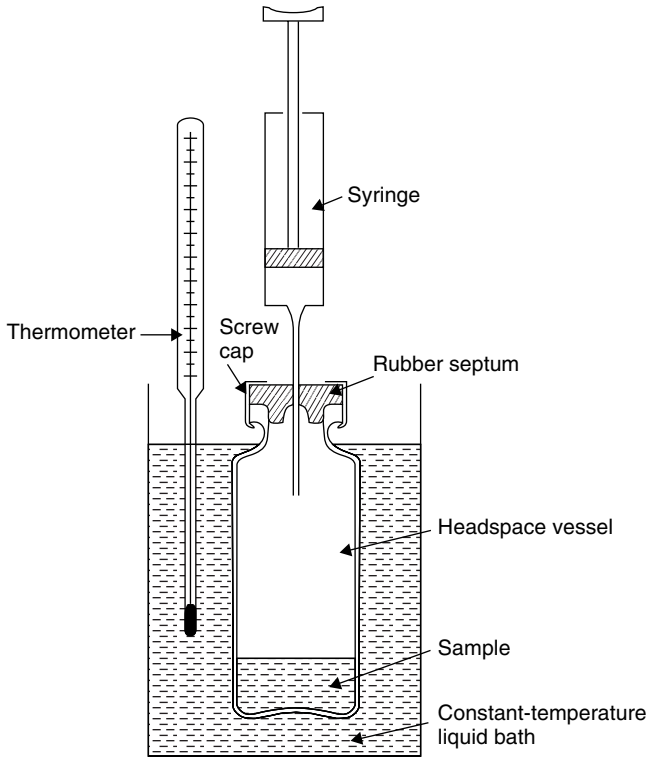


Figure 4.1. Typical static headspace vial, showing the location of the analytical sample and vial headspace. (Reprinted with permission from Ref. 10.)

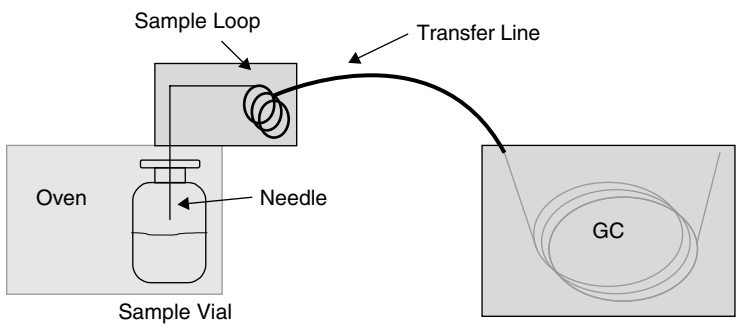


Figure 4.2. Schematic diagram of headspace extraction autosampler and GC instrument.

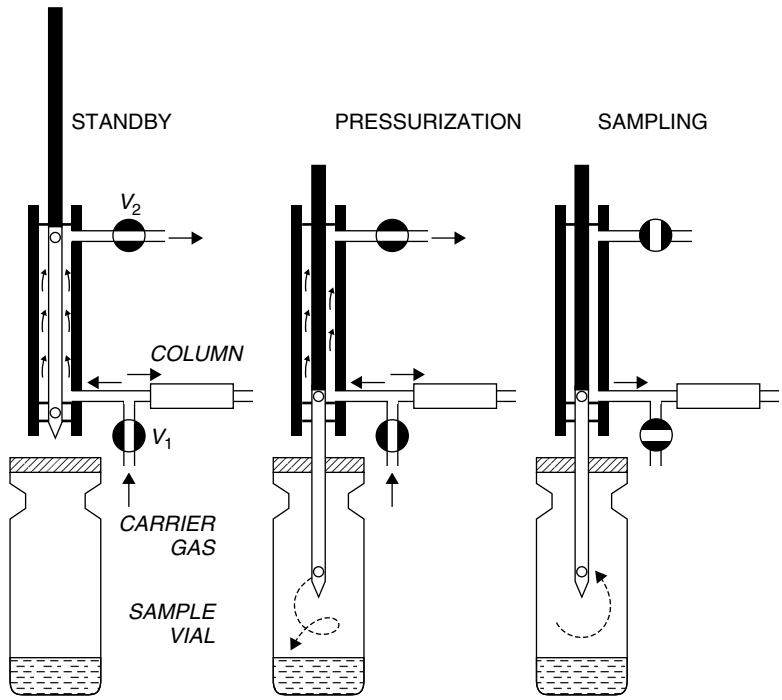


Figure 4.3. Steps for balanced pressure sampling in GC headspace analysis. [Reprinted with permission from Ref. 11 (Fig. 6, p. 208). Copyright John Wiley & Sons.]

introduced as a result of balanced pressure sampling, as demonstrated in Figure 4.3. In this example, the sample vial is brought to a constant temperature and pressure, with both typically being elevated from ambient conditions. Once equilibrium is reached, the vial is connected to the GC column head through a heated transfer line, which is left connected for a given period of time while the sample is transferred to the column by a pressure drop between the vial and the GC inlet pressure. Following this transfer, the vial is again isolated. For automated systems this sampling process can be repeated with the same or the next vial.

4.2.1. Sample Preparation for Static Headspace Extraction

The ease of initial sample preparation is one of the clear advantages of static headspace extraction. Often, for qualitative analysis, the sample can be placed directly into the headspace vial and analyzed with no additional

preparation. However, for quantitation, it may be necessary to understand and optimize the matrix effects to attain good sensitivity and accuracy. For quantitative analysis of volatile compounds from solid particles, equilibrium between the analyte concentration in the headspace and in the sample matrix must be reached in a sensible period of time, typically a matter of minutes. For large solid samples it may be necessary to change the physical state of the sample matrix. Two common approaches are crushing or grinding the sample and dissolving or dispersing the solid into a liquid. The first approach increases the surface area available for the volatile analyte to partition into the headspace. However, the analyte is still partitioning between a solid and the headspace. The second approach is preferred since liquid or solution sample matrices are generally easier to work with than solids since the analyte partitioning process into the headspace usually reaches equilibrium faster. Also, analyte diffusion in liquids eliminates unusual diffusion path problems, which often occur with solids and can unpredictably affect equilibration time.

Solid Sample Matrices

One example of suspending or dissolving a solid in solution is seen in USP method 467, which provides an approach for the analysis of methylene chloride in coated tablets. The sample preparation procedure calls for the disintegration of 1 g of tablets in 20 mL of organic-free water via sonication. The solution is centrifuged after sonication, and 2 mL of the supernatant solution is transferred to a HSAS vial and then analyzed by HSGC [12].

Preparation of Liquids for Static Headspace Extraction

In static headspace extraction, sample preparation for liquid samples is usually quite simple—most often, the sample can just be transferred to the headspace sample vial and sealed immediately following collection of sample to minimize storage and handling losses [13].

4.2.2. Optimizing Static Headspace Extraction Efficiency and Quantitation

There are many factors involved in optimizing static headspace extraction for extraction efficiency, sensitivity, quantitation, and reproducibility. These include vial and sample volume, temperature, pressure, and the form of the matrix itself, as described above. The appropriate choice of physical conditions may be both analyte and matrix dependent, and when there are multiple analytes, compromises may be necessary.

Liquid Sample Matrices

The major factors that control headspace sensitivity are the analyte partition coefficient (K) and phase ratio (β). This was demonstrated by Ettre and Kolb [14]:

$$A \approx C^G = \frac{C^0}{K + \beta} \tag{4.1}$$

where A is the GC peak area for the analyte, C^G the concentration of the analyte in the headspace, C^0 the initial concentration of the analyte in the liquid sample, K the partition coefficient, and β the phase volume ratio. The effect of the parameters K, controlled by the extraction temperature and β, controlled by the relative volume of the two phases, on static headspace extraction analysis sensitivity depends on the solubility of the analyte in the sample matrix. For analytes that have a high partition coefficient, temperature will have a greater influence than the phase ratio. This is because the majority of the analyte stays in the liquid phase, and heating the vial drives the volatile into the headspace. For volatile analytes with a low partition coefficient, the opposite will be true. The volumes of sample and headspace have a greater influence on sensitivity than does the temperature. Essentially, the majority of the volatile analyte is already in the headspace of the vial and there is little analyte left to drive out of the liquid matrix. This is illustrated in Figure 4.4, where a plot of detector response versus temperature for a headspace analysis shows that in an aqueous matrix, increasing the temperature increases the area counts for polar analytes, while the area for nonpolar analytes remains essentially the same [15].

The influence of analyte solubility in an aqueous matrix can also be seen in Figure 4.5, where the influence of sample volume is presented. For a polar analyte in an aqueous matrix, the sample volume will have minimal effect on the area response and a dramatic effect on less polar analytes. The example presented in Figure 4.5 shows the effect of increasing the sample volume from 1 (*a*) to 5 (*b*) mL on area response for analytes cyclohexane and 1,4-dioxane [15]. Salt may also be added to both direct immersion and headspace SPME (discussed later) samples to increase extraction recovery by the classical "salting-out" effect. This effect is demonstrated in Figure 4.5(*b*) and (*c*). Typically, sodium chloride is added to generate a salt concentration of over 1 M. When examining Figure 4.5, one must remember that the concentration of the analytes has not changed, only the volume in the sample and the amount of salt added. Adding salt results in an increase in peak area of 1,4-dioxane (peak 2) and no change in cyclohexane (peak 1). Meanwhile, the result of changing sample volume is an increase in the

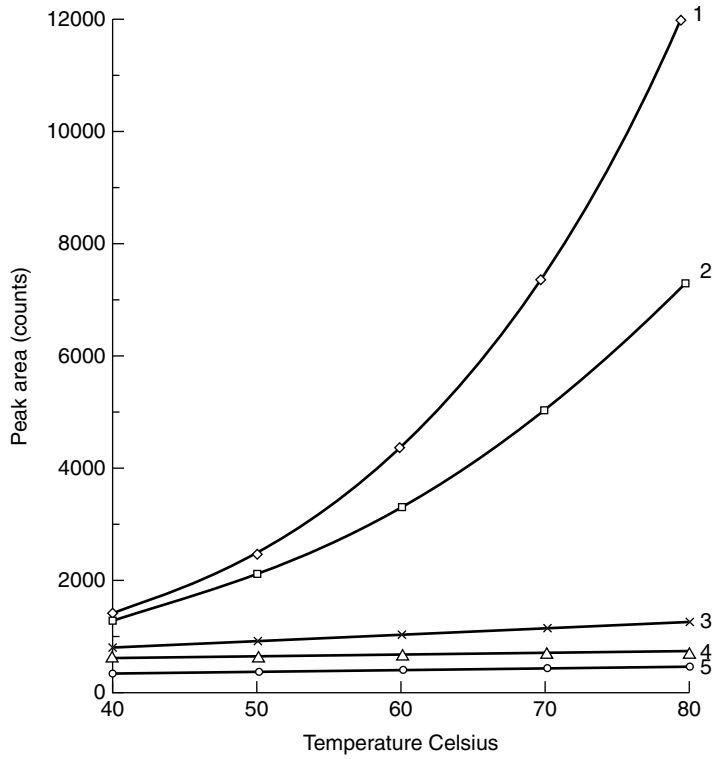


Figure 4.4. Influence of temperature on headspace sensitivity (peak area values, counts) as a function of the partition coefficient K from an aqueous solution with $\beta = 3.46$. The volatiles plotted above are ethanol (1), methyl ethyl ketone (2), toluene (3), *n*-hexane (4), and tetrachloroethylene (5). [Reprinted with permission from Ref. 15 (p. 26). Copyright John Wiley & Sons.]

area for cyclohexane (peak 1) and no change in 1,4-dioxane (peak 2). For an analyte with a large partition coefficient, the impact of β is insignificant on the area. For example, ethanol has a K value around 1000. For a 10-mL headspace vial filled with 1 or 5 mL of the analyte solution, $C_G = C_0/(1000 + 9)$ or $C_G = C_0/(1000 + 1)$, respectively. The difference in the results of these two calculations will be negligible. One can also see that for analytes where K is small, the effect of β will be significant. This phenomenon is extremely useful for the development chemist when method robustness is more important than sensitivity for a quantitative method. By choosing a matrix solvent that has a high affinity for the volatile analytes, problems with sample and standard transfer from volumetric flasks to the headspace vials are eliminated. Also, in the event that a second analysis of

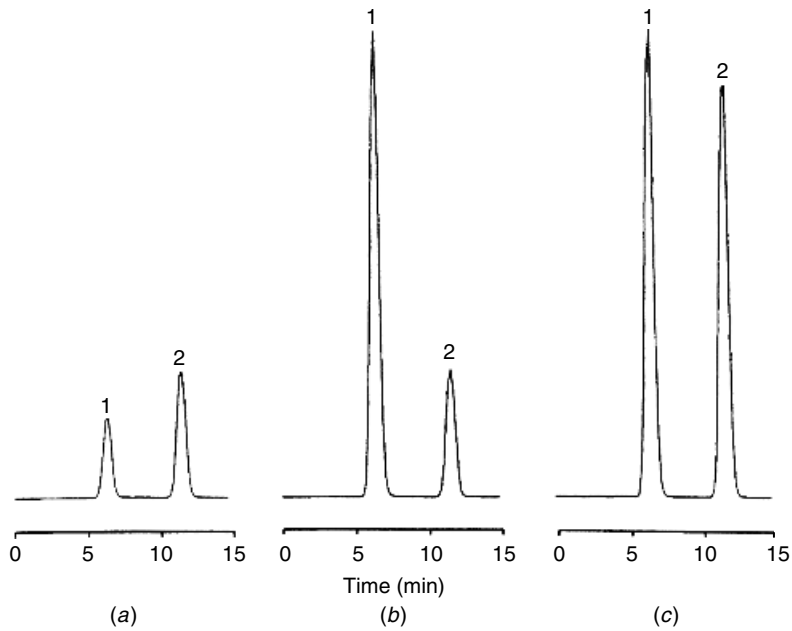


Figure 4.5. Analysis of three samples of an aqueous solution of cyclohexane (0.002 vol %) and 1,4-dioxane (0.1 vol %) in a 22.3-mL vial: (*a*) 1.0 mL of solution ($\beta = 21.3$); (*b*) 5.0 mL of solution ($\beta = 3.46$); (*c*) 5.0 mL of solution ($\beta = 3.46$ to which 2 g of NaCl was added. Headspace conditions: equilibration at 60°C, with shaker. Peaks: 1, cyclohexane; 2, 1,4-dioxane. [Reprinted with permission from Ref. 15 (p. 30). Copyright John Wiley & Sons.]

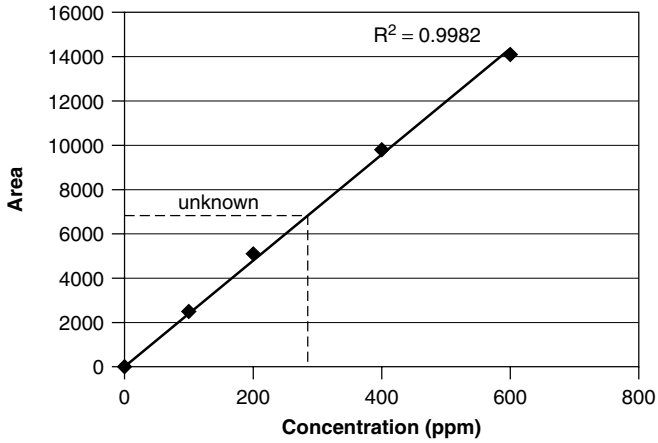
the analytes in the headspace vial is necessary, the drop in signal from the first to the second injection will be minimal. To determine the impact of β when K values are not readily available, simply prepare the analytes in the desired matrix (aqueous or organic) and determine the area counts versus sample volume.

4.2.3. Quantitative Techniques in Static Headspace Extraction

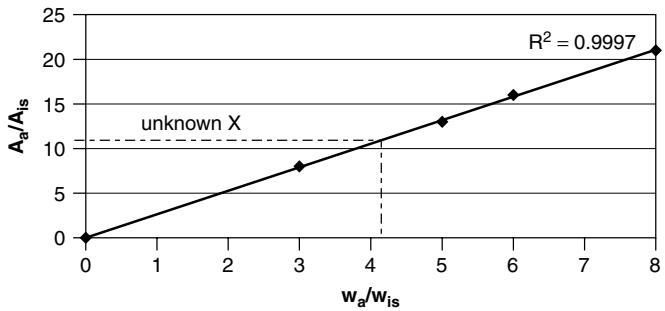
The four most common approaches to quantitative HSGC calibration are classical external standard, internal standard, standard addition, and multiple headspace extraction (MHE). The choice of technique depends on the type of sample being analyzed.

External Standard Calibration

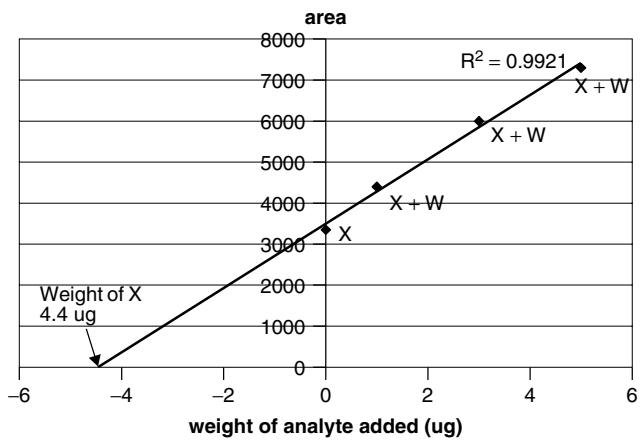
External standard quantitation involves the preparation of a classical calibration curve, as shown in Figure 4.6*a*. Standard samples are prepared at various concentrations over the desired range and analyzed. A calibration



(*a*)



(*b*)



(*c*)

Figure 4.6. Types of calibration curves: (*a*) external standard; (*b*) internal standard; (*c*) standard addition.

curve is then generated, with raw GC peak area plotted versus standard concentration. Peak areas of each analyte are then determined and compared to the curve to generate analyte concentration. This method is best for analytes in liquid samples where the analytes are soluble in the sample matrix and the matrix has no effect on the analyte response. If the analyte has a low solubility in the sample matrix, preparation of standards via serial dilution can be difficult. It is important to match the standard and sample matrix as closely as possible and to demonstrate equivalence in the response between the standards and samples. For solid samples, dissolving or dispersing in a liquid and demonstrating equivalence between standards and samples is preferred to matrix matching, since this simplifies standard preparation. The main difficulty with external standard calibration is that is does not compensate for any variability due to the GC injection or due to variation in the analyte matrix.

Internal Standard Calibration

Internal standard calibration can be used to compensate for variation in analyte recovery and absolute peak areas due to matrix effects and GC injection variability. Prior to the extraction, a known quantity of a known additional analyte is added to each sample and standard. This compound is called an *internal standard*. To prepare a calibration curve, shown in Figure 4.6*b*, the standards containing the internal standard are chromatographed. The peak areas of the analyte and internal standard are recorded. The ratio of areas of analyte to internal standard is plotted versus the concentrations of the known standards. For the analytes, this ratio is calculated and the actual analyte concentration is determined from the calibration graph.

Although internal standard calibration compensates for some errors in external standard quantitation, there are several difficulties in method development. First, choosing an appropriate internal standard can often be difficult, as this compound must be available in extremely pure form and it must never appear in the samples of interest. Second, it cannot interfere in either the extraction or the chromatography of the analytes. Finally, it must be structurally similar to the analytes, so that it undergoes similar extraction and chromatography, otherwise, the compensation will be lost.

Standard Addition

In standard addition calibration, an additional known quantity of the analyte is added directly to the samples, following an initial analysis. By adding one or more aliquots of standard, a calibration curve can be prepared.

The concentration of analyte in the sample can then be determined by extrapolating the calibration curve, as shown in Figure 4.6*c*. For this method, analyte response must be linear throughout the range of concentrations used in the calibration curve. A practical approach to standard addition is to divide up the sample into several equal portions, then add increasing levels of standard. The samples are analyzed and area response versus the final concentration is plotted. The final concentration of the standard is the concentration of the standard after it is added to the sample. The original concentration is then determined by extrapolation to the *x*-axis. Alternatively, a single additional sample can be prepared and the original concentration the analyte can be determined from the following equation:

$$\frac{\text{original concentration of analyte}}{\text{final concentration of analyte (sample + standard)}} = \frac{\text{area from original sample}}{\text{area from (sample + standard)}} \tag{4.2}$$

To calculate the original concentration of the sample using Figure 4.6*c*, the final (diluted) concentration of the sample is expressed in terms of the initial concentration of the sample. Then the initial concentration of the sample is determined [16]. It is important to remember that the sample and the standard are the same chemical compound.

Multiple Headspace Extraction

Multiple headspace extraction (MHE) is used to find the total peak area of an analyte in an exhaustive headspace extraction, which allows the analyst to determine the total amount of analyte present in the sample. This technique, along with the mathematical models behind it, was originally presented by McAuliffe [17] and Suzuki et al. [18]. Kolb and Ettre have an in-depth presentation of the mathematics of MHE in their book [15], and the reader is encouraged to reference that work for further information on the mathematical model.

The advantage to MHE is that sample matrix effects (which are mainly an issue only with solid samples) are eliminated since the entire amount of analyte is examined. This examination is done by performing consecutive analyses on the same sample vial. With the removal of each sample aliquot from the vial, the partition coefficient K will remain constant; however, the total amount of analyte remaining in the sample will decline as each analysis is performed and more of the analyte is driven up into the vial headspace for removal and analysis. Chromatograms of each injection of sample show

declining peak areas as the amount of analyte declines in the sample, and when the peak area eventually falls to zero, one knows that the amount of analyte in the sample has been completely exhausted.

The process described above is, however, not in common practice. MHE has been simplified through laboratory use, and in practice, a limited number of consecutive extractions, usually three to four [15], are taken. Then a linear regression analysis is used to determine mathematically the total amount of analyte present in the sample.

4.3. DYNAMIC HEADSPACE EXTRACTION OR PURGE AND TRAP

For the analysis of trace quantities of analytes, or where an exhaustive extraction of the analytes is required, *purge and trap*, or *dynamic headspace extraction*, is preferred over static headspace extraction. Like static headspace sampling, purge and trap relies on the volatility of the analytes to achieve extraction from the matrix. However, the volatile analytes do not equilibrate between the gas phase and matrix. Instead, they are removed from the sample continuously by a flowing gas. This provides a concentration gradient, which aids in the exhaustive extraction of the analytes.

Purge and trap is used for both solid and liquid samples, which include environmental (water and soil) [19–21], biological [21,22] industrial, pharmaceutical, and agricultural samples. This technique is used in many standard methods approved by the EPA [23–25]. Figure 4.7 shows a chromatogram obtained using a purge and trap procedure described in EPA method 524.2 [26]. The detection limits suggested by the EPA are listed in Table 4.1 [23]. Quantitation is easily performed by external standard calibration.

4.3.1. Instrumentation

Figure 4.8 shows a schematic diagram of a typical purge and trap system [27]. It consists of a purge vessel, a sorbent trap, a six-port valve, and transfer lines. The water sample is placed in the purge vessel. A purge gas (typically, helium) passes through the sample continuously, sweeping the volatile organics to the trap, where they are retained by the sorbents. Once the purging is complete, the trap is heated to desorb the analytes into the GC for analysis.

Three types of purge vessels are most prevalent: frit spargers, fritless spargers, and needle spargers. Frit spargers create uniformed fine bubbles with large surface area that facilitate mass transfer (Figure 4.8*a*). However, these spargers can be used only for relatively clean water samples, not for complex samples that may foam or have particles that can clog the frits.

1. Dichlorodifluoromethane
2. Chloromethane
3. Vinyl chloride
4. Bromomethane
5. Chloroethane
6. Trichlorofluoromethane
7. 1,1-Dichloroethylene
8. Methylene chloride
9. trans-1,2-Dichloroethylene
10. 1,1-Dichloroethane
11. 2,2-Dichloropropane
12. cis-1,2-Dichloroethylene
13. Chloroform
14. Bromochloromethane
15. 1,1,1-Trichloroethane
16. 1,1-Dichloropropene
17. Carbon tetrachloride
18. 1,2-Dichloroethane
19. Benzene
20. Trichloroethylene
21. 1,2-Dichloropropane
22. Bromodichloromethane
23. Dibromomethane
24. cis-1,3-Dichloropropene
25. Toluene
26. trans-1,3-Dichloropropene
27. 1,1,2-Trichloroethane
28. 1,3-Dichloropropane
29. Tetrachloroethylene
30. Dibromochloromethane
31. 1,2-Dibromoethane
32. Chlorobenzene
33. 1,1,1,2-Tetrachloroethane
34. Ethylbenzene
35. m-Xylene
36. p-Xylene
37. o-Xylene
38. Styrene
39. Isopropylbenzene
40. Bromoform
41. 1,1,2,2-Tetrachloroethane
42. 1,2,3-Trichloropropane
43. n-Propylbenzene
44. Bromobenzene
45. 1,3,5-Trimethylbenzene
46. 2-Chlorotoluene
47. 4-Chlorotoluene
48. tert-Butylbenzene
49. 1,2,4-Trimethylbenzene
50. sec-Butylbenzene
51. p-Isopropyltoluene
52. 1,3-Dichlorobenzene
53. 1,4-Dichlorobenzene
54. n-Butylbenzene
55. 1,2-Dichlorobenzene
56. 1,2-Dibromo-3-chloropropane
57. 1,2,4-Trichlorobenzene
58. Hexachlorobutadiene
59. Napthalene
60. 1,2,3-Trichlorobenzene

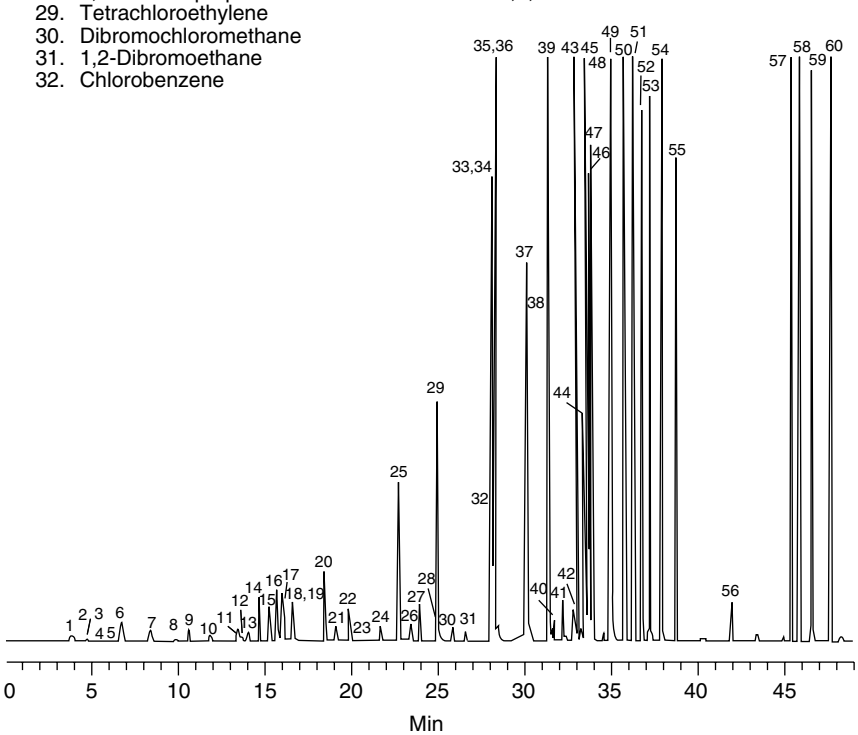


Figure 4.7. Chromatogram obtained using a purge-and-trap procedure as described in EPA method 524.2. (Reproduced from Ref. 26, with permission from Supelco Inc.)

Table 4.1. Detection Limits of the Volatile Organics in EPA Method 524.2[a]

Analyte	MDL (μg/L)	Analyte	MDL (μg/L)
Benzene	0.04	1,3-Dichloropropane	0.04
Bromobenzene	0.03	2,2-Dichloropropane	0.35
Bromochlorobenzene	0.04	1,1-Dichloropropane	0.10
Bromodichlorobenzene	0.08	*cis*-1,2-Dichloropropene	N/A
Bromoform	0.12	*trans*-1,2-Dichloropropene	N/A
Bromomethane	0.11	Ethylbenzene	0.06
n-Butylbenzene	0.11	Hexachlorobutadiene	0.11
sec-Butylbenzene	0.13	Isopropylbenzene	0.15
tert-Butylbenzene	0.14	4-Isopropyltoluene	0.12
Carbon tetrachloride	0.21	Methylene chloride	0.03
Chlorobenzene	0.04	Naphthalene	0.04
Chloroethane	0.10	*n*-Propylbenzene	0.04
Chloroform	0.03	Styrene	0.04
Chloromethane	0.13	1,1,1,2-Tetrachloroethane	0.05
2-Chlorotoluene	0.04	1,1,2,2-Tetrachloroethane	0.04
4-Chlorotoluene	0.06	Tetrachloroethene	0.14
Dibromochloromethane	0.05	Toluene	0.11
1,2-Dibromo-3-chloropropane	0.26	1,2,3-Trichlorobenzene	0.03
1,2-Dibromoethane	0.06	1,2,4-Trichlorobenzene	0.04
Dibromoethane	0.24	1,1,1-Trichloroethane	0.08
1,2-Dichlorobenzene	0.03	1,1,2-Trichloroethane	0.10
1,3-Dichlorobenzene	0.12	Trichloroethene	0.19
1,4-Dichlorobenzene	0.03	Trichlorofluoromethane	0.08
Dichlorodifluoromethane	0.10	1,2,3-Trichloropropane	0.32
1,1-Dichloroethane	0.04	1,2,4-Trimethylbenzene	0.13
1,2-Dichloroethane	0.06	1,3,5-Trimethylbenzene	0.05
1,1-Dichloroethene	0.12	Vinyl chloride	0.17
cis-1,2-Dichloroethene	0.12	*o*-Xylene	0.11
trans-1,2-Dichloroethene	0.06	*m*-Xylene	0.05
1,2-Dichloropropane	0.04	*p*-Xylene	0.13

[a]This method uses purge and trap with GC-MS (with a wide-bore capillary column, a jet separator interface, and a quadrupole mass spectrometer).

Fritless spargers and needle spargers (Figure 4.8*b*) are recommended for these samples, which include soils, slurries, foaming liquids, polymers, pharmaceuticals, and foods. The purging is less efficient, but clogging and foaming problems are eliminated. The most common sizes of the purge vessel are 25 and 5 mL.

In general, the trap should do the following: retain the analytes of interest, not introduce impurities, and allow rapid injection of analytes into the

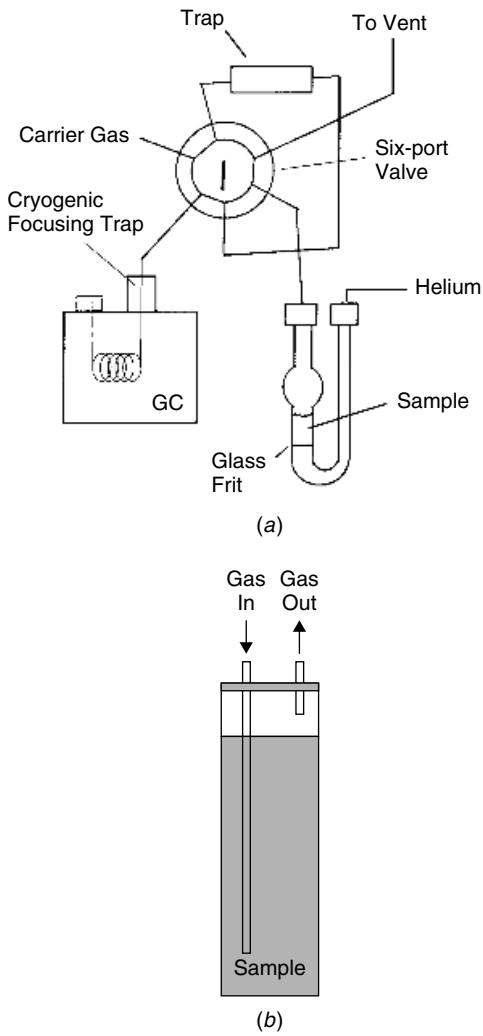


Figure 4.8. (*a*) Schematic diagram of a typical purge and trap–GC system. (Reprinted with permission from Nelson Thornes, Ref. 27.) (*b*) Needle sparger for purge and trap.

column. The trap is usually a stainless steel tube 3 mm in inside diameter (ID) and 25 mm long packed with multiple layers of adsorbents, as shown in Figure 4.9. The sorbents are arranged in layers in increasing trapping capacity. During purging/sorption, the purge gas reaches the weaker sorbent first, which retains only less volatile species. More volatile species break through this layer and are trapped by the stronger adsorbents. During

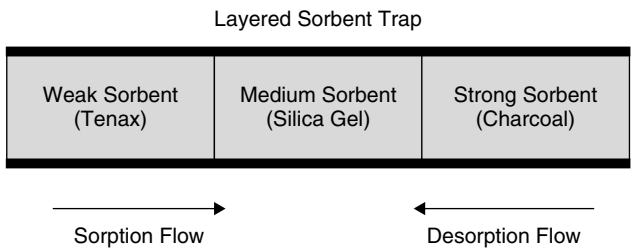


Figure 4.9. Schematic diagram of a multilayer sorbent trap.

desorption, the trap is heated and back-flushed with the GC carrier gas. In this way, the less volatile compounds never come in contact with the stronger adsorbents, so that irreversible adsorption is avoided.

The materials commonly used for trapping volatile organics include Tenax, silica gel, activated charcoal, graphitized carbon black (GCB or Carbopack), carbon molecular sieves (Carbosieve), and Vocarb. Tenax is a porous polymer resin based on 2,6-diphenylene oxide. It is hydrophobic and has a low affinity for water. However, highly volatile compounds and polar compounds are poorly retained on Tenax. To avoid decomposition, Tenax should not be heated to temperatures above 200°C. There are two grades of Tenax: Tenax TA and Tenax GC. The former is of higher purity and is preferred for trace analysis. Silica gel is a stronger sorbent than Tenax. It is hydrophilic and therefore an excellent material for trapping polar compounds. However, water is also retained. Charcoal is another sorbent that is stronger than Tenax. It is hydrophobic and is used mainly to trap very volatile compounds (such as dichlorodifluromethane, a.k.a Freon 12) that can break through Tenax and silica gel. Conventional traps usually contain Tenax, silica gel, and charcoal in series. If the boiling points of the analytes are above 35°C, Tenax itself will suffice, and silica gel and charcoal can be eliminated. Graphitized carbon black (GCB) is hydrophobic and has about the same trapping capacity as Tenax. It is often used along with carbomolecular sieves, which serve as an alternative to silica gel and charcoal for trapping highly volatile species. Vocarb is an activated carbon that is very hydrophobic. It minimizes water trapping and can be dry purged quickly. Vocarb is often used with an ion-trap mass spectrometer, which can be affected by trace levels of water or methanol. GCB, carbon molecular sieves, and Vocarb have high thermal stability and can be operated at higher desorption temperatures than traps containing Tenax.

The transfer line between the trap and the GC is made of nickel, deactivated fused silica, or silica-lined stainless steel tubing. Active sites that can interact with the anlaytes are eliminated on these inert materials. The line is

maintained at a temperature higher than 100°C to avoid the condensation of water and volatile organics. The six-port valve that controls the gas flow path is also heated above 100°C to avoid condensation.

4.3.2. Operational Procedures in Purge and Trap

A purge and trap cycle consists of several steps: purge, dry purge, desorb preheat, desorb, and trap bake. Each step is synchronized with the operation of the six-port valve and the GC [or GC-MS (mass spectrometer)]. First, a sample is introduced into the purge vessel. Then the valve is set to the purge position such that the purge gas bubbles through the sample, passes through the trap, and then is vented to the atmosphere. During purge, dry purge, and preheat, the desorb (carrier) gas directly enters the GC. Typically, the purge time is 10 to 15 minutes, and the helium flow rate is 40 mL/min. The trap is at the ambient temperature. After purging, the purge gas is directed into the trap without going through the sample, called *dry purge*. The purpose of dry purging is to remove the water that has accumulated on the trap. Dry purge usually takes 1 to 2 minutes. Then the purge gas is turned off, and the trap is heated to about 5 to 10°C below the desorption temperature. Preheat makes the subsequent desorption faster. Once the preheat temperature is reached, the six-port valve is rotated to the desorb position to initiate the desorption step. The trap is heated to 180 to 250°C and back-flushed with the GC carrier gas. Desorption time is about 1 to 4 minutes. The flow rate of the desorb gas should be selected in accordance with the type of GC column used. After desorption, the valve is returned back to the purge position. The trap is reconditioned/baked at (or 15°C above) the desorption temperature for 7 to 10 minutes. The purpose of trap baking is to remove possible contamination and eliminate sample carryover. After baking, the trap is cooled, and the next sample can be analyzed. The operational parameters (temperature, time, flow rate, etc.) in each step should be the same for all the samples and calibration standards.

4.3.3. Interfacing Purge and Trap with GC

The operational conditions of the purge and trap must be compatible with the configuration of the GC system. A high carrier gas (desorb gas) flow rate can be used with a packed GC column. The trap desorption time is short at the high flow rate, producing a narrowband injection. The optimum flow is about 50 mL/min. Capillary columns are generally preferred over packed columns for better resolution, but these columns require lower flow rate.

Megabore capillary columns (0.53 mm ID or larger) are typically used at a flow rate of 8 to 15 mL/min. Desorption is slower at such flow rates, and

the column is often cooled to subambient temperature (typically, 10°C or lower) at the beginning of the GC run to retain the highly volatile species. Sub-ambient cooling may be avoided by using a long (60- to 105-m) column with a thick-film stationary phase (3 to 5 μm). Nevertheless, this flow rate is still too high for a GC-MS. A jet separator or an open split interface can be used at the GC/MS interface to reduce the flow into the MS. However, an open split interface decreases the analytical sensitivity because only a portion of the analytes enters the detector.

Narrow-bore capillary columns (0.32 mm ID or smaller) with MS detector are typically operated at a lower flow rate (less than 5 mL/min). There are two ways to couple purge and trap with this type of column. One is to desorb the trap at a high flow rate and then split the flow into the GC using a split injector. A fast injection is attained at the expense of loss in analytical sensitivity. The other approach is to use a low desorb flow rate, which makes desorption time too long for a narrow bandwidth injection. The desorbed analytes need to be refocused on a second trap, usually by cryogenic trapping (Figure 4.8*a*). A cryogenic trap is made of a short piece of uncoated, fused silica capillary tubing. It is cooled to −150°C by liquid nitrogen. After refocusing, the cryogenic trap is heated rapidly to 250°C to desorb the analytes into the GC. Cryogenic trapping requires a dedicated cryogenic module and a liquid-nitrogen Dewar tank.

Without a moisture control device, water can go into the GC from purge and trap. The gas from the purge vessel is saturated with water, which can be collected on the trap and later released into the GC during trap heating. Water reduces column efficiency and causes interference with certain detectors (especially PID and MS), resulting in distorted chromatograms. The column can also be plugged by ice if cryofocusing is used. Therefore, water needs to be removed before entering the GC. Two water management methods are commonly used. One is to have a dry purge step prior to the desorption. However, some hydrophilic sorbents (such as silica gel) are not compatible with dry purging. The other approach is to use a condenser between the trap and the GC. The condenser is made of inert materials such as a piece of nickel tubing. It is maintained at ambient temperature, serving as a cold spot in the heated transfer line. During desorption, water is condensed and removed from the carrier gas. After desorption is complete, the condenser is heated and water vapor is vented.

4.4. SOLID-PHASE MICROEXTRACTION

Solid-phase microextraction (SPME) is a relatively new method of sample introduction, developed by Pawliszyn and co-workers in 1989 [28,29] and

made commercially available in 1993. This technique has already been described in Chapter 2. The additional discussion here pertains mainly to the analysis of volatile organics. SPME is a solventless extraction method that employs a fused silica fiber coated with a thin film of sorbent, to extract volatile analytes from a sample matrix. The fiber is housed within a syringe needle that protects the fiber and allows for easy penetration of sample and GC vial septa. Most published SPME work has been performed with manual devices, although automated systems are also available.

There are two approaches to SPME sampling of volatile organics: direct and headspace. In *direct sampling* the fiber is placed directly into the sample matrix, and in *headspace sampling* the fiber is placed in the headspace of the sample [30,31]. Figure 4.10 illustrates the two main steps in a typical SPME analysis, analyte extraction (adsorption or absorption, depending on the fiber type) and analysis (thermal desorption into a GC inlet). To extract the analytes from a sample vial, the needle containing the fiber is placed in the sample by piercing the septa, the fiber is exposed to the sample matrix (extraction step), retracted into the housing, and removed from the vial. The injection process is similar: Pierce the GC septum with the needle, expose the fiber (desorption step), and then retract the fiber and remove the needle. A high-performance liquid chromatograph interface for SPME is available [32] and SPME has been interfaced to capillary electrophoresis [33] and FT-IR [34]. These have also been described in Chapter 2.

SPME has several advantages in the analysis of volatile organics. First, no additional instruments or hardware are required. Second, the cost of fibers is low compared to the cost of other methods for volatile analyte extraction. Fibers can be reused from several to thousands of times, depending on extraction and desorption conditions. SPME requires minimal training to get started, although there may be many variables involved in a full-method development and validation. SPME is also easily portable, and field sampling devices are readily available. Finally, with a variety of fiber coating chemistries available, SPME can be applied to a wide variety of volatile organic analytes. Table 4.2 shows a list of available SPME fibers, with their usual applications. A complete bibliography of SPME applications has been published by Supelco [35]. SPME has been used to extract volatile organic compounds from a wide variety of sample matrixes, such as air, foods, beverages, pharmaceuticals, natural products, and biological fluids [35].

4.4.1. SPME Method Development for Volatile Organics

The simplest way to begin developing an SPME method is to consult the applications guide provided by Supelco. This allows the analyst to quickly

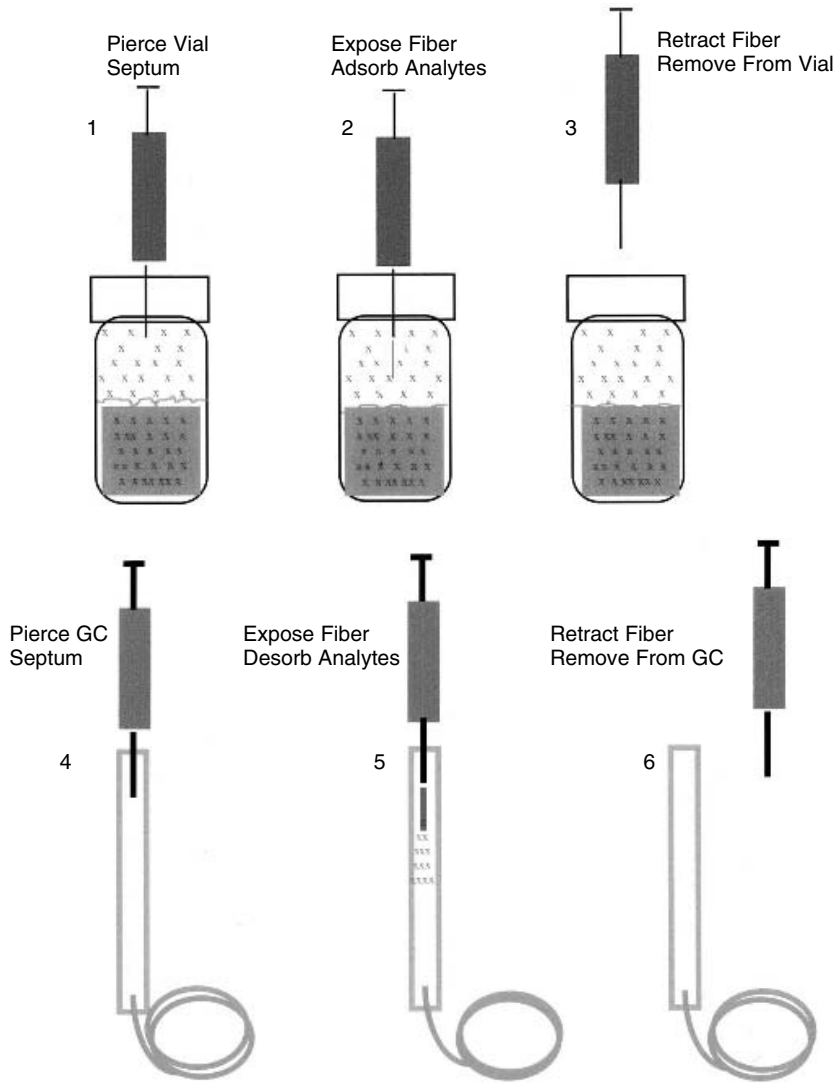


Figure 4.10. Steps in a SPME headspace analysis: 1–3, extraction; 4–6, desorption. (Drawings courtesy of Supelco, Inc.)

Table 4.2. Commercially Available SPME Fibers and Applications

Coating Material	Coating Thickness (μm)	Applications
Polydimethyl siloxane (PDMS)	100	GC/HPLC for volatiles
PDMS	30	GC/HPLC for nonpolar semi-volatiles
PDMS	7	GC/HPLC for nonpolar high-molecular-weight compounds
PDMS/divinylbenzene (PDMS/DVB)	65	GC/HPLC for volatiles, amines, notroaromatics
Polyacrylate (PA)	85	GC/HPLC for polar semivolatiles
Carbowax/divinylbenzene (CW/DVB)	65, 70	GC/HPLC for alcohols and polar compounds
Carboxen/PDMS	75, 85	GC/HPLC for gases and low-molecular-weight compounds
Divinylbenzene/Carboxen	50/30	GC/HPLC for flavor compounds
PDMS/DVB	60	HPLC for amines and polar compounds
Carbowax/templated resin	50	HPLC for surfactants

determine initial extraction and chromatographic conditions for several hundred frequently analyzed compounds from a wide variety of sample matrices [35]. For unique compounds or sample matrices, there are three basic steps to be considered when developing a SPME method analyte extraction, injection into the GC, and chromatographic conditions. A complete list of variables involved in SPME analysis is given in Table 4.3. Not all of these are usually considered by all method developers, but they may become issues in validation, transfer, or troubleshooting. The discussion that follows centers on optimizing the most important variables in SPME extractions of volatile organics and GC analysis.

The optimization of the extraction process, along with SPME extraction theory for both direct and headspace SPME extraction has been described thoroughly by Louch and co-workers [37]. The key issues involved in developing an extraction procedure include: extraction mode (direct or headspace), choice of fiber coating, agitation method, length of extraction, extraction temperature, and matrix modification. Choosing between direct immersion SPME and headspace SPME is relatively straightforward. Direct immersion SPME is warranted for liquid samples or solutions for which other solid-phase or liquid–liquid extraction methods would be considered.

Table 4.3. Variables Involved in Generating Reproducible SPME Results

Extraction	Desorption
Volume of the fiber coating	Geometry of the GC inlet
Physical condition of the fiber coating (cracks, contamination)	GC inlet liner type and volume
Moisture in the needle	Desorption temperature
Extraction temperature	Initial GC column temperature and column dimensions
Sample matrix components (salt, organics, moisture, etc.)	Fiber position in the GC inlet
Agitation type	Contamination of the GC inlet
Sampling time (especially important if equilibrium is not reached)	Stability of GC detector
Sample volume and headspace volume	Carrier gas flow rate
Vial shape	
Time between extraction and analysis	
Adsorption on sampling vessel or components	

Source: Adapted from Ref. 36.

Headspace SPME would be considered for the same analytes as static headspace extraction or purge and trap. Therefore, headspace SPME should be considered for extracting volatile compounds from solid or liquid samples, in which the normal boiling point of the analyte(s) of interest is less than about 200°C. For higher-boiling analytes, direct immersion SPME will probably be necessary. Also, the nature of the sample matrix should be considered. Headspace SPME is preferred for especially complex or dirty samples, as these may foul the fiber coating in a direct immersion analysis. However, SPME fibers have been shown to be usable for about 50 direct immersions into urine [38]. Some laboratories have reported using a fiber for thousands of extractions from drinking water.

4.4.2. Choosing an SPME Fiber Coating

SPME fibers have different coatings for the same reason that GC capillary columns have different coatings: There is no single coating that will extract and separate all volatile organics from a sample, therefore, different types of coatings with different polarities are used on SPME fibers. Currently, three classes of fiber polarity coatings are commercially available: nonpolar, semipolar, and polar coatings [39]. There are several advantages of using different fiber polarities. For one, using a matched-polarity fiber (i.e., polar-coated for a polar analyte) offers enhanced selectivity. Also, there is less of a

chance of extracting interfering compounds along with the analyte of interest, and an organic matrix is not a problem—polar compounds can still be extracted [39].

As shown in Table 4.2, there are several SPME fiber coatings commercially available. These range in polarity from polydimethylsiloxane (PDMS), which is nonpolar, to Carbowax–divinylbenzene (CW-DVB), which is highly polar. The overall application of each is shown in the table. Throughout the literature, about 80% of SPME work is done using PDMS fibers, which are versatile and selective enough to obtain some recovery of most organic compounds from water. In most method development schemes, a PDMS fiber is attempted first, followed by a more polar fiber if necessary. Figure 4.11 provides a graphical scheme for choosing a SPME fiber based on analyte polarity and volatility. The nonpolar fibers are more commonly used for headspace SPME as the majority of volatile analytes tend to be non- or slightly polar. Also, as described below, the fiber coating thickness affects extraction recovery in both direct immersion and headspace SPME. The PDMS fiber is the only one available in more than one thickness.

Fiber coating thickness is a second consideration in selecting a fiber for both direct immersion and headspace SPME. The PDMS coating is avail-

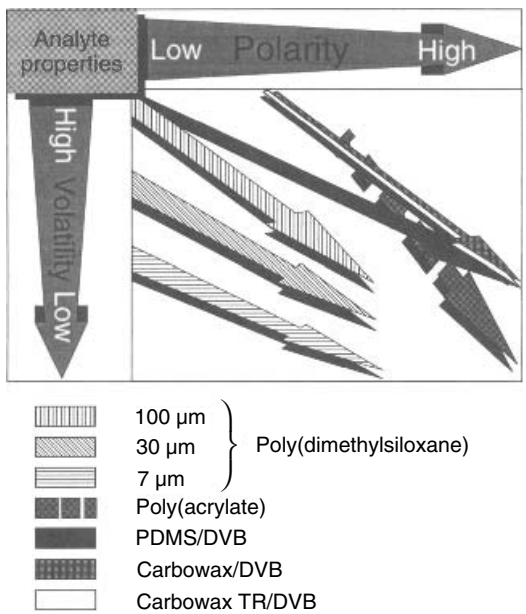


Figure 4.11. Graphical scheme for choosing a SPME fiber coating. [Reprinted with permission from Ref. 36 (Fig. 4.3, p. 99). Copyright John Wiley & Sons.]

able in three thicknesses: 100, 30, and 7 μm. The 100-μm fiber is generally used for highly volatile compounds or when a larger organic extraction volume is needed to improve recovery. Oppositely, the 7-μm-thick fiber is used for less volatile compounds that may present difficulty in thermal desorption in the GC inlet. The 30-μm fiber represents a compromise. For headspace work, the 100-μm fiber is most commonly used, as the larger organic volume enhances partitioning from the headspace.

4.4.3. Optimizing Extraction Conditions

Once the fiber is chosen, extraction conditions must be optimized. As shown in Table 4.3, there are many variables, with extraction time, sample volume, agitation, temperature, and modification of the sample matrix being most important. Extraction time is optimized by extracting a standard using a range of extraction times and plotting the analyte GC peak area versus the extraction time. As extraction time is increased, a plateau in peak area is reached. This represents the time required for the system to reach equilibrium and is the optimized extraction time. This has been presented in detail in Chapter 2. If the extraction time can be controlled carefully, and if sensitivity is adequate, shorter extraction time can be used without fully reaching equilibrium. Due to more rapid kinetics, headspace SPME generally reaches equilibrium faster than does direct immersion SPME. Most SPME headspace extractions are completed in less than 5 minutes, while direct immersion may require more than 30 minutes, although this is highly matrix dependent.

The sample volume also has an effect on both the rate and recovery in SPME extractions, as determined by extraction kinetics and by analyte partition coefficients. The sensitivity of a SPME method is proportional to n, the number of moles of analyte recovered from the sample. As the sample volume (V_s) increases, analyte recovery increases until V_s becomes much larger than the product of K_{fs}, the distribution constant of the analyte, and V_f, the volume of the fiber coating (i.e., analyte recovery stops increasing when $K_{\text{fs}}V_f \ll V_s$) [41]. For this reason, in very dilute samples, larger sample volume results in slower kinetics and higher analyte recovery.

As with any extraction, the agitation method will affect both the extraction time and recovery and should be controlled as closely as is practical. In direct-immersion SPME, agitation is usually accomplished using magnetic stirring, so the stirring rate should be constant. Also, the fiber should not be centered in the vial, as there is little to no liquid velocity there; the fiber should always be off-centered so that liquid is moving quickly around it. Agitation can also be achieved by physical movement of the fiber or by

movement of the sample vial. Sonication is also used. Typically, headspace SPME sample vials are not agitated.

Extraction temperature can also be an important factor, especially in headspace SPME analyses. However, in SPME, unlike in GC headspace analysis, increasing the temperature in SPME can result in a maximum usable temperature for the method (i.e., going from 25°C to 30°C may result in a reduction in sensitivity [42].

The sample matrix may also be modified to enhance extraction recovery. This is typically done by either dissolving a solid sample in a suitable solvent, usually water or a strongly aqueous mixture, or by modifying the pH or salt content of a solution. Modifying the pH to change the extraction behavior works the same way in SPME as it does for classical liquid–liquid extraction. At low pH, acidic compounds will be in the neutral form and will be extracted preferentially into the fiber coating; at high pH, basic compounds are extracted favorably. Neutral compounds are not affected appreciably by solution pH.

4.4.4. Optimizing SPME–GC Injection

The GC injection following SPME is typically performed under splitless conditions. Since no solvent is present, the GC inlet liner does not need to have a large volume to accommodate the sample solvent, so special small-internal-diameter glass liners are often used. Optimizing SPME–GC injections has been discussed in detail by Langenfeld et al. [43] and Okeyo and Snow [44]. The main considerations involve transferring the analytes in the shortest possible time out of the fiber coating, through the inlet and onto the capillary GC column and in focusing the analytes into the sharpest bands possible. Thus, both inlet and chromatographic conditions play roles.

For semivolatile compounds, inlet optimization is very simple. Classical splitless inlet conditions, followed by an initial column temperature cool enough to refocus the analyte peaks following the desorption, work well. Thus, a typical condition would be a temperature of about 250°C, a head pressure sufficient to maintain optimum GC column flow and an initial column temperature at least 100°C below the normal boiling point of the analyte. For semivolatile analytes, a classical splitless inlet liner can be used, as the cool column will refocus these peaks. The desorption time in the inlet must be determined by experimentation, but typically, runs between 1 and 5 minutes.

For volatile analytes, optimizing the inlet is more difficult, as making the initial column temperature low enough to refocus these analytes is often not possible without cryogenics. The inlet must therefore be optimized to pro-

vide the fastest-possible desorption and transfer to the GC column, while the GC column is maintained as cool as possible to achieve any focusing that is possible. First, a low-volume SPME inlet liner should be used in place of the classical splitless liner. Second, a pulsed injection, with the inlet pressure higher than usual during the desorption, should be used to facilitate rapid analyte transfer. With an electronically controlled inlet, the pressure can be returned to the optimum for the GC column following the desorption. Finally, it may be necessary to use a thicker-film GC column to aid in retaining the volatile analytes.

As an example, Figure 4.12 shows the effect of inlet liner diameter on the separation of a hydrocarbon sample. In the first chromatogram, a 0.75-mm-ID liner was used and all of the peaks are sharp. In the second and third chromatograms, 2- and 4-mm liners are used. Significant peak broadening of the early peaks is seen in the 4-mm case especially. Also in the 4-mm case, however, the later eluting peaks are not significantly broadened, indicating that the liner diameter is not important for these compounds.

4.5. LIQUID–LIQUID EXTRACTION WITH LARGE-VOLUME INJECTION

Classical liquid–liquid and liquid–solid extractions are recently receiving additional examination, as new injection techniques for GC have made very simple, low-volume extractions feasible. Recently, several commercial systems for large-volume liquid injections (up to 150 μL all at once, or up to 1 to 2 mL over a short period of time) have become available. When combined with robotic sampling systems, these have become powerful tools in the trace analysis of a variety of sample types. Due to its simplicity, classical liquid–liquid extraction is often the method of choice for sample preparation. Some of the robotic samplers available for this type of analysis, such as the LEAP Technologies Combi-PAL robotic sampler, which has been licensed by several instrument vendors, are also capable of performing automated SPME and SHE.

4.5.1. Large-Volume GC Injection Techniques

The techniques for injecting large volumes into a capillary GC column were developed in the 1970s but not widely commercialized until the 1990s, when electronic control of the GC pneumatics became available. Two methods are used for large-volume injection: solvent vapor exit (SVE), which is based on a classical on-column inlet and programmed temperature vaporization (PTV), which was originally built into a split/splitless inlet. For relatively clean samples, both are capable of satisfactory large-volume injections,

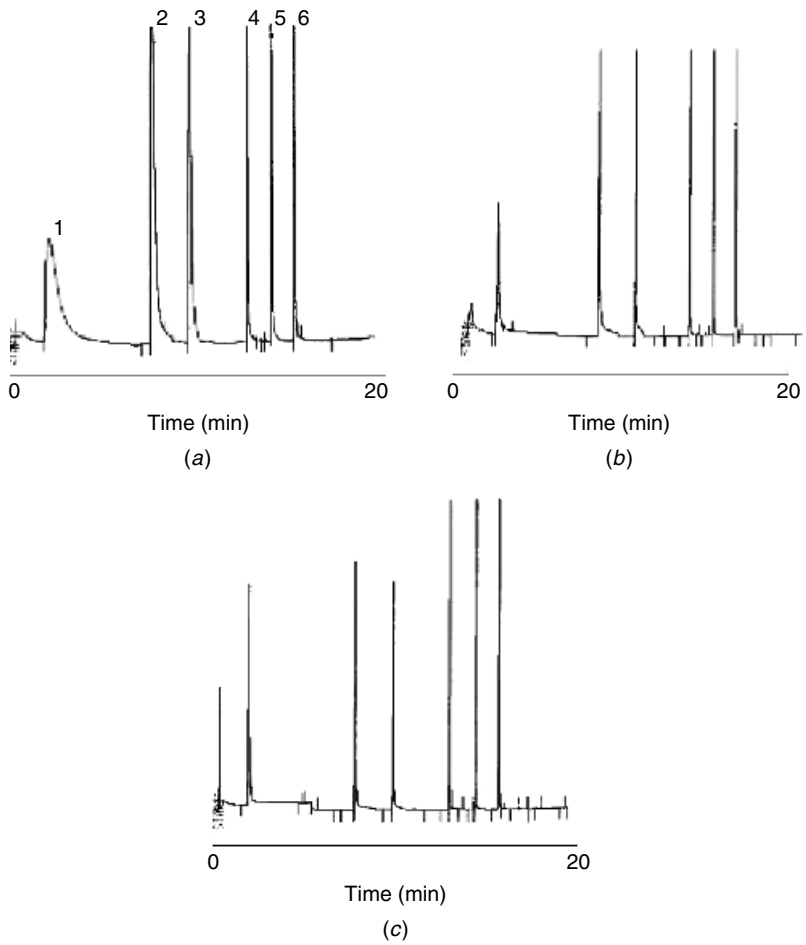


Figure 4.12. Effect of inlet liner diameter on SPME injection of hydrocarbons. (*a*) 4-mm-diameter liner; (*b*) 2-mm-diameter liner; (*c*) 0.75-mm-diameter liner. Analytes: 1, octane; 2, decane; 3, undecane; 4, tridecane; 5, tetradecane; 6, pentadecane. [Reprinted with permission from Ref. 44 (Fig. 3). Copyright Advanstar Communications.]

while for dirty samples, the SVE inlet is prone to fouling. These two inlets are pictured schematically in Figure 4.13.

The SVE configuration begins with a classical cool on-column inlet. A retention gap consisting of a length (usually about 5 m) of uncoated fused silica tubing is connected to the inlet. Following the retention gap is a short length (2 m) of coated analytical column that serves as a retaining pre-

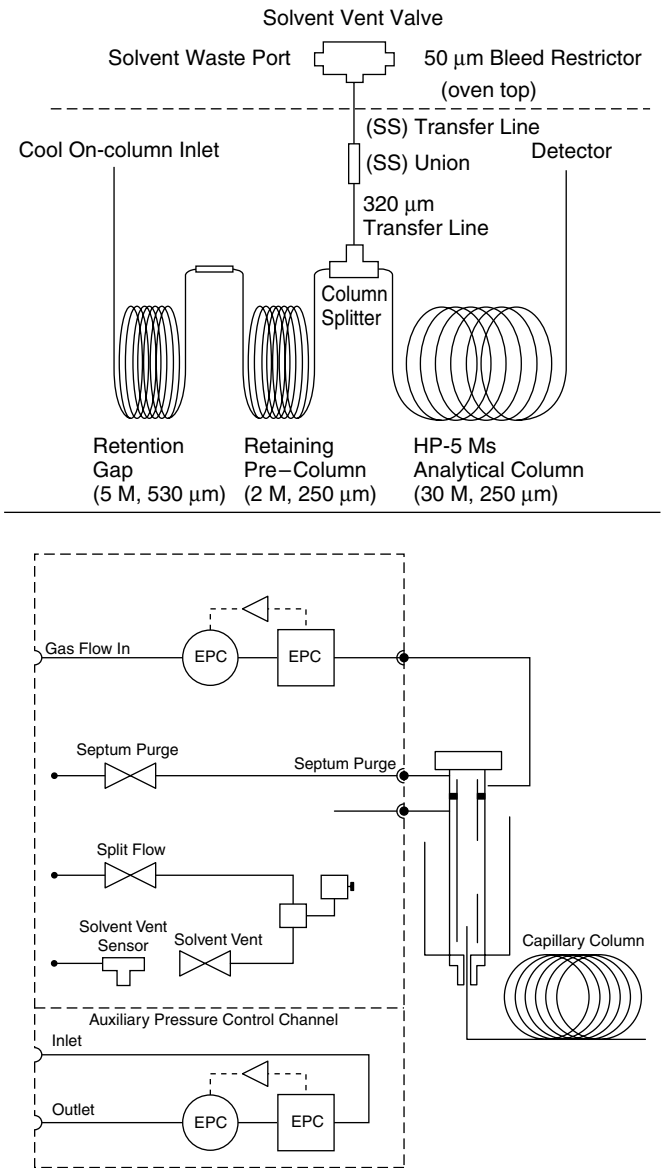


Figure 4.13. Schematic diagrams of large-volume injection systems. Top: on-column configuration with solvent vapor exit. (Drawing courtesy of Agilent Technologies.) Bottom: programmed temperature vaporization configuration. (Drawing courtesy of ATAS, International.)

column. Following the retaining precolumn, the flow is split to the analytical column and to the solvent vapor exit. The solvent vapor exit consists of a transfer line (uncoated tubing) and an electronically controlled solenoid valve that opens and closes. A restrictor is used to maintain a small permanent flow through the vapor exit so that back-flushing of solvent does not occur. Prior to injection, the vapor exit valve is opened and it remains opened during the injection process. Following injection, liquid solvent enters the retention gap, where it is evaporated and ejected through the vapor exit. After evaporation of about 95% of the solvent vapor, with the analytes being retained in the retaining precolumn, the vapor exit is closed and the analytical run is started. This allows the injection of sample amounts of up to 100 μL all at once, or up to several milliliters of sample using a syringe pump. SVE large-volume injection is generally used for relatively ''clean'' samples, such as drinking water or natural water extracts, since as in on-column injection, the entire sample reaches the retention gap, making fouling a common occurrence. Commercial systems generally include software that assists in optimizing the many injection variables.

The PTV large-volume inlet is, essentially, a temperature-programmable version of the classical split/splitless GC inlet. The main design change is that the glass liner within the inlet and the inlet itself is of low thermal mass, so that the temperature can be programmed rapidly. The PTV inlet can operate in several modes, including the classical split and splitless, cold split solvent vent, and hot split solvent vent. In the cold injection modes, the inlet begins at a relatively low temperature, below the normal boiling point of the sample solvent. The sample is injected, usually into a packed glass sleeve within the inlet. The solvent vapor is then vented through the open split vent, while the inlet is cool and the analytes remain behind in the liner. When about 95% of the solvent vapor has exited through the vent, the vent is closed, the inlet is heated rapidly, and the analytes are thermally desorbed into the GC column. This method also allows rapid injection of up to 150 μL of liquid sample, with the benefit that nonvolatile or reactive material will remain in the inlet sleeve rather than in the GC column or retention gap. The analysis of a lake water extract using liquid–liquid extraction followed by PTV injection is shown in Figure 4.14. A thorough and readable manual for PTV large-volume injection that is freely available on the Internet has been written by Janssen and provided by Gerstel [45].

4.5.2. Liquid–Liquid Extraction for Large-Volume Injection

The ability to inject 100 or more microliters of a liquid sample rapidly and automatically into a capillary gas chromatograph necessitates another look at liquid–liquid extraction. Sensitivity of the analysis is a common problem

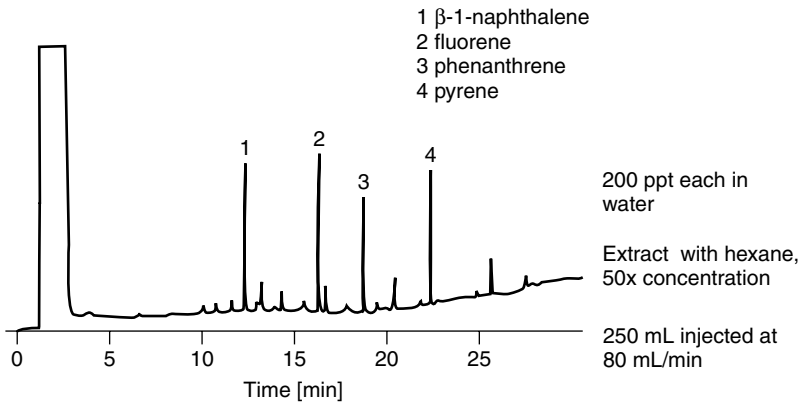


Figure 4.14. Chromatogram of lake water extract analyzed using liquid–liquid extraction with large-volume injection. (Drawing courtesy of ATAS, International.)

with all extraction methods, as sample concentration is often difficult. In SPME, sample concentration occurs automatically. In liquid–liquid extraction, however, an evaporation step is often required, which greatly increases the possibility of contamination and sample losses. For example, in a trace analysis, 1.0 L of water is often extracted with several hundred milliliters of organic solvent, which is then evaporated down to 1 mL prior to classical splitless injection of 1 μL of the remaining extract. If a 100-μL large-volume injection is available, the same concentration amount can be achieved by extracting 10 mL of water with 1 mL of solvent and injecting 100 μL of the extract, without an evaporation step. The same 1000-fold effective sample concentration is achieved without the potentially counterproductive concentration and with over a 99% reduction in solvent use and with less sample requirement.

4.6. MEMBRANE EXTRACTION

Membrane extraction has emerged as a promising alternative to conventional sample preparation techniques. It has undergone significant developments in the last two decades and is still evolving. It has been used for the extraction of a wide variety of analytes from different matrices. Only the extraction of volatile organics is discussed in this chapter. Figure 4.15 shows the concept of membrane separation. The sample is in contact with one side of the membrane, which is referred to as the *feed* (or *donor*) *side*. The membrane serves as a selective barrier. The analytes pass through to the other

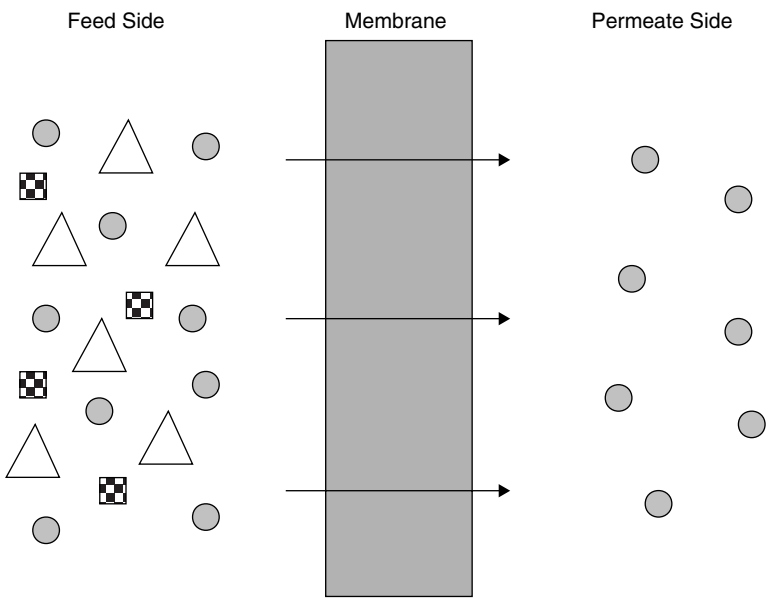


Figure 4.15. Concept of membrane separation; the circles are the analytes.

side, referred to as the *permeate side*. Sometimes, the permeated species are swept by another phase, which can be either a gas or a liquid.

A major advantage of membrane extraction is that it can be coupled to an instrument for continuous online analysis. Typically, a mass spectrometer [46–56] or gas chromatograph [57–66] is used as the detection device. Figure 4.16 shows the schematic diagrams of these systems. In membrane introduction mass spectrometry (MIMS), the membrane can be placed in the vacuum compartment of the MS. The permeates enter the ionization source of the instrument directly. In membrane extraction coupled with gas chromatography (Figure 4.16*b*), a sorbent trap is used to interface the membrane to the GC. The analytes that have permeated across the membrane are carried by a gas stream to the trap for preconcentratin. The trap is heated rapidly to desorb the analytes into the GC as a narrow injection band. For complex samples, GC has been the method of choice, due to its excellent separation ability. Tandem MS is emerging as a faster alternative to GC separation, but such instruments are more expensive. Detection limits of the membrane-based techniques are typically in the ppt to ppb range.

Membrane pervaporation (permselective "evaporation" of liquid molecules) is the term used to describe the extraction of volatile organics from an aqueous matrix to a gas phase through a semipermeable membrane.

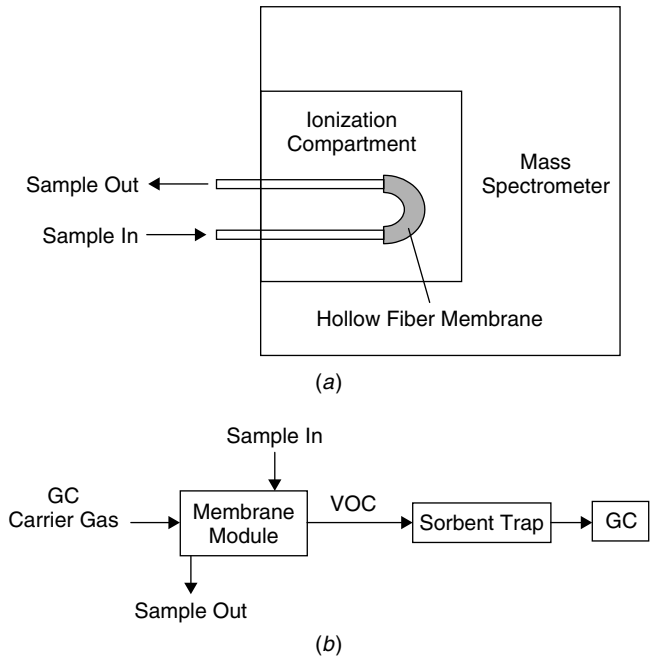


Figure 4.16. (*a*) Mass introduction mass spectrometry. (*b*) Hyphenation of membrane extraction with online GC.

The extraction of volatiles from a gas sample to a gaseous acceptor across the membrane is called *permeation*, which is the mechanism of extraction from the headspace of an aqueous or solid sample. For both pervaporation and permeation, the transport mechanism can be described by the solution–diffusion theory [67]. In pervaporation, the organic analytes first move through the bulk aqueous sample to the membrane surface and then dissolve/partition into it. After diffusing through the membrane to the permeate side, the analytes evaporate into the gas phase. In headspace sampling, an additional step of transporting the analytes from the bulk aqueous phase into the headspace is involved. In both cases, the extraction is driven by the concentration gradient across the membrane.

Steady-state permeation is governed by *Fick's first law:*

$$J = -AD\frac{dC}{dx} = AD\frac{\Delta C}{l} \tag{4.3}$$

where J is the analyte flux, A the membrane surface, D the diffusion coeffi-

cient, C the solute concentration, x the distance along the membrane wall, and l the membrane thickness. It can be seen from the equation that mass transfer is faster across a thin, large-surface-area membrane. In pervaporation, the overall mass transfer resistance is the sum of the mass transfer resistance of the bulk aqueous phase on the feed side, the membrane, and the gas on the permeate side. In headspace sampling, the overall mass transfer resistance is the sum of the mass transfer resistance of the bulk aqueous sample, the liquid–gas interface, the gas phase on the feed side, the membrane, and the gas on the permeate side. Non-steady-state permeation can be described by *Fick's second law:*

$$\frac{dC(x,t)}{dt} = -D\frac{d^2C(x,t)}{dt^2} \tag{4.4}$$

where $C(x,t)$ is the solute concentration at position x and time t.

4.6.1. Membranes and Membrane Modules

Membranes can be classified as porous and nonporous based on the structure or as flat sheet and hollow fiber based on the geometry. Membranes used in pervaporation and gas permeation are typically hydrophobic, nonporous silicone (polydimethylsiloxane or PDMS) membranes. Organic compounds in water dissolve into the membrane and get extracted, while the aqueous matrix passes unextracted. The use of mircoporous membrane (made of polypropylene, cellulose, or Teflon) in pervaporation has also been reported, but this membrane allows the passage of large quantities of water. Usually, water has to be removed before it enters the analytical instrument, except when it is used as a chemical ionization reagent gas in MS [50]. It has been reported that permeation is faster across a composite membrane, which has a thin (e.g., 1 μm) siloxane film deposited on a layer of microporous polypropylene [61].

As the name suggests, flat-sheet membranes are flat, like a sheet of paper, and can be made as thin as less than 1 μm. However, they need special holders to hold them in place. Hollow-fiber membranes are shaped like tubes (200 to 500 μm ID), allowing fluids to flow inside as well as on the outside. Hollow fibers are self-supported and offer the advantage of larger surface area per unit volume and high packing density. A large number of parallel fibers can be packed into a small volume.

There are two ways to design a membrane module [66]. The membrane can be introduced into the sample, referred to as *membrane in sample* (MIS), or the sample can be introduced into the membrane, referred to as *sample in membrane* (SIM). Figure 4.17*a* is a schematic diagram of the MIS configu-

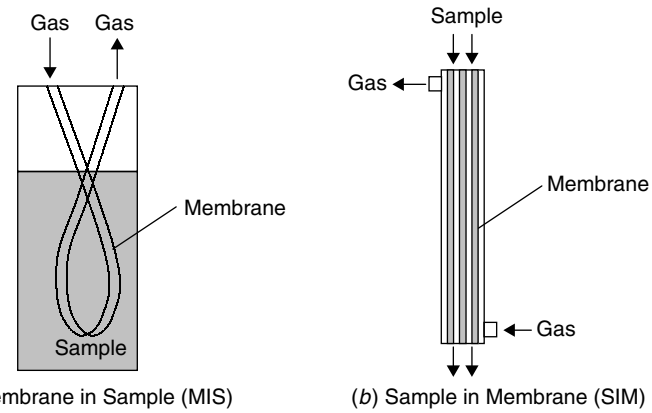


Figure 4.17. Configurations of membrane modules using hollow-fiber membranes. (*a*) Membrane in sample (MIS). (*b*) Sample in membrane (SIM).

ration. A hollow-fiber membrane is shown here, although a flat membrane fitted on the tip of a probe can also be used. The membrane is submerged in the sample, and the permeated analytes are stripped by a flowing gas (or vacuum) on the other side of the membrane. At any time, only a small fraction of the sample is in direct contact with the membrane. The ratio of membrane surface area to sample volume is quite low. The sample is usually stirred to enhance analyte diffusion through the aqueous phase. The membrane can also be placed in the headspace of a sample. The analytes first vaporize and then permeate through the membrane. In the MIS configuration, the time to achieve exhaustive extraction can be rather long. On the other hand, this configuration is simple and does not require the pumping of samples. It can also be used for headspace extraction where the membrane is not in direct contact with the sample. In this way, possible contamination of the membrane can be avoided, and the extraction can be applied to solid samples as well.

Figure 4.17*b* shows a schematic diagram of the SIM configuration. The membrane module has the classical shell-and-tube design. The aqueous sample is either made to "flow through" or "flow over" the hollow fiber, while the stripping gas flows countercurrent on the other side. In both cases, the sample contact is dynamic, and the contact surface/volume ratio is much higher than in the MIS extraction. Consequently, extraction is more efficient. The flow-through mode provides higher extraction efficiency than the flow-over mode. This is because tube-side volume is smaller than the shell-

side volume, which results in higher surface/volume ratio for the aqueous sample. Comparison studies show that under similar experimental conditions, flow-through extraction provides the highest sensitivity among all available membrane module configurations [59].

4.6.2. Membrane Introduction Mass Spectrometry

The use of membrane introduction mass spectrometry (MIMS) was first reported in 1963 by Hoch and Kok for measuring oxygen and carbon dioxide in the kinetic studies of photosynthesis [46]. The membrane module used in this work was a flat membrane fitted on the tip of a probe and was operated in the MIS mode. The permeated anaytes were drawn by the vacuum in the MS through a long transfer line. Similar devices were later used for the analysis of organic compounds in blood [47]. Memory effects and poor reproducibility plagued these earlier systems. In 1974, the use of hollow-fiber membranes in MIMS was reported, which was also operated in the MIS mode [48]. Lower detection limits were achieved thanks to the larger surface area provided by hollow fibers. However, memory effects caused by analyte condensation on the wall of the vacuum transfer line remained a problem.

In the late 1980s, Bier and Cooks [49] introduced a new membrane probe design, which was operated in the SIM mode. The schematic diagram of such a system is shown in Figure 4.16*a*. The sample flowed though the hollow-fiber membrane, which was inserted directly in the ionization chamber of the mass spectrometer. This eliminated memory effects and increased sensitivity and precision. Sample introduction was accomplished using flow injection, which increased the speed of analysis. Instruments based on this design were commercialized in 1994 by MIMS Technology, Inc. (Palm Bay, FL). MIMS in its modern forms has several advantages. Sample is directly introduced into the MS through the membrane, without additional preparation. The sensitivity is high, with detection limits in the sub-ppb (parts per billion) range. The analysis is fast, typically from 1 to 6 minutes. This technique is especially attractive for online, real-time analysis. It has been used in environmental monitoring [51–53], bioreactor monitoring [54,55], and chemical reaction monitoring [56].

The absence of chromatographic separation makes MIMS a fast technique. It is advantageous in some applications where only select compounds are to be detected or the total concentration of a mixture is to be determined. For instance, the total concentration of trihalomethanes (THMs, including chloroform, bromoform, bromodichloromethane, and dibromochloromethane) in drinking water can be determined by MIMS in less than

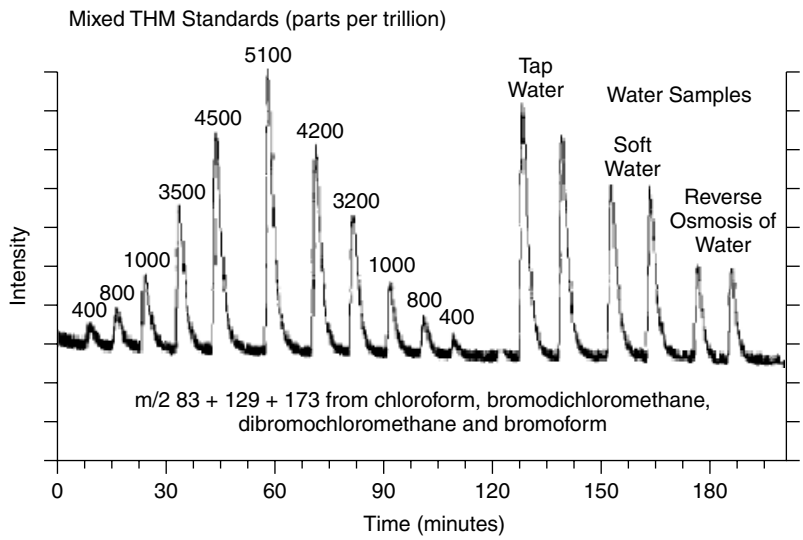


Figure 4.18. Ion current summation chromatogram for m/z $83 + 129 + 173$ from trihalomethane analysis. (Reproduced from Ref. 51, with permission from the American Chemical Society.)

10 minutes, without identifying the individual species [51]. Figure 4.18 shows the ion current chromatogram obtained using this method, where the peak area is proportional to the total THM concentration. MIMS works best for nonpolar, volatile organics with small molecular weight (<300 amu). In recent years efforts have been made to extend the application of MIMS to semivolatiles. This is beyond the scope of this chapter and is not discussed here. More details on MIMS can be found in several review articles [68,69].

4.6.3. Membrane Extraction with Gas Chromatography

The hyphenation of membrane extraction with gas chromatography is more complex. The analytes pervaporate into the GC carrier gas, which is at a positive pressure, thus reducing the partial pressure gradient. A sorbent trap is used to concentrate the analytes prior to GC analysis. Continuous monitoring can be carried out by pumping the water through the membrane module continuously, and heating the sorbent trap intermittently to desorb the analytes into the GC for analysis [57,58]. Although this works for the monitoring of a water stream, discrete, small-volume samples cannot be

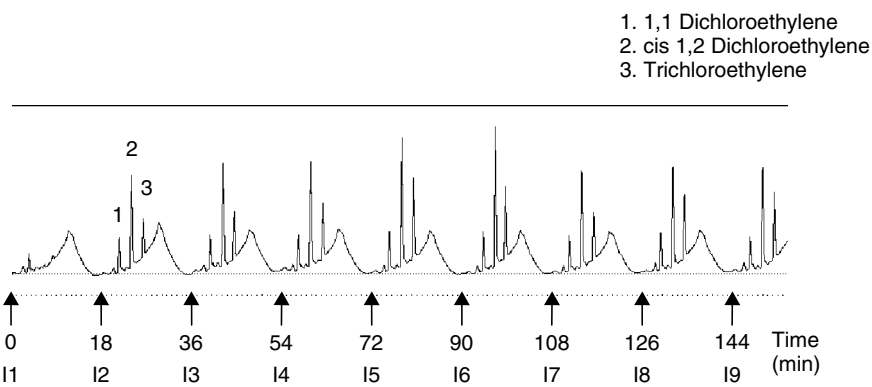


Figure 4.19. Chromatograms obtained during continuous monitoring of a contaminated groundwater well. Sample Injections were made every 18 minutes (I1, injection 1; and so on). (Reproduced from Ref. 65, with permission from Wiley-VCH.)

analyzed in this fashion. Moreover, it may take a relatively long time for the permeation to reach steady state. In other words, the membrane response to the concentration change in the stream can be slow. Any measurement during the transition period provides erroneous results.

A non-steady-state membrane extraction method referred to as pulse introduction membrane extraction (PIME) has been developed to avoid these problems [62]. PIME resembles a flow-injection operation. Deionized water (or an aqueous solution) serves as a carrier fluid, which introduces the sample into the membrane as a pulse. Analyte permeation does not have to reach steady state during extraction. Once the extraction is complete, the analytes are thermally desorbed from sorbent trap into the GC. A chromatogram is obtained for each sample that reflects its true concentration. PIME can be used for the analysis of multiple discrete samples, as well as for the continuous monitoring of a stream by making a series of injections. Figure 4.19 shows chromatograms obtained during continuous monitoring of contaminated groundwater using PIME [65]. The sample injections were made every 18 minutes.

The greatest challenge in membrane extraction with a GC interface has been the slow permeation through the polymeric membrane and the aqueous boundary layer. The problem is much less in MIMS, where the vacuum in the mass spectrometer provides a high partial pressure gradient for mass transfer. The time required to complete permeation is referred to as *lag time*. In membrane extraction, the lag time can be significantly longer than the sample residence time in the membrane. An important reason is the bound-

ary layer effects. When an aqueous stream is used as the carrier fluid, a static boundary layer is formed between the membrane and the aqueous phase. The analytes are depleted in the boundary layer, and this reduces the concentration gradient for mass transfer and increases the lag time. In a typical analytical application, mass transfer through the boundary layer is the rate-limiting step in the overall extraction process [63,64].

Sample dispersion is another cause of the long lag time in flow injection techniques where an aqueous carrier fluid is used [63,64]. Dispersion is caused by axial mixing of the sample with the carrier stream. This increases the sample volume, resulting in longer residence time in the membrane. Dilution reduces the concentration gradient across the membrane, which is the driving force for diffusion. The overall effects are broadened sample band and slow permeation.

Gas Injection Membrane Extraction

Gas injection membrane extraction (GIME) of aqueous samples has been developed to address the issues of boundary layer effects and sample dispersion [66]. This is shown in Figure 4.20. An aqueous sample from the loop

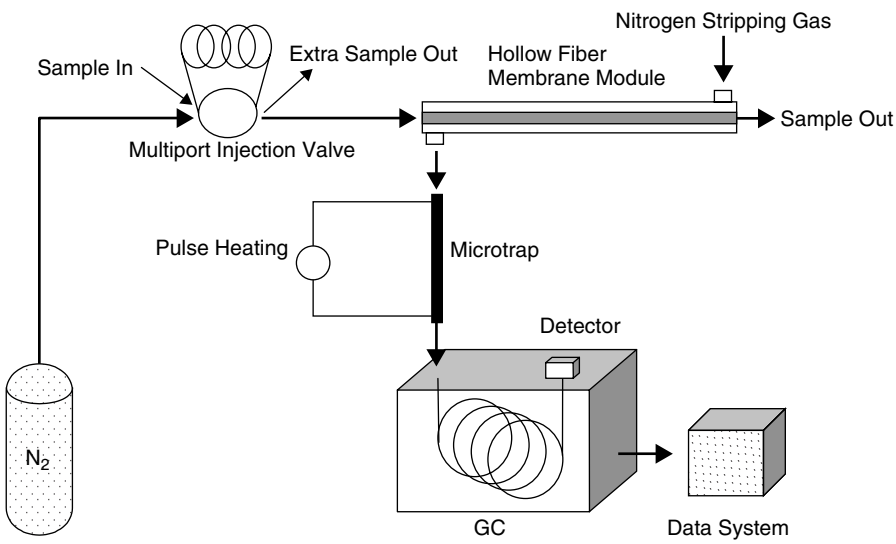


Figure 4.20. Schematic diagram of gas injection membrane extraction. (Reproduced from Ref. 66, with permission from the American Chemical Society.)

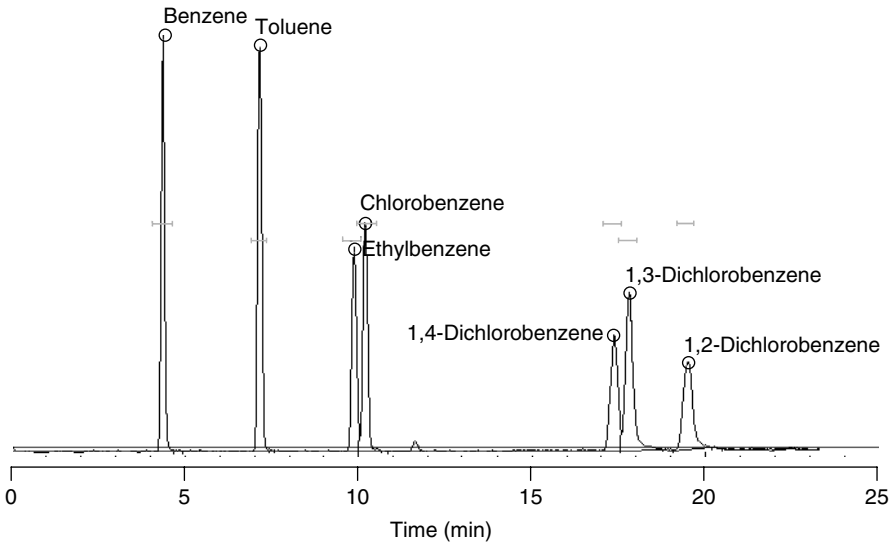


Figure 4.21. Chromatogram of an aqueous sample containing ppb-level purgable aromatics as listed in EPA standard method 602 by GIME. (Reproduced from Ref. 66, with permission from the American Chemical Society.)

of a multiport injection valve is injected into the hollow fiber membrane module by an N_2 stream. The gas pushes the sample through the membrane fibers, while the organic analytes permeate to the shell side, where they are swept by a countercurrent nitrogen stream to a microsorbent trap. After a predetermined period of time, the trap is electrically heated to desorb the analytes into the GC. Figure 4.21 shows a chromatogram of ppb-level volatile organic compounds, as listed in EPA method 602, obtained by GIME [66].

The permeation profiles obtained by aqueous elution and GIME are shown in Figure 4.22. It can be seen that the lag time was reduced significantly by gas injection of aqueous samples. There is no mixing between the eluent gas and the sample; thus dispersion is eliminated. The boundary layer is also greatly reduced, as the gas cleans the membrane by removing any water sticking on the surface. GIME is a pulsed introduction technique that can be used for the analysis of individual samples by discrete injections or for continuous on-line monitoring by sequentially injecting a series of samples. This technique is effective in speeding up membrane extraction. It can significantly increase sample throughput in laboratory analysis and is desirable for online water monitoring.

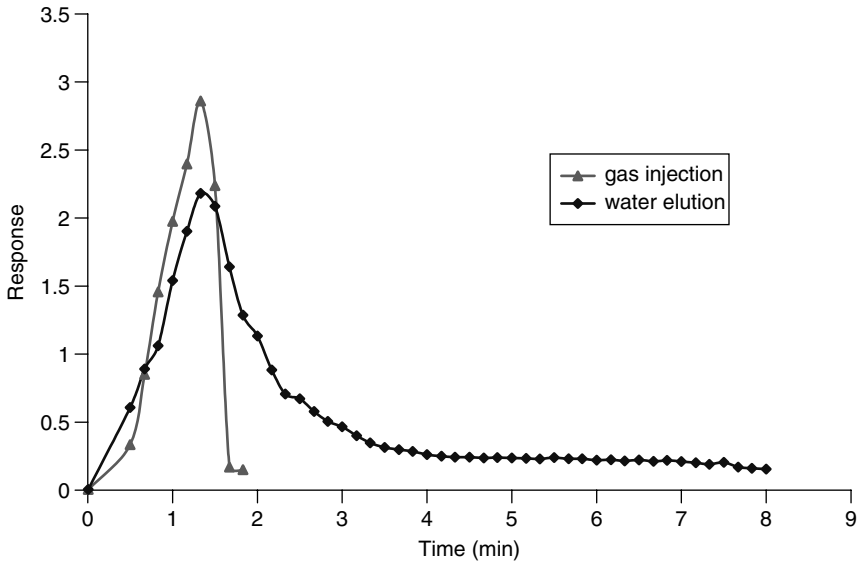


Figure 4.22. Permeation profiles for 1 mL of a 500-ppb benzene sample at an eluent (gas or liquid) flow rate of 1 mL/min. (Reproduced from Ref. 66, with permission from the American Chemical Society.)

4.6.4. Optimization of Membrane Extraction

Several factors affect the efficiency of membrane extraction and hence the sensitivity of the analysis: temperature, membrane surface area, membrane thickness, geometry, sample volume, and sample flow rate. These parameters need to be optimized for specific applications. Higher temperature facilitates mass transfer by increasing diffusion coefficient, but at the same time decreases analyte partition coefficient in the membrane. The temperature of the membrane module needs to be controlled to avoid fluctuation in extraction efficiency and sensitivity. Extraction efficiency can also be improved by using thinner membranes, which provide faster mass transfer. In the case of hollow fiber membranes, extraction efficiency can be increased by using longer membranes and multiple fibers, which provide lager contact area between the membranes and the sample. It has been reported that spiraled membranes provide more efficient extraction than straight membranes, because the former facilitates turbulent flow in the membrane module and reduces the boundary layer effects. The larger the sample volume, the more analytes it has and the higher is the sensitivity. However, larger volumes take longer to extract. Lower sample flow rates increase the extraction efficiency but prolong the extraction time.

4.7. CONCLUSIONS

There are many techniques available for the preparation of volatile analytes prior to instrumental analysis. In this chapter the major techniques, leading primarily to gas chromatographic analysis, have been explored. It is seen that the classical techniques: purge and trap, static headspace extraction, and liquid–liquid extraction still have important roles in chemical analysis of all sample types. New techniques, such as SPME and membrane extraction, offer promise as the needs for automation, field sampling, and solvent reduction increase. For whatever problems may confront the analyst, there is an appropriate technique available; the main analytical difficulty may lie in choosing the most appropriate one.

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