Anal. Chem. **2003,** *75,* 2543−2558

Perspectives

Sample Preparation: Quo Vadis?

Janusz Pawliszyn*

Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

The sample preparation step in an analytical process typically consists of an extraction procedure that results in the isolation and enrichment of components of interest from a sample matrix. Extraction can vary in degree of selectivity, speed, and convenience and depends not only on the approach and conditions used but on the geometric configurations of the extraction phase. Increased interest in sample preparation research has been generated by the introduction of nontraditional extraction technologies. These technologies address the need for reduction of solvent use, automation, and miniaturization and ultimately lead to on-site in situ and in vivo implementation. These extraction approaches are frequently easier to operate but provide optimization challenges. More fundamental knowledge is required by an analytical chemist not only about equilibrium conditions but, more importantly, about the kinetics of mass transfer in the extraction systems. Optimization of this extraction process enhances overall analysis. Proper design of the extraction devices and procedures facilitates convenient on-site implementation, integration with sampling, and separation/quantification, automation, or both. The key to rational choice, optimization, and design is an understanding of the fundamental principles governing mass transfer of analytes in multiphase systems. The objective of this perspective is to summarize the fundamental aspects of sample preparation and anticipate future developments and research needs.

RECENT DEVELOPMENTS IN EXTRACTION TECHNOLOGY

During the past several decades, increased public awareness that environmental contaminants are a health risk has stimulated interest in environmental research and monitoring, resulting in a requirement for determination of toxic contaminants in air, water, and solids, including soil and sediment samples. Despite highly selective separation and sensitive instrumentation for quantification, the simple approach of "dilute and shoot" is not usually compatible with environmental determinations. An extraction step is required to isolate and enrich trace level analytes from sample matrixes. Classical extraction procedures consume large amounts of solvents, thus themselves creating environmental and occupational hazards, and often provide very little selectivity. For example, toxic chemical management and disposal is required when analyzing for the presence of semivolatile compounds in different matrixes using conventional approaches such as Soxhlet extraction for solid samples, liquid−liquid extraction for aqueous matrixes, or the charcoal tube method with carbon disulfide desorption for gas analysis. During the volume reduction step of most extraction procedures, the solvents are frequently disposed of into the atmosphere, which causes pollution and contributes to unwanted atmospheric effects, such as smog and ozone holes. To address this issue the Montreal Protocol' treaty, signed over a decade ago, stipulated reduction of solvent use.[1] The analytical community responded to this challenge by increasing research on sorbent traps, solid-phase extraction (SPE), supercritical fluid extraction (SFE) as other, less-solvent-consuming, alternatives to charcoal tubes, liquid−liquid, and Soxhlet extraction, respectively. The development of new technologies, such as new pressurized fluid extraction (PFE) approaches including hot-solvent (accelerated solvent extraction) and hot-water extraction, microwave-assisted extraction, and microextraction approaches such as solid-phase microextraction (SPME) followed by modern versions of solvent microextraction, including single solvent drop approaches and other related techniques also reduced solvent use.[2] It is interesting to note that these new developments in extraction technologies had an impact on other areas of analytical science. For example, introduction of hot-water extraction[3] led to use of hot water as a solvent in liquid chromatography (LC).[4] Development of poly(dimethylsiloxane) (PDMS)-coated SPME fibers led to the development of PDMS-based sensors.[5] Considering the progress made by the analytical research community, the disappointing response of the environmental protection agencies with continued certified method development has been attributed to reduced government environmental funding at the end of the 1990s.

During the past decade, active research on sample preparation has also been fueled by interest in rapid analysis of combinatorial chemistry and biological samples requiring high-level automation

* E-mail: janusz@uwaterloo.ca.

(1) Noble, D. *Anal. Chem.* **1993**, *65*, 693A−695A.
(2) Pawliszyn, J., Ed. *Sampling and Sample Preparation for Field and Laboratory*; Elsevier: Amsterdam 2002.
(3) Hawthorne, S.; Yang, Y.; Miller, D. *Anal. Chem.* **1994**, *66*, 2912−2920.
(4) Smith, R.; Burgess, R. *Anal. Commun.* **1996**, *33*, 327−329.
(5) Stahl, D.; Tilotta, D. Infrared Spectroscopic Detection for SPME. Burck, J. SPME in Near-IR Fiber-optics Evanescent Field Absorption Spectroscopy. In *Application of Solid-Phase Microextraction*; Pawliszyn, J., Ed.; Royal Society of Chemistry: Letchworth, Hertfordshire, U.K., 1999; pp 625−653.

10.1021/ac034094h CCC: $25.00
Published on Web 05/03/2003

with robots able to process multiwell plates containing an ever increasing number of samples. These new developments resulted in miniaturization of the extraction process, resulting in new micro SPE configurations in which extraction is performed using pipets. The robot, able to control several pipets simultaneously, can therefore perform parallel extraction also.[6] In addition, several nonextraction rapid sample preparation approaches have been developed. For example, analysis of drugs and metabolites in blood frequently involves removal of interferences, e.g., protein by precipitation, followed by direct injection of the remaining sample matrix into the LC−MS instrument. Also, electrofocusing and stacking concentration methods taking advantage of the interaction of charged analytes with electrical fields have been developed. These approaches will not be covered in this perspective but have been discussed elsewhere.[2] Sample preparation research remains very active with growing interest in the application of on-site analytical technologies for homeland security, a direction that presents new challenges for the community of analytical chemists.[7] Several specialized meetings dedicated to sample preparation—for example, ExTech, International Symposium on Advances in Extraction Technologies—have been initiated to encourage and accelerate evolution of the technologies.

In parallel with the development of new technologies, fundamental understanding of extraction principles has advanced. This progress has been very important in the development of novel approaches resulting in new trends in sample preparation, e.g., microextraction, miniaturization, and integration of the sampling and separation or quantification steps of the analytical process. The fundamentals of the sampling and sample preparation processes are substantially different from those related to chromatographic separations or other traditional disciplines of analytical chemistry.

Sampling and sample preparation frequently resemble engineering approaches on a smaller scale. Some analytical scientists feel uncomfortable when working in the engineering discipline. Engineering progress, however, often drives development of new analytical technologies. For example, an optical fiber manufacturing process is presently used to produce GC capillary columns. The availability of coated fused-silica fibers originally developed for telecommunication applications was instrumental in the practical implementation of the SPME approach. Similarly, recent advancements in micromachining and wireless communication are expected to have a profound impact on future analytical devices. These engineering developments do not preclude analytical scientists from making substantial contributions to the evolution and implementation of the new technologies, rather these developments generate new opportunities for them. For example, over the last several decades, our engineering colleagues have developed micromachining technologies including microelectromechanical systems and components currently used to construct micro total analysis system devices. As analytical researchers investigate in detail these new technologies developed by engineers, they will continue to find new and unique opportunities

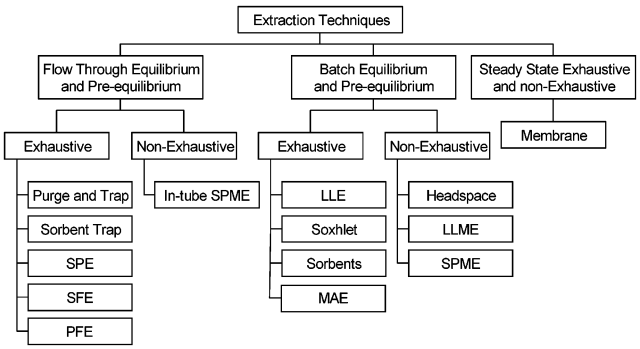

Figure 1. Classification of extraction techniques.

and applications for their science.[8] Currently there is increased interest in the incorporation of sample preparation into miniaturized devices to enable on-site deployment, automation, or both. The key to rational choice, design, and optimization of sample preparation components to facilitate this objective is based on an understanding of fundamental principles governing the mass transfer of analytes in multiphase systems. The objective of this perspective is to emphasize common principles among different extraction techniques, describe a unified theoretical treatment, and discuss future research opportunities in integration and miniaturization trends.

CLASSIFICATION OF EXTRACTION TECHNIQUES

Figure 1 provides a classification of extraction techniques and unifies the fundamental principles behind the different extraction approaches. In principle, exhaustive extraction approaches do not require calibration, because most analytes are transferred to the extraction phase by employing overwhelming volumes of it. In practice, however, confirmation of satisfactory recoveries is implemented in the method by using surrogate standards. To reduce the amounts of solvents and time required to accomplish exhaustive removal, batch equilibrium techniques (for example, liquid−liquid extractions) are frequently replaced by flow-through techniques. For example, a sorbent bed can be packed with extraction phase dispersed on a supporting material; when sample is passed through, the analytes in the sample are retained on the bed. Large volumes of sample can be passed through a small cartridge, and the flow through the well-packed bed facilitates efficient mass transfer. The extraction procedure is followed by desorption of analytes into a small volume of solvent, resulting in substantial enrichment and concentration of the analytes. This strategy is used in sorbent trap techniques and in SPE.[9] Alternatively, sample (typically a solid sample) can be packed in the bed and the extraction phase can be used to remove and transport the analytes to the collection point. In SFE, compressed gas is used to wash analytes from the sample matrix; an inert gas at atmospheric pressure performs the same function in purge-and-trap methods. In dynamic solvent extraction, for example, in a Soxhlet apparatus, the solvent continuously removes the analytes from the matrix at the boiling point of the solvent. In more recent PFE techniques, smaller volumes of organic solvent or even water are used to achieve greater enrichment at the same time as

(6) Wells, D.; Lloyd, T. In Automated of Sample Preparation for Pharmaceutical and Clinical Analysis. *Sampling and Sample Preparation for Field and Laboratory*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, 2002.
(7) Smith, W. *Anal. Chem.* **2002**, *74*, 463A−466A.
(8) Reyes, D.; Iossifidis, D.; Auroux, P.-A.; Manz, A. *Anal. Chem.* **2002**, *74*, 2623−2652.
(9) Thurman, E.; Mills, M. *Solid-Phase Extraction*; John Wiley: New York, 1998.

extraction, because of the increased solvent capacity and elution strength at high temperatures and pressures.[10]

Alternatively nonexhaustive approaches can be designed on the basis of the principles of equilibrium, preequilibrium, and permeation.[11] Although equilibrium nonexhaustive techniques are fundamentally analogous to equilibrium-exhaustive techniques, the capacity of the extraction phase is smaller and is usually insufficient to remove most of the analytes from the sample matrix. This is because of the use of a small volume of the extracting phase relative to the sample volume, such as is employed in microextraction (solvent microextraction[12] or SPME[13]) or a low sample matrix−extraction phase distribution constant, as is typically encountered in gaseous headspace techniques.[14]

Preequilibrium conditions are accomplished by breaking the contact between the extraction phase and the sample matrix before equilibrium with the extracting phase has been reached. Although the devices used are frequently identical with those of microextraction systems, shorter extraction times are employed. The preequilibrium approach is conceptually similar to the flow injection analysis approach[15] in which quantification is performed in a dynamic system and system equilibrium is not required to obtain acceptable levels of sensitivity, reproducibility, and accuracy. In permeation techniques, e.g., membrane extraction,[16] continuous steady-state transport of analytes through the extraction phase is accomplished by simultaneous re-extraction of analytes. Membrane extraction can be made exhaustive by designing appropriate membrane modules and optimizing the sample and stripping flow conditions,[17] or it can be optimized for throughput and sensitivity in nonexhaustive open-bed extraction.[18]

As the above discussion and Figure 1 indicate, there is a fundamental similarity among the extraction techniques used in the sample preparation process. In all techniques, the extraction phase is in contact with the sample matrix and analytes are transported between the phases. To ensure quantitative transfer of the analyte in an exhaustive technique, the phase ratio is higher and geometries are more restrictive than for nonexhaustive approaches. The thermodynamics of the process are defined by the extraction phase/sample matrix distribution constant. It would be instructive to consider in more detail the kinetics of processes occurring at the extraction phase/sample matrix interface, because this controls the time taken by the analytical procedure. The analytes are often re-extracted from the extraction phase, but this step is not discussed here, because this process is analogous and more basic in principle than removing analytes from a more complex sample matrix. Common fundamental principles among different extraction techniques are outlined below to aid selection of extraction technique, device geometry, and operating conditions for a given application.

THERMODYNAMICS

Distribution Constant. The fundamental thermodynamic principle common to all chemical extraction techniques involves the distribution of analyte between the sample matrix and the extraction phase. When a liquid is used as the extraction medium, the distribution constant, K_{es}

$$K_{es} = a_e/a_s = C_e/C_s \quad (1)$$

defines the equilibrium conditions and ultimate enrichment factors achievable by use of the technique; a_e and a_s are the activities of analytes in the extraction phase and matrix, respectively, and can be approximated by the appropriate concentrations. This physicochemical constant, which reflects the chemical composition of the extraction phase, has been discussed in detail in fundamental chromatographic literature, because it determines the retention and selectivity of a separation column. Although chromatography is frequently used to determine distribution constants, convenient sample preparation techniques, e.g., SPME, can also be used to provide information about the thermodynamics of the partitioning process.[19]

K_{es} can be estimated by use of a variety of the properties characteristic of matrix and analyte[20] analogues, as is typically performed when octanol−water distribution constants (K_{ow}) are estimated.[21] Chromatographic retention times obtained by use of appropriate mobile and stationary phases corresponding to the extractant and the sample matrix, respectively, can occasionally be used to estimate the distribution constant.[22] The extraction phase−sample matrix distribution constants are thermodynamic constants that depend on a variety of conditions including temperature, pressure, and sample matrix conditions such as pH, salt, and organic component concentration.

For solid extractant, adsorption equilibria can be explained by use of the equation

$$K^s_{es} = S_e/C_s \quad (2)$$

where S_e is the solid extraction phase surface concentration of adsorbed analytes. The above relationship is similar to eq 1 except for replacement of the extraction phase concentration with the surface concentration. The S_e term in the numerator indicates that the sorbent surface area available for adsorption must also be considered. This complicates calibration under equilibrium conditions, because of displacement effects and the nonlinear adsorption isotherm.[23]

The equations given above can be used to calculate the amount of analyte in the extraction phase under equilibrium conditions. For equilibrium liquid microextraction techniques and large

(10) Dean, J. *Extraction Methods for Environmental Analysis*; John Wiley: New York, 1998.
(11) Handley, A., Ed.; *Extraction Methods in Organic Analysis;* Sheffield Academic Press: Sheffield, UK., 1999.
(12) Cantwell, F.; Losier, M. Liquid−Liquid Extraction. In *Sampling and Sample Preparation for Field and Laboratory*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, 2002.
(13) Pawliszyn, J. *Solid-Phase Microextraction*; Wiley-VCH: New York, 1997.
(14) Ioffe, B.; Vitenberg, A. *Headspace Analysis and Related Methods in Gas Chromatography*; John Wiley: New York, 1984.
(15) Ruzicka, J.; Hansen, E. *Flow Injection Analysis*, 1st ed., Wiley: New York, 1981.
(16) Stern, S. *Membrane Separation Technology*; Elsevier: Amsterdam, 1995.
(17) Pratt, K.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 2101−2106.
(18) Yang, M.; Adams, M.; Pawliszyn, J. *Anal. Chem.* **1996**, *68*, 2782−2789.
(19) Zhang, Z.; Pawliszyn, J. *J. Phys. Chem.* **1996,** *100*, 17648−17654.
(20) Poole, S.; Poole, C. *Analyst* **1995**, *120*, 1733−1739.
(21) *Environmental Organic Chemistry*; Schwanrzenbach, R., Gschwed, P., Imboden, D., Eds.; Wiley: New York, 1993.
(22) Saraullo, A.; Martos, P.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 1992−1998.
(23) Gorecki, T.; Yu, X.; Pawliszyn, J. *Analyst* **1999**, *124*, 643−649.

samples, including direct extraction from an entire investigated system, the appropriate expression is very simple:[24]

$$n = K_{es} V_e C_s \quad (3)$$

where K_{es} is the extraction phase/sample matrix distribution constant, V_e is the volume of the extraction phase, and C_s is the concentration of the sample. This equation is valid when the amount of analytes extracted is insignificant compared with the amount of analytes present in a sample (large V_s, small K_{es}, or both), resulting in negligible depletion of analyte concentration in the original sample. In eq 3, K_{es} and V_e determine the sensitivity of the microextraction method whereas K_{es} determines its selectivity. The sample volume can be neglected, thus integrating sampling and extraction without the need for a separate sampling procedure, as discussed in more detail later. The nondepletion properties of the small dimensions typically associated with microextraction systems result in minimum disturbance of the investigated system, facilitating convenient speciation, investigation of multiphase distribution equilibria, and repeated sampling from the same system to follow a process of interest.

When significant depletion occurs, the sample volume, V_s, has some impact on the amount extracted and, therefore, on sensitivity.[25] This effect can be calculated by use of the equation

$$n = K_{es} V_e C_0 V_s / K_{es} V_e + V_s \quad (4)$$

Matrix Effects. Two potential complications are typically observed when analytes are extracted from complex matrixes. One is associated with competition among different phases for the analyte and the other with the fouling of the extraction phase, because of adsorption of macromolecules such as proteins and humic materials at the interface. The components of heterogeneous samples (including headspace, immiscible liquids, and solids) partition in the multiphase system and are less available for extraction. This effect depends on analyte affinity and the volume of the competing phases and can be estimated if appropriate volumes and distribution constants are known. The mass of an analyte extracted by an extraction phase in contact with a multiphase sample matrix can be calculated by use of the equation

$$n = \frac{K_{es} V_e C_0 V_s}{K_{es} V_e + \sum_{i=1}^{i=m} K_{is} V_i + V_s} \quad (5)$$

where $K_{is} = C_i^{\infty}/C_s^{\infty}$ is the distribution constant of the analyte between the *i*th phase and the matrix of interest.[26] Equation 5 simplifies to eq 4 if there are no competing phases in the sample matrix.

The typical approach used to reduce fouling involves introduction of a barrier between the sample matrix and the extraction phase to restrict transport of high molecular weight interferences (Figure 2). For example, the extraction phase can be surrounded

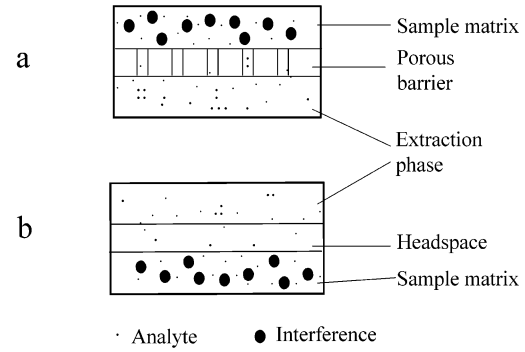

Figure 2. Integrated cleanup and extraction using selective barrier approaches based on size exclusion with a porous membrane (a) and based on volatility with a headspace gap (B).

by a porous membrane with pores smaller than the size of the interfering macromolecules (Figure 2a), e.g., use of a dialysis membrane with the appropriate molecular cutoff. This approach is conceptually similar to membrane dialysis from complex matrixes, in which the porous membrane is used to prevent large molecules from entering the dialyzed solution.[27] Membrane separation has been used to protect SPME fibers from humic material.[28] More recently, hollow fiber membranes have been used in solvent microextraction both to support the small volume of solvent and to eliminate interferences when biological fluids are extracted.[29] This concept has been further explored by integrating a protective structure and the extraction phase in individual sorbent particles, resulting in restricted-access material.[30] The chemical nature of the small inner pore surface of the particles is hydrophobic, facilitating extraction of small target analytes, whereas the outer surface is hydrophilic, thus preventing adsorption of excluded large proteins. In practice, fouling of the hydrophobic interface occurs to large extent only when the interfering macromolecules are hydrophobic in nature.

A gap made of gas is also a very effective separation barrier (Figure 2b). Analytes must be transported through the gaseous barrier to reach the coating, thus resulting in exclusion of nonvolatile components of the matrix. This approach is practically implemented by placing the extraction phase in the headspace above the sample; it results in a technique such as headspace SPME, which is suitable for extraction of complex aqueous and solid matrixes.[31] The major limitation of this approach is that rates of extraction are low for poorly volatile or polar analytes, because of their small Henry's law constants. In addition, sensitivity for highly volatile compounds can suffer, because these analytes have high affinity for the gas phase, where they are concentrated. The effect of the headspace on the amount of analytes extracted and, therefore, on sensitivity can be calculated by use of eq 5, which indicates that reducing its gaseous volume minimizes the effect.

Extraction at elevated temperatures enhances Henry's law constants by increasing the concentrations of the analytes in the

(24) Lord, H.; Pawliszyn, J. *J. Chromatogr., A* **2000**, *885*, 153−193.
(25) Gorecki, T.; Pawliszyn, J. *Analyst* **1997**, *122*, 1079−1086.
(26) Pawliszyn, J. *Solid-Phase Microextraction, Theory and Practice*; Wiley: New York, 1997; pp 44−47.
(27) Mulder, M. *Basic Principles of Membrane Technology*; Kluwer: Dordrecht, 1991.
(28) Zhang, Z.; Poerschmann, J.; Pawliszyn, J. *Anal. Commun.* **1996**, *33*, 129−131.
(29) Rasmussen, K.; Pedersen-Bjergaard, S.; Krogh, H.; Ugland, H.; Gronhaug *J. Chromatogr., A* **2000**, *873*, 3−11.
(30) Boos, K.; Grimm, C.-H. *Trends Anal. Chem.* **1999**, *18*, 175−180.
(31) Zhang, Z.; Pawliszyn, J. *Anal. Chem.* **1993**, *65*, 1843−1852.

headspace; this results in rapid extraction by the extraction phase. The coating/sample distribution coefficient also decreases with increasing temperature, however; this results in diminution of the equilibrium amount of analyte extracted. To prevent this loss of sensitivity, the extraction phase can be cooled simultaneously with sample heating. This "coldfinger" effect results in increased accumulation of the volatilized analytes on the extraction phase. This additional enhancement in the sample matrix−extraction phase distribution constant associated with the temperature gap present in the system can be described by the equation[32]

$$K_T = K_0 \frac{T_s}{T_e} \exp\left[\frac{C_p}{R}\left(\frac{\Delta T}{T_e} + \ln\frac{T_e}{T_s}\right)\right] \quad (6)$$

where $K_T = C_e(T_e)/C_s(T_s)$ is the distribution constant of the analyte between cold extraction phase on the fiber having temperature T_e and hot headspace at temperature T_s, C_p is the constant-pressure heat capacity of the analyte, $\Delta T = T_s - T_e$, and K_0 is the coating/headspace distribution constant of the analyte when both coating and headspace are at temperature T_e. Because of enhancement of the sample matrix−extraction phase distribution constant, quantitative extraction of many analytes, including volatile compounds, is possible with this method.

Characteristics of the Extraction Phase. The properties of the extraction phase should be carefully optimized, because they determine the selectivity and reliability of the method. Properties include both bulk physicochemical properties, e.g., polarity, and physical properties, e.g., thermal stability and chemical inertness. Solvents and liquid polymeric phases, e.g., poly(dimethylsiloxane),[33] are very popular because they have wide linear dynamic ranges associated with linear absorption isotherms. They also facilitate "gentle" sample preparation, because chemisorption and catalytic properties, frequently associated with solid surfaces, are absent. No loss or modification of the analyte occurs during extraction or desorption. Despite these attractive properties of liquid extraction media, solid phases are frequently used because of their superior selectivity and sensitivity for some groups of compounds. For example, carbon-based sorbents are effective for extraction of volatile analytes.

The development of selective extraction materials often parallels that of the corresponding selective chemical sensors.[34] Similar manufacturing approaches and structures similar to those sensor surfaces have been implemented as extraction phases. For example, phases with specific properties such as molecularly imprinted polymers[35] and immobilized antibodies[36] have recently been developed for extraction. An interesting concept based on differences between bulk properties of the extraction phase and the highly specific molecular recognition centers dissolved in it facilitate high-selectivity extraction with minimum nonspecific adsorption.[37] In addition, chemically tunable properties of the extraction phase have been controlled during the preparation procedure. For example, polypyrrole has been used successfully for a range of applications from ion-exchange extraction to hydrophobic extraction based on selective interaction between the polymer and the target analytes.[38] In addition, tunable properties of the polymer, e.g., the oxidation/reduction equilibrium in conductive polypyrrole, can be explored to control adsorption and desorption.[39]

Demands on the specificity of extraction phases are, typically, less stringent than for sensor surfaces, because a powerful separation and quantification technique, e.g., GC/MS or LC/MS, is typically used after extraction, facilitating accurate identification of the analyte. More demand is, however, placed on the thermal stability and chemical inertness of the extraction phase, because the extraction materials are frequently exposed to high temperatures and different solvents during extraction and introduction to the analytical separation instruments. New coating chemistries, for example, the sol−gel polymerization approach, have recently been developed to address these needs.[40]

To optimize sensitivity, the choice of the extraction phase is frequently based on its affinity toward the target analyte. In practice, however, kinetic factors defined by dissociation constants, diffusion coefficients, and agitation conditions frequently determine the amounts of analytes extracted from complex samples. Because overall extraction rates are slow, the amount of analytes extracted during experiments of limited duration do not reach equilibrium values.

KINETICS

Extraction of Solids. The most challenging extractions occur when a solid is present as a part of the sample matrix. This situation will be considered as the most general example of extraction, because it involves several fundamental processes occurring during the extraction procedure. If we assume that a matrix particle consists of an organic layer on an impermeable but porous core and the analyte is adsorbed on the pore surface, the extraction process can be modeled by considering several basic steps, as shown in Figure 3. To remove the analyte from the extraction vessel, the compound must first be desorbed from the surface (A(M,S), see Figure 3); it must then diffuse through the organic part of the matrix (A(M,L)) to reach the matrix/fluid interface (A(M,I)). At this point, the analyte must be solvated by the extraction phase (A(EP,P)) and it must then diffuse through the static phase present inside the pore to reach the portion of the extraction phase that is affected by convection. The analyte is then transported through the interstitial pores of the matrix, eventually reaching the bulk of the extraction phase (A(EP,B)). The simplest way to design a kinetic model for this problem is to adopt equations developed by engineers[41] to investigate mass transport through porous media.[42]

The leaching approach can be performed directly in a vessel (for example, Soxhlet, sonication, or microwave extraction) or can

(32) Zhang, Z.; Pawliszyn, J. *Anal. Chem.* **1995**, *67*, 34−43.
(33) Louch, D.; Motlagh, S.; Pawliszyn, J. *Anal. Chem.* **1992**, *64*, 1187−1199.
(34) Eggins, B. *Chemical Sensors and Biosensors*, 2nd ed.; Wiley-VCH: New York, 2002.
(35) Sellegren, B., Ed. *Molecularly Imprinted Polymers: Man-made Mimics of Antibodies and Their Applications in Analytical Chemistry*; Elsevier: Amsterdam, 2001.
(36) Pichon, V.; Bouzige, M.; Miege, C.; Hennion, M.-C. *Trends Anal. Chem.* **1999**, *18*, 219−235.
(37) Li, S.; Weber, S. *Anal. Chem.* **1997**, *69*, 1217−1222.
(38) Wu, J.; Pawliszyn, J. *J. Chromatogr., A* **2001**, *909*, 37−52.
(39) Wu, J.; Mullett, W.; Pawliszyn, J. *Anal. Chem.* **2002**, *74*, 4855−4859.
(40) Chong, S.-L.; Wang, D.-X.; Hayes, J.; Wilhite, B. Malik, A. *Anal. Chem.* **1997**, *69*, 4566−4576.
(41) Wankat, P. *Rate-controlled Separations*; Elsevier Applied Science: New York, 1990. Dullien, F. *Porous Media*; Academic Press: San Diego, 1992.
(42) Horvath, C.; Lin, H. *J. Chromatogr.* **1978**, *149*, 43−65.

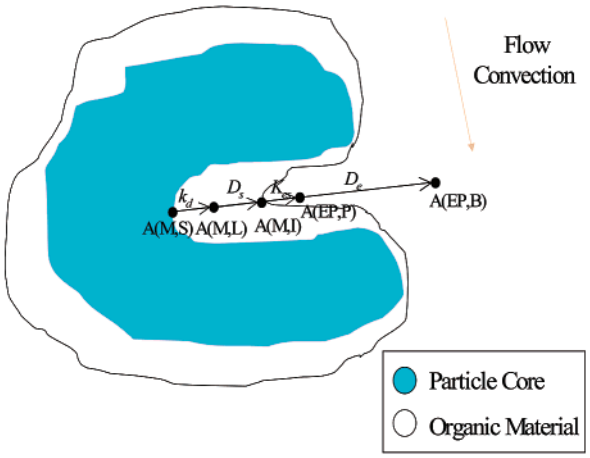

Figure 3. Processes involved in the extraction of heterogeneous samples containing porous solid particles. K_{es} is the extraction phase/sample matrix distribution constant, k_d is the dissociation rate constant of the analyte−matrix complex, and D_s and D_e are analyte diffusion in the sample matrix and the extraction phase, respectively. The other terms in the figure are discussed in the text.

be combined with elution from the packed tube (SFE, PFE). For the purpose of further discussion, we will consider the efficient and frequently applied experimental arrangement for removing solid-bound semivolatile analytes, which involves use of a piece of stainless steel tubing as the extraction vessel. The sample is typically placed inside the tubing and a linear-flow restrictor is attached to maintain the pressure at the end of the vessel. During the process, the extraction phase continuously removes analytes from the matrix; these are then transferred to the collection vessel after expansion of the fluid. This leaching process is very similar to chromatographic elution with packed columns. In particular, chromatographic frontal analysis and the corresponding equations can be used to model this process.[43] The main difference is that in sample preparation analytes are dispersed in the matrix at the beginning of the experiment, whereas in chromatographic frontal analysis a long plug is introduced into the column at the initial stage of the separation process. The principal objective of the extraction is to remove analytes from the vessel as quickly as possible; this requires elution conditions under which the analytes are released. In chromatography, on the other hand, the ultimate goal is separation of components of the sample, which requires retention of the analytes on the column. Another major difference between extraction and chromatography is that the packing is usually well characterized in chromatography (support with the dispersed stationary phase) whereas in sample preparation the matrix is often unknown (sample matrix).

Convolution Model of Extraction. The discussion above applies only when the analytes are initially present in a fluid phase, in which flow-through techniques correspond to elution of uniform spikes from the extraction vessel or weakly adsorbed native analytes are removed from an organic-poor matrix such as sand. In other words, the frontal chromatography approach is suitable for systems in which the partitioning equilibrium between the matrix and extraction fluid is reached quickly compared with the rate of fluid flow. It is also suitable for modeling static and dynamic extractions, under conditions of good solubility ($k = 0$), in which the sample is initially exposed to the static extraction phase (vessel is capped) for a time required to achieve equilibrium before elution by fluid flow. If dynamic extraction is performed from the beginning of extraction, in most practical circumstances the system is not expected to achieve the initial equilibrium conditions. This is because of the slow mass transport between the matrix and the fluid (for example, slow desorption kinetics or slow diffusion in the matrix). The expected relationship between the amount of analyte removed from the vessel and elution time can be obtained in this instance by convoluting the function describing the rate of mass transfer between the phases, $F(t)$, with the elution time profile, $m/m_o(t)$:[44]

$$\int_{\tau=0}^{\tau=t} \frac{m(t-\tau)}{m_o} F(\tau)\, d\tau \qquad (7)$$

The resulting function describes a process in which elution and the mass transfer between the phases occur simultaneously. In this discussion, we will refer to this function as the "extraction time profile" to emphasize that for most extractions these two processes are expected to be combined. $F(t)$ describes the kinetics of the process, which defines the rate of release of analyte from the sample matrix and can include, for example, the matrix−analyte complex dissociation rate constant (assuming linear adsorption isotherm), the diffusion coefficient, the time constant that describes swelling of the matrix that will facilitate removal of analyte, or a combination of the above. Detailed discussion, graphical representations, and applications of this model to describe or investigate processes in supercritical fluid extraction have been described elsewhere.[45]

The conclusion reached above can be stated more generally. Convolution among functions describing individual processes occurring during extraction describes the overall extraction process and is a unified way of describing the kinetics of these complex processes. The exact mathematical solution of the convolution integral is frequently difficult to obtain, but the solution can be represented graphically by use of Fourier transform or by numerical approaches. It is frequently possible to incorporate mathematical functions that describe a combination of the unit processes. In the flow-through system discussed above, the frontal elution function describes the effects on the extraction rate of porosity and of analyte affinity for the extraction matrix. It should be emphasized that the convolution approach considers all processes equivalently. In practice, however, a small number and frequently just one unit process controls the overall rate of extraction, enabling simplification of the equation.

Determination of the limiting step is not possible exclusively by qualitative agreement with the mathematical model, because the effect on recovery of most of the unit processes has an exponential yield curve. For proper recognition of all unit processes, quantitative agreement, the effect of extraction conditions, or both must be examined. Identification of the limiting process provides valuable insight into the most effective approach to optimization of the extraction.

(43) Pawliszyn, J. *J. Chromatogr. Sci.* **1993**, *31*, 31−37.

(44) Cadzow, J.; van Landingham, F. *Signals, Systems, and Transforms*; Prentice Hall, Inc.: Englewoods Cliffs, NJ, 1985.

(45) Langenfeld, J.; Hawthorne, S.; Miller, D.; Pawliszyn, J. *Anal. Chem.* **1995**, *67*, 1727−1736.

Fundamental understanding of the extraction process leads to better strategies for optimization of performance. In heterogeneous samples, for example, release of solid-bound analytes from the sample matrix, by reversal of chemisorption or inclusion, frequently controls the rate of extraction. Recognition of this enables extraction conditions to be changed to increase the rates of extraction. For example, dissociation of the chemisorbed analytes can be accomplished either by use of high temperature or by application of additives, facilitating desorption. This led to the development of high-temperature supercritical fluid extraction[46] and then to evolution of both the pressurized fluid extraction approach[47] and microwave extraction, with more selective energy focusing at the sample matrix/extraction phase interface.[48] There is also an indication that milder conditions can be applied by taking advantage of the catalytic properties of the extraction phase or additives.[49] To realize this opportunity, however, more research must be performed to gain more insight into the nature of interactions between analytes and matrixes. Deconvolution of experimental data can be used to investigate the matrix effect associated with slow release of analytes from the matrix. Benefits include not only improved speed but also selectivity resulting from application of appropriate conditions. This strategy of simultaneous extraction and cleanup has been successfully applied to the very difficult extraction of polychlorinated dibenzo-*p*-dioxins from fly ash.[50]

If the rate of extraction is controlled by mass transport of analytes in the pores of the matrix, the process can be successfully enhanced by application of sonic and microwave energy, which induce convection even in the small dimensions of the pore. Diffusion through all or some of a sample matrix containing natural or synthetic polymeric material frequently controls the rate of extraction.[51] In such circumstances, swelling of the matrix and increasing the temperature result in increased diffusion coefficients and, therefore, increased extraction rates.

Batch Techniques. Mathematical modeling equations for systems involving convection caused by flow through a tube, as discussed above, are frequently not appropriate for modeling other means of agitation or other geometric configurations. In these circumstances, the most successful approach is to consider the boundary layer approach used to model interfacial heat and mass transport. Irrespective of the level of agitation, fluid in contact with the extraction phase surface is always stationary, and as the distance from the extraction phase surface increases, fluid movement gradually increases until it corresponds to bulk flow in the sample. To model mass transport, the gradation in fluid motion and convection of molecules in the space surrounding the extraction phase can be simplified as a zone of defined thickness in which no convection occurs; perfect agitation occurs elsewhere in the bulk of the fluid. This static layer zone is called the *Prandtl* boundary layer (see Figure 4).[52]

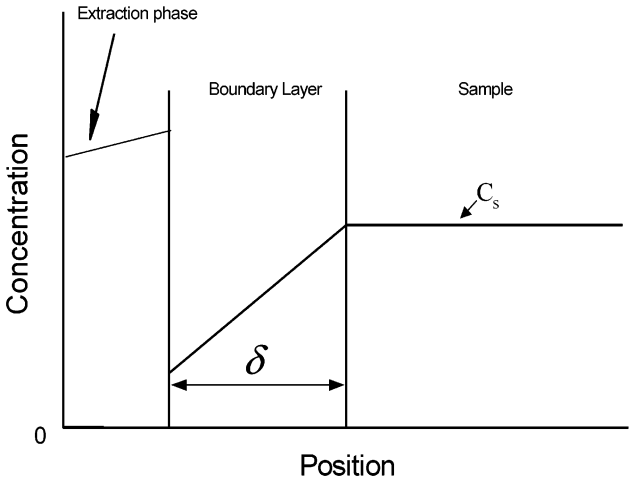

Figure 4. Boundary layer model.

Boundary Layer Model. A precise understanding of the definition and thickness of the boundary layer in this sense is useful. The thickness of the boundary layer (δ) is determined by both the rate of convection (agitation) in the sample and the diffusion coefficient of the analyte. Thus, in the same extraction process, the boundary layer thickness will be different for different analytes. Strictly speaking, the boundary layer is a region where analyte flux is progressively more dependent on analyte diffusion and less on convection, as the extraction phase is approached. For convenience, analyte flux in the bulk of the sample (outside of the boundary layer) is assumed to be controlled by convection, whereas analyte flux within the boundary layer is assumed to be controlled by diffusion. δ is defined as the position where this transition occurs or the point at which convection toward the extraction phase is equal to diffusion away from the extraction phase. At this point, analyte flux from δ toward the extraction phase (diffusion controlled) is equal to analyte flux from the bulk of the sample toward δ, controlled by convection.

Often when the extraction phase is dispersed well, forming a thin coating, diffusion of analytes through the boundary layer controls the rate of extraction. The equilibration time, t_e, can be estimated as time required to extract 95% of the equilibrium amount and can be calculated under these conditions from the equation[4]

$$t_e = B_1(\delta b K_{es}/D_s) \qquad (8)$$

where b is the extraction phase thickness, D_s is the diffusion coefficient of the analyte in the sample matrix, and K_{es} is the distribution constant of the analyte between the extraction phase and the sample matrix. B_1 is a geometric factor referring to the geometry of the supporting material on which the extraction phase is dispersed; the value is 3 for cylindrical geometry. The boundary layer thickness can be calculated for given convection conditions by use of engineering principles and is discussed in more detail later. Equation 8 can be used to predict equilibration times when the extraction rate is controlled by diffusion in the boundary layer, which is valid for thin extraction phase coatings ($b < 200\ \mu$m) or high distribution constants ($K_{es} > 100$).

(46) Langenfeld, J.; Hawthorne, S.; Miller, D.; Pawliszyn, J. *Anal. Chem.* **1993**, *65*, 338−344.
(47) Richter, B. E.; Jones, B. A.; Ezzell, J. L.; Porter, N. L.; Avdalovic, N.; Pohl, C. *Anal. Chem.* **1996**, *68*, 1033−1039.
(48) Pare, J.; Belanger, J.; Li, K.; Stafford, S. *J. Microcolumn Sep.* **1995**, *7*, 37−41.
(49) Alexandrou, N.; Pawliszyn, J. *Anal. Chem.* **1989**, *61*, 2770−2776.
(50) Miao, Z.; Zhang, Z.; Pawliszyn, J. *J. Microcolumn Sep.* **1994**, *6*, 459−465.
(51) Bartle, K.; Boddington, T.; Clifford, A.; Cotton, N. *Anal. Chem.* **1991**, *63*, 2371−2377.
(52) Young, A. *Boundary Layers*; BSP Professional Books, Oxford, 1989.

For a thicker stationary extraction phase and smaller distribution constants, mass transfer in the extraction phase controls the rate of extraction and the extraction time can then be obtained from

$$t_e = B_2(b^2/D_e) \quad (9)$$

where D_e is the diffusion coefficient of the analyte in the extraction phase and B_2 is the geometric factor, which is unity for cylindrical geometry. The optimum agitation conditions are sufficient to minimize the boundary layer to the extent that the rate of extraction is controlled by diffusion in the polymer coating, the fastest extraction possible. In other words, the extraction time obtained by use of eq 8 is smaller than the value obtained by use of eq 9. By combining both equations, the boundary layer conditions required to ensure the extraction is controlled by diffusion of the analytes in the extraction phase can be defined as

$$\delta < \frac{B_2}{B_1}\frac{D_s}{D_e}\frac{K_{es}}{b} \quad (10)$$

The thickness of the boundary layer can be related to required agitation conditions by using appropriate semiempirical equations.[53] By use of eq 9, it can be estimated that the shortest extraction time for 0.1-mm-thick liquid phase is <30 s. In practice, however, it is possible to achieve such short times only for analytes characterized by low K_{es}, as mentioned above. To extend this short extraction time to analytes characterized by higher K_{es}, very energetic agitation of the sample matrix is required, e.g., direct probe sonication resulting in increased extraction temperature and poor precision. The energetic agitation approach is practical only for monitoring of flowing streams, which are self-cooled.[54] Other approaches to agitation are available that eliminate the need for additional stirring devices, e.g., vortex mixing and approaches in which the extraction device itself performs agitation, for example, up−down movement,[33] vibration, and rotation[55] of the fiber in SPME and rotation of the magnetic stir bars coated with the extraction phase in stir bar sorptive extraction.[56]

Solid versus Liquid Sorbents. There is a substantial difference between the performance of liquid and solid coatings (Figure 5). With liquid coatings, the analytes partition into the extraction phase, in which the molecules are solvated by the coating molecules. The diffusion coefficient in the liquid coating enables the molecules to penetrate the whole volume of the coating within a reasonable extraction time if the coating is thin (see Figure 5a). With solid sorbents (Figure 5b), the coating has a glassy or a well-defined crystalline structure which, if dense, substantially reduces diffusion coefficients within the structure. Within the time of the experiment, therefore, sorption occurs only on the porous surface of the coating (see Figure 5b). During extraction by use of a solid phase and high analyte/interference concentration, after long extraction times, compounds with poor affinity toward the

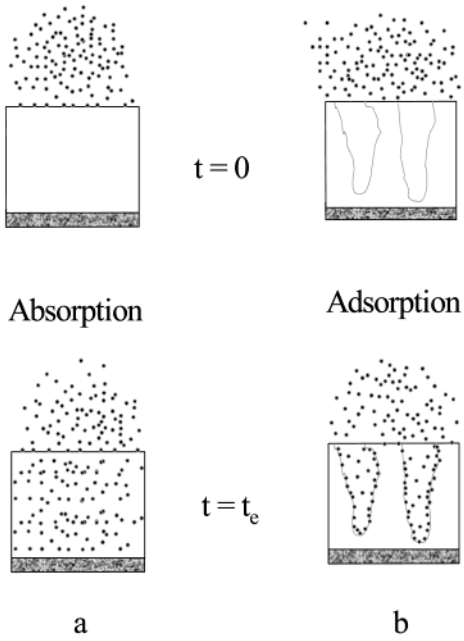

Figure 5. Extraction using absorptive (a) and adsorptive (b) extraction phases immediately after exposure of the phase to the sample ($t = 0$) and after completion of the extraction ($t = t_e$).

phase are frequently displaced by analytes characterized by stronger binding or those present in the sample at high concentrations. This is because only a limited surface area is available for adsorption. If this area is substantially occupied competition occurs and the equilibrium amount extracted can vary with the concentrations of both the target and other analytes.[57] In extraction with liquid phases, on the other hand, partitioning between the sample matrix and extraction phase occurs. Under these conditions, equilibrium extraction amounts vary only if the bulk coating properties are modified by the extracted components; this occurs only when the amount extracted is a substantial portion (a few percent) of the extraction phase, resulting in a possible source of nonlinearity. This is rarely observed, because extraction/enrichment techniques are typically used for analysis of trace contaminants.

Diffusion-Based Calibration. One way to overcome this fundamental limitation of porous coatings in a microextraction application is, as Figure 5 suggests, use of an extraction time much less than the equilibration time, so that the total amount of analytes accumulated by the porous coating is substantially below the saturation value. At saturation, all surface sites available for adsorption are occupied. When such experiments are performed, not only it is critical to control extraction times precisely, convection conditions must also be controlled, because they determine the thickness of the diffusion layer. One way of eliminating the need to compensate for differences in convection is to normalize (i.e., use consistent) agitation conditions. For example, by use of stirring (i.e., a well-defined rate of rotation in the laboratory) or use of fans for field air monitoring, consistent convection will be ensured.[58,59] The short-term exposure measurement described above has an advantage in that the rate of

(53) Crank, J. *Mathematics of Diffusion*; Clarendon Press: Oxford, 1989.
(54) Motlagh, S.; Pawliszyn, J. *Anal. Chim. Acta* **1993**, *284*, 265−273.
(55) Geppert, H, *Anal. Chem.* **1998**, *70*, 3981−3982.
(56) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. *J. Microcolumn Sep.* **1999**, *11*, 737−747.
(57) Ruthven, D. *Principles of Adsorption and Adsorption Processes*; Wiley: New York, 1984.
(58) Augusto, F.; Koziel, J.; Pawliszyn, J. *Anal. Chem.* **2001**, *73*, 481−486.

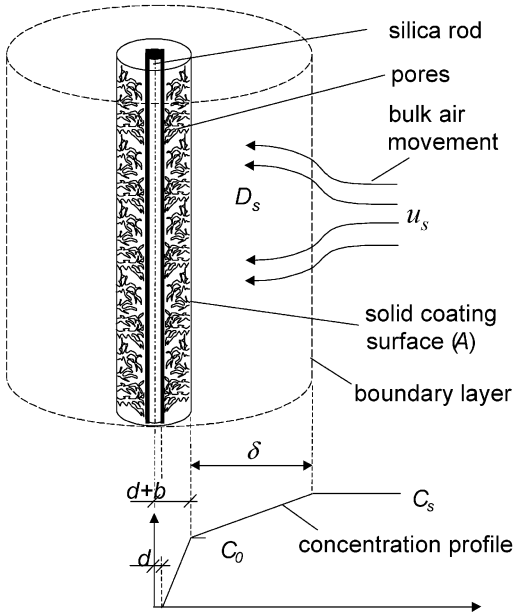

Figure 6. Schematic diagram of the diffusion-based calibration model for cylindrical geometry. The terms are defined in the text.

extraction is defined by the diffusivity of analytes through the boundary layer of the sample matrix and, thus, the corresponding diffusion coefficients rather than by distribution constants. This situation is illustrated in Figure 6 for cylindrical geometry of the extraction phase dispersed on the supporting rod.

The analyte concentration in the bulk of the matrix can be regarded as constant when a short sampling time is used and there is a constant supply of analyte as a result of convection. These assumptions are true for most types of sampling in which the volume of sample is much greater than the volume of the interface and the extraction process does not affect the bulk sample concentration. In addition, the solid coating can be treated as a "perfect sink" for analytes. Adsorption binding is frequently instantaneous and essentially irreversible. The analyte concentration on the coating surface is far from saturation and can be assumed to be negligible for short sampling times and the relatively low analyte concentrations in a typical sample. The analyte concentration profile can be assumed to be linear from C_s to C_0. In addition, the concentration of analyte on the coating surface (C_0) can be assumed to be zero when extraction begins. Diffusion of analytes inside the pores of a solid coating controls mass transfer from the outer to inner surfaces of the coating.

The function describing the mass of extracted analyte with sampling time can be derived[60] by use of the equation

$$n(t) = \frac{B_3 A D_s}{\delta} \int_0^t C_s(t)\, dt \qquad (11)$$

where n is the mass of analyte extracted (ng) in a sampling time (t), D_s is the gas-phase molecular diffusion coefficient, A is the outer surface area of the sorbent (for example, outer surface area of the coated rod in Figure 1 defined as $6.28(d + b)L$, where L is the length of the coated portion of the rod), δ is the thickness of the boundary layer surrounding the extraction phase, B_3 is a geometric factor, and C_s is the analyte concentration in the bulk of the sample. It can be assumed that the analyte concentration is constant for very short sampling times and, therefore, eq 11 can be further reduced to

$$n(t) = (B_3 D_s A/\delta) C_s t \qquad (12)$$

where t is the sampling time.[61]

It can be seen from eq 12 that the mass extracted is proportional to the sampling time, D_s for each analyte, and the bulk sample concentration and inversely proportional to δ. This is consistent with the fact that an analyte with a greater D_s will cross the interface and reach the surface of the coating more quickly. Values of D_s for each analyte can be found in the literature or estimated from physicochemical properties.[25] This relationship enables quantitative analysis. As mentioned above, the discussion assumes nonreversible adsorption. Equation 12 can be modified to enable estimation of the concentration of analyte in the sample for rapid sampling with solid sorbents:

$$C_s = n\delta / B_1 D_s A t \qquad (13)$$

The amount of extracted analyte (n) can be estimated from the detector response.

The thickness of the boundary layer (δ) is a function of sampling conditions. The most important factors affecting δ are the geometric configuration of the extraction phase, sample velocity, temperature, and D_s for each analyte. The effective thickness of the boundary layer can be estimated for the coated fiber geometry (see Figure 6) by use of eq 14, an empirical equation adapted from heat transfer theory:

$$\delta = 9.52(d/Re^{0.62} Sc^{0.38}) \qquad (14)$$

where Re is the Reynolds number $= 2u_s d/\nu$, u_s is the linear sample velocity, ν is the kinematic viscosity of the matrix, Sc is the Schmidt number $= \nu/D_s$, and d is the fiber diameter. The effective thickness of the boundary layer in eq 14 is a surrogate (or average) estimate and does not take into account changes of the thickness that can occur when the flow separates, a wave is formed, or both. Equation 14 indicates that the thickness of the boundary layer will decrease with increasing linear sample velocity (Figure 6). Similarly, when sample temperature (T_s) increases, the kinematic viscosity decreases. Because the kinematic viscosity term is present in the numerator of Re and in the denominator of Sc, the overall effect on δ is small. Reduction of the boundary layer and an increased rate of mass transfer for an analyte can be achieved in two ways—by increasing the sample velocity and by increasing the sample temperature. Increasing the temperature will, however, reduce the efficiency of the solid sorbent (reduced K^s_{es}). As a result, the sorbent coating might not be able to adsorb all molecules reaching its surface and it might, therefore, stop behaving as a "perfect sink" for all the analytes.

(59) Sukola, K.; Koziel, J.; Augusto, F.; Pawliszyn, J. *Anal. Chem.* **2001**, *73*, 13−18.

(60) Carslaw, H.; Jaeger, J. *Conduction of Heat in Solids*; Clarendon Press: Oxford, 1986.

(61) Koziel, J.; Jia, M.; Pawliszyn, J. *Anal. Chem.* **2000**, *72*, 5178−5186.

Equation 11 indicates that the initial extraction rate is proportional to the planar surface area of the extraction phase. The equilibration time can therefore be reduced by increasing the interfacial contact between the phases, by designing the extraction phases with appropriate configurations—thin flat films with high surface area.[62]

Headspace Extraction. Equations 11 and 12 indicate that use of the headspace above the sample as an intermediate phase might be an interesting means of accelerating extraction for analytes characterized by high Henry's law constants. When a thin extraction phase is used, the initial rate of extraction, and hence the extraction time, is controlled by diffusion of analytes present in the sample matrix through the boundary layer. Addition of a gaseous headspace facilitates enhanced transport into the extraction phase, because of the high diffusion coefficients of the analytes into the gas phase. To increase transport from the sample matrix into the headspace, the system can be designed to produce a well-agitated, large sample/headspace interface. This can be accomplished by use of large-diameter vials with good agitation, by purging, or even by use of spray systems. At room temperature, only volatile analytes are transported through the headspace. For low-volatility compounds, heating of the sample is a good approach, if loss in magnitude of the distribution constant can be accepted. The ultimate approach is to heat the sample and cool the extraction phase at the same time. Heating the sample not only increases the Henry's law constant but also induces convection in the headspace, because density gradients associated with temperature gradients present in the system result in higher mass transport rates. The cooling of the sorbent increases its adsorption capacity. Collection of analytes can be performed in the same vial, as discussed previously,[32] or can be separated in space, similarly to the purge-and-trap technique. In the heating−cooling experiments, both kinetic and thermodynamic factors are addressed simultaneously. Headspace approaches are also interesting because, as discussed above, adverse affects associated with the presence of solids, or oily or high molecular weight interferences, which can cause fouling of the extraction phase, are eliminated.

Extraction Combined with Derivatization. The selectivity and capacity of the extraction phase for analytes such as polar or ionic species, which are difficult to extract, can be frequently enhanced by introducing a derivatization step.[63] The objective of derivatization is not only to convert the native analytes into less polar derivatives that are extracted more efficiently but also to label them for better detection or chromatography. The most interesting implementation of this approach is simultaneous extraction/derivatization. In this technique, the derivatization reagent is present in the extraction phase during the extraction. The main advantage of this approach is that two steps are combined. Two limiting cases describe the combination of extraction and derivatization. The first occurs when mass transfer to the fiber is slow compared with the reaction rate. Under these conditions, eq 11, as discussed above, describes the rate of accumulation of the analytes, assuming that the derivative is trapped in the extraction phase.

In the second limiting case, the situation is reversed in that the reaction rate is slow compared with transport of analytes to the extraction phase. In other words, at any time during the extraction procedure the extraction phase is at equilibrium with the analyte in a well-agitated sample, resulting in a uniform reaction rate throughout the coating. This is typical for thinly dispersed extraction phases, because the equilibration time for well-agitated conditions is very short compared with a typical reaction rate constant. The accumulation rate of the product in the extraction phase n/t can then be defined by

$$n = V_e k_r K_{es} \int C_s(t)\, dt \qquad (15)$$

where C_s is the initial concentration of analyte in the sample and k_r is chemical reaction rate constant. In short, when the sample is of large volume, e.g., direct sampling in the field, the reaction and accumulation of analyte in the extraction phase proceeds with the same rate as long as reagent is present in excess. It is worth noting that the rate is also proportional to the extraction phase/sample matrix distribution constant. If the analyte concentration varies during accumulation, the amount collected corresponds to the integral over concentration and time, as will be discussed later for time-weighted average sampling. For limited sample volume, however, the concentration of analyte in the sample phase decreases with time as it becomes partitioned into the coating and converted to trapped product, resulting in a gradual decrease of the rate. The time required to extract analytes exhaustively from a limited volume can be estimated from the experimental conditions.[13]

Solvent Extraction. The approaches described above for method optimization can be implemented for both solvent- and polymer-based extraction. Effects of agitation on mass transfer in solvent extraction systems can be calculated by use of the two-film boundary layer model.[64,65] In typical liquid−liquid extraction with solvents, agitation helps increase both interfacial contact area and convection, thus increasing transport rate and resulting in a dramatically reduced extraction time. It was even observed in the solvent microextraction approach that convection within the solvent drop can be induced by interfacial friction, resulting in much faster than expected rates of extraction. This extraction enhancement is associated with low solvent viscosity and is not attainable in extraction with polymeric liquids, e.g., PDMS. As in polymeric systems, mass transfer is better for thin films than for round drops, because of greater surface area.[66] Volumes of extraction phase in solvent microextraction can be conveniently controlled and can be as small as picoliters,[67] making it compatible with microseparation technologies, and well suited to speciation studies.[68] Solvent microextraction processes can be performed practically in several different ways. The most convenient ones yet tested involve the falling drop arrangement,[69] use of a syringe to deliver a single drop of solvent at the tip of the needle,[70] use of

(62) Liu, X.; Bruheim, I.; Wu, J.; Pawliszyn, J. *Anal. Chem.* **2003**, *75*, 1002−1010.
(63) Rosenfeld, J. Recent Developments in the Chemistry and Application of Analytical Derivatizations. In *Sampling and Sample Preparation for Field and Laboratory*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, 2002.
(64) Furman, W. *Continuous Flow Analysis: Theory and Practice*; Marcel Dekker: New York, 1976.
(65) Ma, M.; Cantwell, F. *Anal. Chem.* **1999**, *71*, 388−393.
(66) Cardoso, A.; Dasgupta, P. *Anal. Chem.* **1995**, *67*, 2562−2566.
(67) Kogi, O.; Kim, H.; Kitamura, J. *Anal. Chim. Acta* **2000**, *418*, 129−135.
(68) Jeannot, M.; Cantwell, F. *Anal. Chem.* **1997**, *69*, 2935−2940.
(69) Liu, H.; Dasgupta, P.; *Anal. Chem.* **1997**, *67*, 44221−4228.
(70) Jeannot, M.; Cantwell, F. *Anal. Chem.* **1996**, *68*, 2236−2240.

a nonswelling porous solid fiber coating soaked with solvent in an SPME device,[71] and use of a porous hollow fiber membrane to support the solvent.[72]

Flow-Through Techniques. For homogeneous samples and a flowing fluid, description of the extraction process is much simpler and can be based directly on chromatographic theory for liquid stationary phases.[73] Let us consider another example of the flow-through system in which the extraction phase is dispersed as a thin layer inside the extraction bed and the sample flows through the cartridge. The bed can be constructed of a piece of fused-silica capillary or internally coated with a thin film of extracting phase (a piece of open tubular capillary GC column; in-tube SPME),[74] or the bed can be packed with extracting phase dispersed on an inert supporting material (SPE cartridge). In these geometric arrangements, the concentration profile along the axis x of the tubing containing the extracting phase, as a function of time t, can be described by adopting the expression for dispersion of a concentration front:

$$C(x,t) = 0.5C_s\left(1 - \mathrm{erf}\frac{x - (ut/(1+k))}{\sigma\sqrt{2}}\right) \qquad (16)$$

where u_s is linear velocity of the sample through the tube and k is the retention factor defined as

$$k = K_{es}(V_e/V_v) \qquad (17)$$

where K_{es} is a extraction phase/sample matrix distribution constant, V_e is the volume of the extracting phase, and V_V is a void volume of the tubing containing the extracting phase. σ is the root-mean-square dispersion of the front defined as

$$\sigma = \sqrt{Ht\frac{u}{1+k}} \qquad (18)$$

where H is the HETP (height equivalent to a theoretical plate) in chromatographic systems. This can be calculated as a sum of individual contributions to dispersion of the front. These contributions depend on the particular geometry of the extraction system.

Figure 7 illustrates the normalized concentration profiles produced in the bed during extraction.[25] Full breakthrough is obtained for the right-most curve, which corresponds to the appropriate volume of the sample matrix for microextraction. The time required to pass this volume through the extraction system corresponds to the equilibration time of the compound with the bed. In SPE extraction, on the other hand, the optimized extraction time is shorter to prevent loss of analyte. Figure 7 indicates that the extraction capacity per volume of extraction phase in microextraction is substantially greater than for the exhaustive approach.

Equations 16 and 17 and Figure 7 indicate that the analyte front migrates through the capillary/bed at a speed proportional to the linear velocity of the sample and is inversely related to the partition ratio. For in-tube SPME and short capillaries with small dispersion, the minimum extraction time for in-tube SPME under equilibrium conditions can be assumed to be the time required for the center of the front to reach the end of the capillary:

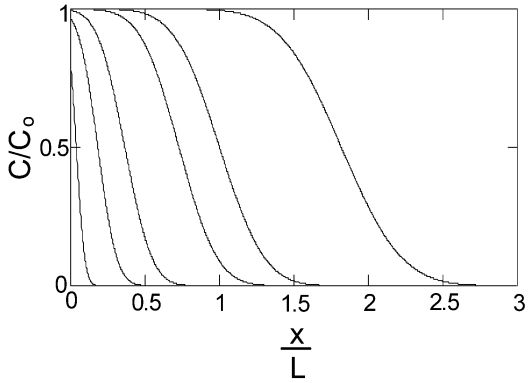

Figure 7. Normalized concentration profiles for in-tube SPME, calculated using the equation discussed in the text.

$$t_e = \frac{L(1 + K_{es}(V/V_v))}{u} \qquad (19)$$

where L is the length of the capillary holding the extraction phase. For packed-bed extractors typical of SPE techniques, analogous equations can be developed. For these, the calculated time corresponds to the maximum extraction time defined as the midpoint of the breakthrough curve. As expected, the extraction time is proportional to the length of the extraction bed and inversely proportional to the linear flow rate of the sample. Extraction time also increases with increasing extraction phase/sample distribution constant and with increasing volume of the extracting phase, but decreases with increasing void volume of the capillary/cartridge.

For example, when a gas or liquid sample is extracted with PDMS, it is important to disperse the extraction phase as a thin film to rapidly reach equilibrium between the phases and minimize resistance to flow. This approach is, however, difficult to apply in exhaustive techniques, because the low surface-to-volume ratio results in a small amount of extraction phase and breakthrough for small sample volumes.[75] The breakthrough volume can be increased by using PDMS-packed capillaries.[76] The open-tubular coated capillaries are, on the other hand, well suited to microextraction of gaseous and aqueous samples, as discussed above. They are very convenient for use in conventional LC automated systems without need for any modification.[77]

Membrane Extraction Techniques. One essential characteristic of membrane-based separation processes is the ease with which they can be adapted for continuous operation. This feature makes membranes very attractive sample preparation tools, because their application enables conversion of analytical separation and detection instruments into sensorlike devices suitable for monitoring operations. In combination with a microanalytical system, membrane extraction is, therefore, an attractive ap-

(71) Jia, C.; Luo, Y.; Pawliszyn, J. *J. Microcolumn Sep.* **1998**, *10*, 167−173.
(72) Pedersen-Bjergaard, S.; Rasmussen, K. *Anal. Chem.* **1999**, *71*, 2650−2656.
(73) Lovkvist, P.; Jonsson, J. *Anal. Chem.* **1987**, *59*, 818−821.
(74) Eisert, R.; Pawliszyn, J. *Crit. Rev. Anal. Chem.* **1997**, *27*, 103−135.
(75) Grob, K.; Habich, A. J. *Chromatogr., A* **1985**, *321*, 45−58.
(76) Baltussen, E.; David, F.; Sandra, P.; Janssen, H.-G.; Cramers, C. *Anal. Chem.* **1999**; *71*, 5193−5198.
(77) Eisert, R.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 3140−3147.

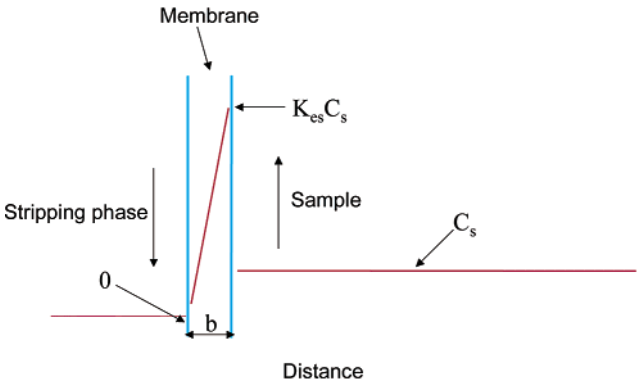

Figure 8. Membrane extraction with good sample agitation and stripping conditions. The terms are defined in the text.

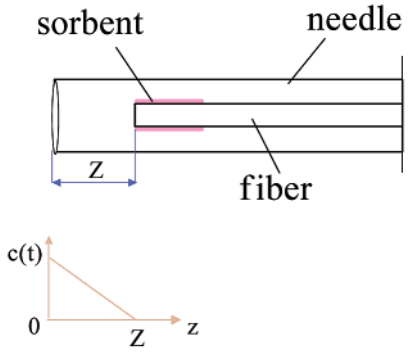

Figure 9. SPME/TWA approaches based on the in-needle coated fiber.

proach.[78] Permeation through a membrane is a unique extraction process in which sorption into and desorption out of the extraction phase occur simultaneously. The sample (donor phase) is in contact with one side of the membrane, where extraction into the membrane material occurs, whereas permeated analytes are removed by the stripping phase (acceptor). For membrane extraction with good flow (agitation) conditions at both acceptor and donor sites, low K_{es} and efficient stripping the effect of the boundary layers can be neglected and the rate of mass transport through the membrane is controlled by the diffusion of analytes through the membrane material. The concentration gradient, which facilitates transport across the membrane, is formed by the difference between the analyte concentrations on the sample side ($K_{es}C_s$) and the stripping phase; this difference is highest for high flow rates of the stripping phase (see Figure 8), under which conditions the concentration in the stripping phase approaches zero. The rate of mass transfer through the membrane, n/t, can be estimated under steady-state conditions by use of the equation

$$n/t = B_4 A D_e K_{es} C_s / b \qquad (20)$$

where A is the surface area of the membrane, D_e is the diffusion coefficient in the membrane material, K_{es} is the membrane material/sample matrix distribution constant, b is the thickness of the membrane, and B_4 is a geometric factor defined by the shape of the membrane. The permeation rate through the membrane is proportional to both the diffusion coefficient (D_e) and the distribution constant (K_{es}) and inversely proportional to b. D_e determines the rate of analyte migration through the membrane and K_{es} the magnitude of the concentration gradient generated in the membrane.[79] This information can be used to calibrate the extraction process a priori, if these values are obtained from tables or experimental data.[80] The concentration of the unknown can be calculated by rearranging eq 20:

$$C_s = bn / B_4 A D_e K_{es} t \qquad (21)$$

The membrane material/sample matrix distribution constant K_{es} determines the sensitivity of membrane extraction (see eq 20), indicating that the membrane is a physical barrier as well as a concentrating medium, analogous to the extraction phase in other configurations. The concentration of analyte in the stripping gas phase is, however, lower than in the sample, because a gradient must exist in the membrane to generate diffusive mass transfer through the membrane material (see Figure 8). Incorporating sorbent trapping after the membrane would therefore enable enrichment of the extract and increase the sensitivity of the analysis. When the membrane is in direct contact with the aqueous phase, mass transfer through the boundary layer surrounding the membrane could contribute to overall mass transfer in the system. For analytes characterized by high Henry's law constants, it is therefore important to consider the arrangement used for headspace membrane extraction.[81] In membrane extraction, the sample matrix/membrane material distribution constant is chosen to be a moderate value, to enable stripping of analytes at the receiving end. It is also possible to facilitate efficient and selective re-extraction by controlling chemical potential of the analyte on either side of the membrane, by taking advantage of analyte acid−base equilibria. Transport selectivity can also be achieved by incorporating a specific carrier in the extraction phase constituting the membrane.[82]

Passive Time-Weighted Average (TWA) Sampling. Consideration of different arrangements of the extraction phase, including the protective barriers discussed earlier, is beneficial. For example, extension of the boundary layer by a protective shield that restricts convection would result in a time-weighted average measurement of analyte concentration (see eq 11). A variety of diffusive samplers have been developed based on this principle. One system consists of an externally coated fiber with the extraction phase withdrawn into the needle (Figure 9). When the extracting phase in an SPME device is not exposed directly to the sample, but is contained within protective tubing (a needle) without any flow of sample through it, diffusive transfer of analytes occurs via the static sample (gas phase or other matrix) trapped in the needle. This geometric arrangement is a very simple method, capable of generating a response proportional to the integral of the analyte concentration over time and space (when the needle is moved through space).[83] Under these conditions, the only mechanism of analyte transport to the extracting phase is diffusion through the matrix contained in the needle. During

(78) Bao, L.; Dasgupta, P. *Anal. Chem.* **1992**, *64*, 991−996.
(79) Luo, Y.; Adams, M.; Pawliszyn, J. *Anal. Chem.* **1998**, *70*, 248−254.
(80) Luo, Y.; Pawliszyn, J. *Anal. Chem.* **2000**, *72*, 1064−1071.
(81) Luo, Y.; Pawliszyn, J. *Anal. Chem.* **2000**, *72*, 1058−1062.
(82) Jonsson, J.; Mathiasson, L. *Trends Anal. Chem.* **1999** *18*, 318−334.
(83) Chai, M.; Pawliszyn, J. *Environ. Sci. Technol.* **1995**, *29*, 693−701.

this process, a linear concentration profile (shown in Figure 9) is established in the tubing between the small needle opening, characterized by a surface area A and the distance, Z, between the needle opening and the position of the extracting phase. The amount of analyte extracted, dn, during time interval, dt, can be calculated by considering Fick's first law of diffusion:[4]

$$dn = AD_m \frac{dc}{dz} dt = AD_m \frac{\Delta C(t)}{Z} dt \qquad (22)$$

where $\Delta C(t)/Z$ is an expression of the gradient established in the needle between the needle opening and the position of the extracting phase, Z; $\Delta C(t) = C(t) - C_Z$, where $C(t)$ is the time-dependent concentration of analyte in the sample in the vicinity of the needle opening and C_Z is the concentration of the analyte in the vicinity of the coating. C_Z is close to zero for a high extraction phase/matrix distribution constant, then: $\Delta C(t) = C(t)$. The concentration of analyte, C_Z, at the coating position in the needle will increase with integration time, but it will remain low compared with the sample concentration, because of the presence of the extraction phase. The amount of analyte accumulated over time can therefore be calculated as

$$n = D_m \frac{A}{Z} \int C_s(t)\, dx \qquad (23)$$

As expected, the amount of analyte extracted is proportional to the integral of sample concentration over time, the diffusion coefficient of analyte in the matrix filling the needle, D_m, and the area of the needle opening, A, and inversely proportional to the distance, Z, of the coating from the needle opening. It should be emphasized that eqs 22 and 23 are valid only when the amount of analyte extracted on to the sorbent is a small fraction (below the RSD of the measurement, typically 5%) of the equilibrium amount for the lowest concentration in the sample. To extend integration times, the coating can be placed further into the needle (larger Z), the opening of the needle can be reduced by placing an additional orifice over the needle (smaller A), or a higher capacity sorbent can be used. The first two solutions will result in low measurement sensitivity. Increasing the sorbent capacity is a more attractive proposition. It can be achieved either by increasing the volume of the coating or by changing its affinity for the analyte. Because increasing the coating volume would require an increase in the size of the device, the optimum approach to increasing the integration time is to use sorbents characterized by large coating/gas distribution constants. If the matrix filling the needle is something other than the sample matrix, an appropriate diffusion coefficient should be used in eq 23, as discussed below for membrane extraction.

In the system described, the length of the diffusion channel can be adjusted to ensure that mass transfer in the narrow channel of the needle controls overall mass transfer to the extraction phase, irrespective of convection conditions.[84] This is a very desirable feature of TWA sampling, because the performance of this device is independent of the flow conditions in the system investigated. This is difficult to ensure for high surface area membrane permeation-based TWA devices, for example, passive diffusive badges[85] and semipermeable membrane devices.[86] For analytes characterized by moderate to high distribution constants, mass transport is controlled by diffusive transport in the boundary layer. The performance of these devices therefore depends on the convection conditions in the investigated system.[87]

SIGNIFICANCE OF FUNDAMENTAL DEVELOPMENTS

Optimization. Whenever a new type of complex sample is considered, a small research project is frequently conducted to find optimum extraction conditions enabling the most efficient and most complete release of native analytes from the matrix and their partitioning into the extraction phase. Typically, an empirical approach is used and several parameters are varied, for example, the chemical properties of the extraction phase or types of additive/reagent used. Better understanding of the analyte/matrix interaction would facilitate more rational choice of extraction conditions on the basis of models, when the characteristics of the analyte and the matrix are known. With solid matrixes, however, greater research effort is required to reach the level of fundamental knowledge necessary for practical implementation of such an approach.

Calibration. Reliance on physicochemical constants in calibration results in rapid and cost-effective procedures but might seem unconventional or even uncomfortable to some researchers. As theory indicates, however, these constants define the extraction process, and there is an opportunity to take advantage of this. Physicochemical constants can be frequently estimated from simple experiments or calculated by considering the molecular structures of analytes, extraction phase, and matrix; this adds to the attractiveness of this approach. For equilibrium microextraction techniques, the extraction phase/sample matrix distribution constant is used to quantify the concentration of analytes in the sample matrix (see eq 3). For extraction approaches controlled by mass transfer, calibration can be based on the diffusion coefficient in the sample matrix for constant extraction time under well-defined convection conditions (see eqs 12 and 23). When the derivatization reaction kinetics control the rate of extraction, the rate constant can provide a means of calibration (see eq 15). Occasionally, for example, in membrane extraction, a combination of constants defines the rate of extraction and can be used for calibration also (see eq 20). The major argument against using this approach is that physicochemical constants are affected by many experimental conditions, for example, temperature and the properties of the matrix. The impact of temperature change can, however, be compensated by monitoring the temperature, using correction factors, and allowing for direct calibration for simple matrixes.[88] For more complex matrixes, internal standard or standard addition calibrations, routinely applied in exhaustive techniques to monitor recoveries, can be used to compensate for matrix variations.[89] In future research, correlations between

(84) Chen, Y.; Pawliszyn, J. *Anal. Chem.* **2003**, *75*, 2004−2010.

(85) Koziel, J. Sampling and Sample Preparation for Indoor Air Analysis. In *Sampling and Sample Preparation for Field and Laboratory*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, 2002.

(86) Petty, J.; Orazio, C.; Huckins, J.; Gale, R.; Lebo, J.; Echols, K.; Cranor, W. *J. Chromatogr., A* **2000**, *879*, 83−95.

(87) Vrana, B.; Schuurmann, G. *Environ. Sci. Technol.* **2002**, *36*, 290−296.

(88) Martos, P.; Pawliszyn, J. *Anal. Chem.* **1997**, *69*, 206−215.

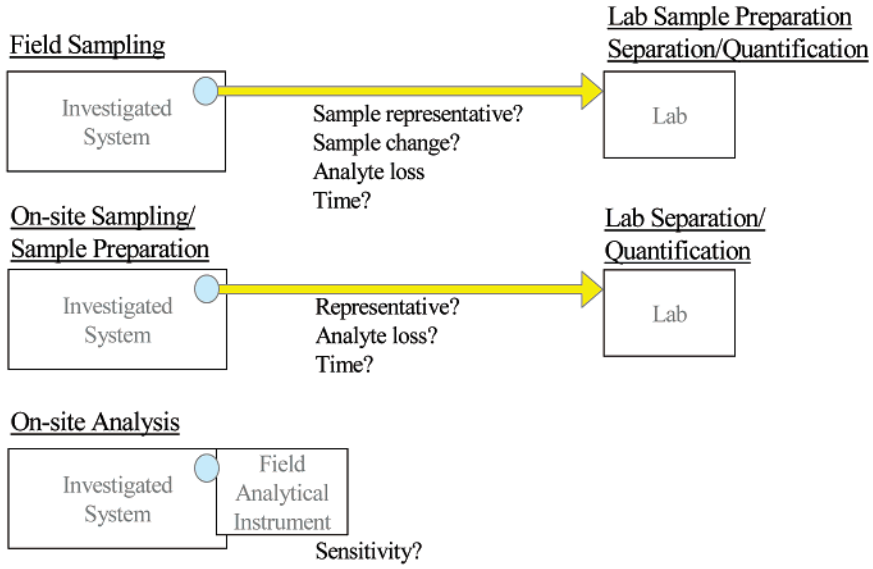

Figure 10. Benefits of on-site analysis.

distribution constants and simple measurements such as turbidity and pH might be found to account for matrix variations for a given type of matrix and, therefore, eliminate the need for internal calibration.

One can draw several parallels between developments and applications of extraction techniques with electrochemical methods. For instance, the coulometric technique corresponds to total or exhaustive extraction methods. Although the most precise, this technique is not used frequently because of the time required to complete it. Equilibrium potentiometric techniques are more frequently used (pH electrode), particularly when the sample is a simple mixture or the selectivity of the membrane in an ion-selective electrode is sufficient to quantify the target analyte in complex matrixes. The equilibrium microextraction approach has further advantages in selectivity, because the extraction is coupled with separation, specific detection (e.g., mass spectrometry), or both, which enables identification and quantification of many components simultaneously. The advantage of electrochemical methods is a short response time, because of the low capacities of electrodes. Design of microsystems with cylindrical geometry facilitates rapid extraction, as in microelectrodes.[90] Some electrochemical methods, e.g., amperometry, are based on mass transport through the boundary layers, as in preequilibrium extraction techniques (e.g., TWA and diffusion based). Analogously, extraction calibration based on diffusion coefficients can be accomplished as long as the agitation conditions are constant, the extraction times are short, and the coating has high affinity for the analytes.

On-Site Implementation. The advantages of nonexhaustive extraction are its fundamental simplicity and fewer geometric restrictions. This facilitates several interesting on-site implementations by integrating sampling and sample preparation. For instance, sample introduction to miniaturized analytical field instrumentation should be more convenient. More information about the investigated system can also be obtained. It is, for example, possible to speciate and determine the distribution of analytes in multiphase systems, because the extraction process does not disturb the equilibria naturally present in the systems. Different forms of an analyte are therefore extracted and quantified according to their corresponding distribution constants, diffusion coefficients, or both.

Simplification of sample preparation technologies and their integration with sampling or separation/quantification steps in the analytical processes are significant challenges to, and opportunities for, the contemporary analytical chemist. These developments will eventually enable attainment of a major goal of the analytical chemist—to perform analysis at the place where the sample is taken, rather than moving the sample to a laboratory, as is traditional (see Figure 10). The on-site analysis approach reduces errors and the time associated with sample transport and storage, resulting in more accurate, precise, and faster analytical data. The trend in analytical instrumentation is toward miniaturization, which results in portability and on-site compatibility. Simplification and miniaturization of sampling and sample preparation is a logical next step.

Miniaturization, Automation, and Integration. Practical integration of sample preparation with the rest of the analytical process has been accomplished in several ways. The concept of flow injection analysis (FIA) has facilitated the performance of sequential sample preparation processes and quantification in a single device with the help of a flowing stream.[91] These devices can be made very small by using capillary flowing systems integrated, for example, with small semiconductor light emission and detection devices that use fiber optics,[92] and can be implemented on-site, for example, in combination with single solvent drop detection.[93] Further miniaturization of FIA technology results in a whole sample preparation process being performed in the body of a single valve ("lab on valve").[94]

(89) Grote, C.; Levsen, K. The Application of SPME in Water Analysis. In *Applications of Solid-Phase Microextraction*; Pawliszyn, J., Ed.; Royal Society Chemistry: Cambridge, U.K., 1999.
(90) Heinze, J. Angew. *Chem., Int. Ed. Engl.* **1993**, *32*, 1268−1288.
(91) Fang, Z.-L. *Flow Injection Separation and Preconcentration*; VCH: Weinheim, 1993.
(92) Pawliszyn, J. *Spectrochim. Acta Rev.* **1990**, *13*, 311−354. Pawliszyn, J. *Anal. Chem.* **1986**, *58*, 3207−3215.

The application of micromachining technology to the construction of highly integrated analytical systems (μTAS or "lab on a chip") has recently resulted in sample preparation being performed in machined microchannels.[95] μTAS enables effective coupling of separation/detection processes with sample preparation similar to capillary-based devices but can, potentially, be mass-produced at much lower cost.

Integration of sample preparation in the microdevices with the other steps of the analytical process can be accomplished in two fundamentally different ways. First, analogous to flow injection analysis, sample preparation may be performed directly in the capillaries/microchannels in the flowing systems. This approach would typically use flow-through sample preparation techniques (see Figure 1). These devices are expected to be structurally complex and relatively large, because they must incorporate valves to control flows, pumps, or high-voltage supply, sample, reagent ports, and detection components.[96] In addition, because of the high surface area/volume ratio, there is a possibility of sample losses and carry-over in a complex channel network. Integration of the sampling step with this complex system might be a challenge. It can be addressed by using moisture-repellent sorbents, electromigration focusing mechanisms, membranes, and solvent microextraction when the mobile phase in the separation technique is a solvent. For example, attempts are being made to integrate CE with sampling/concentration[97] and a micromachined GC system with sampling via a microsorbent trap.[98] Membrane sampling interfaced to an investigated system could facilitate sampling of aqueous media, as is currently performed in microdialysis systems coupled to condensed phase separations[99] or membrane extraction with sorbent interface (MESI) coupled to micro gas chromatography.[100] Recent developments in polymer manufacturing of microfluidic systems, including PDMS,[101] will facilitate these approaches, because this material is an excellent extraction phase. In addition, membraneless approaches exploring differences in diffusion coefficients between small and large molecules can be explored in designing interfaces.[102] Because the overall size of the fully integrated device is expected to be relatively large, there will always be some restriction on the dimensions of the object that can be investigated. The most significant limitation of this approach, however, is expected to be the cost, because unique configurations would be required for each specific application. This restriction would make the approach cost-competitive only for very popular applications, when mass production is justified.

The alternative approach involves integration of sampling with sample preparation only, by performing extraction and sample processing directly in the sampling device and then on-site introduction to a microseparation/quantification instrument. The extraction process can be made very selective for target analytes, limiting disturbance of the system investigated. If the sampling/sample preparation device is small enough, it can deliver the prepared samples directly into the separation channel/capillary of the separation/quantification microdevice. For example, in-microneedle and on-fiber microsampling devices could enable such a method, because processing reagents can be either drawn into the needle or delivered on to the fiber by dipping or by use of a spray.[103] Prepared analytes can subsequently be introduced to the micro device for separation and quantification. Because sample preparation is performed directly in the sampling system, external to the separation/quantification device, restrictions applied to one device will not have to be arbitrarily applied to the other. Low-cost generic microseparation/microdetection devices can be used as long as they are designed to accommodate a specific configuration of sampler. The major limitation of this approach is in monitoring and parallel analysis applications for which separate miniaturized automated systems would be required to control the device to perform sample preparation and, occasionally, sampling also. It is, however, sometimes possible to prepare an extraction phase that already contains the required reagents before sampling.[104] In this approach to on-site analysis, optimization of the design of the sampling/sample preparation systems is conducted independently. Much smaller and flexible devices are expected, compared with the previously described fully integrated single microdevice. Several of the sample preparation technologies listed in Figure 1, including batch extraction techniques such as coated microfibers, can be explored for this application.

In Vivo Analysis. The sampling procedures in the integrated on-site microdevices described above are a significant departure from conventional "sampling" techniques in which a portion of the system under study is removed from its natural environment and the compounds of interest are extracted and analyzed in a laboratory environment. There are two main motivations for exploring these types of configuration. The first is the desire to study chemical processes in association with the normal biochemical milieu of a living system; the second is the lack of availability, or the impracticality, frequently associated with size, of removing suitable samples for study from the living system. New approaches of an externally coated extraction phase on a microfiber mounted in a syringelike device, packed microneedles, or on-line sampling from a membrane interface seem to be logical targets for the development of such tools. As with any microextraction or membrane technique, compounds of interest are not exhaustively removed from the investigated system. On the contrary, conditions can be devised in which only a small proportion of the total compounds are removed and none of the matrix is removed, thus avoiding disturbance of the normal balance of chemical components. Second, because it is either a syringelike device that can be physically removed from the laboratory environment for sampling or it is an integrated micromembrane system, it is suitable for monitoring of a living system in its natural environment, rather than trying to move the

(93) Liu, H.; Dasgupta, P. *Anal. Chim. Acta* **2003**, *479*, 151−165.
(94) Wu, C.-H.; Scampavia, L.; Ruzicka, J. *Analyst* **2002**, *127*, 898−905 and references therein.
(95) Greenwood, P.; Greenway, G. *Trends Anal. Chem.* **2002**, *21*, 726−740.
(96) Huang, Y.; Mather, E.; Bell, J. *Anal. Bioanal. Chem.* **2001**, *372*, 49−65.
(97) Zhu, L. Y.; Tu, C. H.; Lee, H. K., *Anal. Chem.* **2001**; *73*, 5655−5660. Wu, X.-Z. *Trends Anal. Chem.* **2003**, *22*, 48−58.
(98) Potkay, J.; Driscoll, J.; Agha, M.; Sacks, R.; Wise, K. A High-Performance Microfabricated Gas Chromatography Column. Proceedings of the Sixteenth Annual IEEE Conference on Micro Electro Mechanical Systems (MEMS), Kyoto, Japan, January 19−23, 2003; pp 395−398.
(99) Blakely, R.; Wages, S.; Justice, J., Jr.; Herndon, J.; Neil, D. *Brain Res.* **1984**, *308*, 1−12.
(100) Segal, A.; Gorecki, T.; Mussche, P.; Lips, J.; Pawliszyn, J. *J. Chromatogr., A* **2000**, *873*, 13−28.
(101) Ng, J.; Stroock, A.; Whitesides, G. *Electrophoresis* **2002**, *23*, 3461−3473.
(102) http://www.micronics.net/technologies/h_filter.php.
(103) Pawliszyn, J. Solid-Phase Microextraction. In *Sampling and Sample Preparation for Field and Laboratory*; Pawliszyn, J., Ed.; Elsevier: Amsterdam, 2002.
(104) Koziel, J.; Noah, J.; Pawliszyn, J. *Environ. Sci. Technol.* **2001**, *35*, 1481−1486.

living system to an unnatural laboratory environment. Microdialysis systems are already used in animal studies,[105] and MESI has been used in breath monitoring.[106] The coated microfiber approach has recently been used in drug biodegradation studies—the components of interest were extracted directly from a peripheral vein of an animal.[107] To further improve the capability of SPME for in vivo sampling, new specific coatings, e.g., affinity phases, should be developed for a range of important target analytes. The ultimate goal is to remove only those compounds required to characterize the system investigated and none of the matrix, using molecular recognition approaches, as it is frequently performed in sensor arrays.[108] This specific direct extraction approach is critical to minimizing interference with the operation of the system investigated. For example, removal of neurotransmitters from the synaptic cleft results in elimination of the signal coming down the nerves, depletion the presynaptic stores of the transmitter, or both. In addition, specific nonequilibrium direct extraction might facilitate sampling at the speed of biological processes. The extraction can be limited to small number of molecules and combined with on-probe amplification approaches, single molecular detection schemes, or both, facilitating investigation of analytes present in the system at low copy number.

Conclusion. Rapid progress in material science, nanotechnology, wireless communication, and low-energy-consumption devices will accelerate development of on-site autonomous analytical instrumentation capable of supplying continuous information about the investigated system. These high-performance monitoring devices, organized in a wireless computerized network, will enable rapid data analysis and immediate decision-making with regard to health, security, and environmental protection issues. Direct coupling of these devices to the investigated system would require the analytical chemist to become very familiar with the investigated system and operation of the sampling interface to generate reliable analytical data. Deeper fundamental understanding of the extraction processes will facilitate exploration of these new opportunities and will make sample preparation research more vital and scientifically rewarding.

ACKNOWLEDGMENT

I thank my co-workers who contributed to sample preparation research in my laboratory and the Natural Sciences and Engineering Council of Canada for financial support. Heather Lord, Carolyn Goodridge, and Jack Rosenfeld provided helpful input.

Received for review January 31, 2003. Accepted April 3, 2003.

AC034094H

(105) Song, Y.; Lunte, C. *Anal. Chim. Acta* **1999**, *400*, 143−152.
(106) Lord, H.; Yu, W.; Segal, A.; Pawliszyn, J. *Anal. Chem.* **2002**, *74*, 5650−5657.
(107) Lord, H.; Grant, R.; Incledon, B.; Walles, M.; Fahie, B.; Pawliszyn, J. Development and Evaluation of a SPME Probe for In−Vivo Pharmacokinetic Studies. Submitted to *Anal. Chem*.
(108) Michael, K.; Taylor, L.; Schultz, S.; Walt, D. *Anal. Chem.* **1998**, *70*, 1242−1248.