Theory of Solid-Phase Microextraction

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Abstract

The main objective of this contribution is to describe the fundamental concepts associated with solid-phase microextraction (SPME). Theory provides insight when developing SPME methods and identifies parameters for rigorous control and optimization. A mathematical model has been developed to understand the principal processes of SPME by applying basic fundamental principles of thermodynamics and diffusion theory. The model assumes idealized conditions and is limited to air, liquid, or headspace above liquid sampling. Theory for ideal cases can be quite accurate for trace concentrations in simple matrices such as air or drinking water at ambient conditions when secondary factors such as thermal expansion of polymers and changes in diffusion coefficients because of solutes in polymers can be neglected. When conditions are more complex, theory for ideal cases still efficiently estimates general relationships between parameters.

Introduction

Solid-phase microextraction (SPME) was developed to address the need to facilitate rapid sample preparation both in the laboratory and on-site where the investigated system is located (1). In the technique, a small amount of extracting phase that is dispersed on a solid support is exposed to the sample for a well-defined period of time. In one approach, a partitioning equilibrium between the sample matrix and the extraction phase is reached. In this case, convection conditions do not affect the amount extracted. In a second approach that uses short-time pre-equilibrium extraction, if convection or agitation or both are constant, then the amount of analyte extracted is related to time. Quantitation can then be performed based on timed accumulation of analytes in the coating. Figure 1 illustrates several implementations of SPME that have been considered. These mainly include open-bed extraction concepts such as coated fibers, vessels, and agitation mechanism disks, but in-tube approaches are also considered. Some implementations better address issues associated with agitation, and others better address the ease of implementing sample introduction to the analytical instrument.

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It should be noted that SPME was originally named after the first experiment that used an SPME device, which involved extraction on solid fused-silica fibers. Then, it was later renamed to be a reference to the appearance of the extracting phase in relation to a liquid or gaseous donor phase, even though it is recognized that the extraction phase is not always technically a solid.

The configurations and operation of SPME devices are very simple. For example, in the coated fiber implementation of the technology, one who knows how to use a syringe is able to operate an SPME device. In the case of automated in-tube extraction for high-performance liquid chromatography (HPLC), fitting a piece of the gas chromatography (GC) capillary into the system and then turning on the autosampler is all that is required to start its operation. The technology is designed to greatly simplify sample preparation. However, this feature creates a false impression that the extraction is a simple, almost trivial process. This misunderstanding frequently results in disappointments. It should be emphasized that the fundamental processes involved in SPME are similar to more traditional techniques, and therefore, challenges to develop successful methods are similar. The nature of target analytes and the complexity of the sample matrix determine the level of difficulties in accomplishing a successful extraction. The simplicity, speed, and convenience of the extraction devices primarily impact the costs of the practical implementation and automation of the developed methods (2). The objective of this

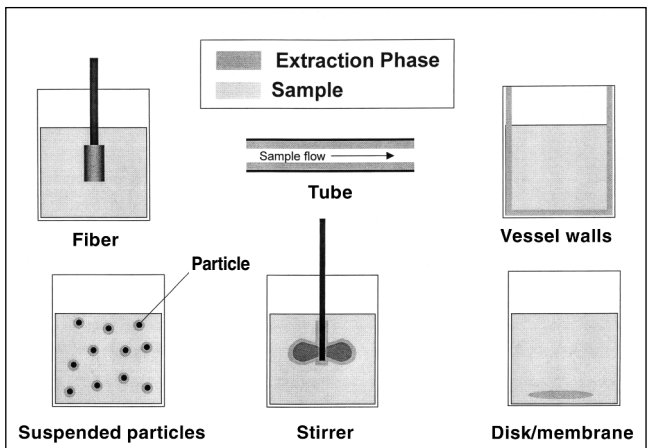

Figure 1. SPME configurations.

contribution is to emphasize the fundamental principles of the technique that define the advantages and limitations of SPME technology.

Discussion

Principles of SPME

In SPME, a small amount of the extracting phase associated with a solid support is placed in contact with the sample matrix for a predetermined amount of time. If the time is long enough, a concentration equilibrium is established between the sample matrix and the extraction phase. When equilibrium conditions are reached, exposing the fiber for a longer amount of time does not accumulate more analytes. There are 2 different implementations of the SPME technique extensively explored to date. One implementation is associated with a tube design, and the other is associated with fiber design. The tube design can use very similar arrangements (such as SPE); however, the primary difference (in addition to the volume of the extracting phase) is that the objective of SPME is never that of an exhaustive extraction. This difference substantially simplifies the design of systems. For example, in the analysis of liquids, in-tube SPME uses 0.25-mm-i.d. tubes and approximately 0.1 µL of the extraction phase; concern for breakthrough is not relevant because exhaustive extraction is not an objective. In fact, the objective of the experiment is to produce full breakthrough as soon as possible, because this indicates that equilibrium extraction has been reached.

A more traditional approach to SPME involves coated fibers. The transport of analytes from the matrix into the coating begins as soon as the coated fiber has been placed in contact with the sample. There is a substantial difference in performance between the liquid and solid coatings (Figure 2). A comparison with adsorptive versus absorptive equilibrium extraction is useful. In both cases, the extraction process begins by the adsorption of analytes at the extraction phase–matrix interface, and then diffusion of analytes into the bulk of the extraction-phase follows. If the diffusion coefficients of the analytes in the extraction phase are high, then the analytes partition fully between the 2 phases, and absorptive extraction is accomplished. This process is aided by thin extraction phase coatings or the convection of the sample matrix (if flowing liquid). However, if the diffusion coefficient is low, the analyte remains at the interface and adsorption results. The principle advantage of absorption extraction (partitioning) is a linear isotherm over a wide range of analyte and interference concentrations, because the property of the extraction phase does not change substantially until the extracted amount is approximately 1% of the extraction phase weight. However, in adsorption extraction, the isotherm is highly nonlinear for higher concentrations when the surface coverage is substantial. This causes a particular problem in the equilibrium methods because the response of the fiber for the analyte at high sample concentrations depends on the concentrations of both analytes and interferences. The advantages of the solid sorbents include higher selectivity and capacity for polar and volatile analytes.

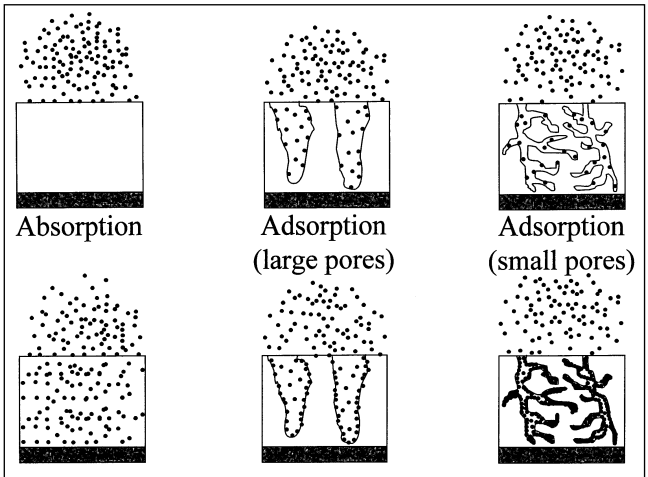

Figure 2. Schematic representation of absorptive versus adsorptive extraction and adsorption in small versus large pores.

Multiphase equilibria

SPME is a multiphase equilibration process. Frequently, the extraction system is complex, such as in a sample consisting of an aqueous phase with suspended solid particles having various adsorption interactions with analytes, plus a gaseous headspace. In some cases, specific factors have to be considered, such as analyte losses by biodegradation or adsorption on the walls of the sampling vessel or stirring mechanism. In this discussion, only 2 phases are considered: (a) the fiber coating and (b) a homogeneous matrix such as pure water or air.

Typically, SPME is considered to be complete when the analyte concentration has reached distribution equilibrium between the sample matrix and the fiber coating. In practice, this means that once equilibrium has been reached, the extracted amount is constant within the limits of experimental error and it is independent of further increases of extraction time. The equilibrium conditions can be described as:

$$n = \frac{(K_{fs} V_f V_s C_0)}{(K_{fs} V_f + V_s)} \quad (1)$$

where n is the mass of analyte extracted by the coating, K_{fs} is a fiber coating–sample matrix distribution constant, V_f is the fiber-coating volume, V_s is the sample volume, and C_0 is the initial concentration of a given analyte in the sample (3).

Strictly speaking, this discussion is limited to the partitioning equilibrium that involves liquid polymeric phases such as polydimethylsiloxane (PDMS). The method of analysis for solid sorbent coatings is the same for the low analyte concentration because the total surface area available for adsorption is proportional to the coating volume if constant porosity of the sorbent is assumed. For high analyte concentrations, saturation of the surface may occur, resulting in nonlinear isotherms (as will be discussed). Similarly, the high concentration of a competitive interference compound can displace the target analyte from the surface of the sorbent.

Equation 1 (which assumes that the sample matrix can be represented as a single homogeneous phase and no headspace is present in the system) can be modified to account for the existence of other components in the matrix by considering the volumes of

the individual phases and the appropriate distribution constants. The extraction can be interrupted and the fiber analyzed prior to equilibrium. However, to obtain reproducible data, constant convection conditions and careful timing of the extraction are necessary.

Simplicity and convenience of operation make SPME a superior alternative to more established techniques in a number of applications. In some cases, the technique facilitates unique investigations. Equation 1 indicates that after equilibrium has been reached, there is a direct proportional relationship between the sample concentration and the amount of analyte extracted. This is the basis for analyte quantification. The most visible advantages of SPME exist at the extremes of sample volumes. Because the setup is small and convenient, coated fibers can be used to extract analytes from very small samples. For example, SPME devices are used to probe for substances emitted by a single flower bulb during its life span—the use of submicrometer-diameter fibers permits the investigation of single cells. Because SPME does not exhaustively extract target analytes, its presence in a living system should not result in a significant disturbance. In addition, the technique facilitates speciation in natural systems, because the presence of a minute fiber that removes small amounts of analyte is not likely to disturb chemical equilibria in the system. However, it should be noted that the fraction of analyte extracted increases as the ratio of coating to sample volume increases. Complete extraction can be achieved for thick coatings and small sample volumes when distribution constants are reasonably high. This observation can be used as an advantage if exhaustive extraction is required. It is very difficult to work with small sample volumes using conventional sample-preparation techniques. Also, SPME allows for the rapid extraction and transfer of a sample volume to an analytical instrument. These features result in an additional advantage when investigating intermediates in the system. For example, SPME was used to study the biodegradation pathways of industrial contaminants (4). The other advantage is that this technique can be used for studies of the distribution of analytes in a complex multiphase system (5) and to speciate different forms of analytes in a sample (6).

In addition, when the sample volume is very large, equation 1 can be simplified to:

$$n = K_{fs} V_f C_0 \qquad (2)$$

This equation points to the usefulness of the technique for field applications. In this equation, the amount of extracted analyte is independent of the volume of the sample. In practice, there is no need to collect a defined sample prior to analysis because the fiber may be exposed directly to ambient air, water, or the production stream. The amount of the extracted analyte corresponds directly to its concentration in the matrix without being dependent on the sample volume. When the sampling step is eliminated, the whole analytical process can be accelerated, thus errors associated with analyte losses through decomposition or adsorption on the sampling container walls can be prevented. This advantage of SPME could be enhanced practically by developing portable field devices on a commercial scale.

Rapid sampling

In the case of solid sorbents, the coating has a well-defined crystalline glass structure, which (if dense) substantially reduces the diffusion coefficients within the structure. Therefore, within the experimental time, the extraction occurs only on the surface of the coating. This can be demonstrated by considering the extraction of proteins (illustrated in Figure 3). The original mixture contains 3 compounds: myoglobin, cytochrome, and lysozyme. During fiber extraction with polyacrylic acid, compounds with weaker affinity are only observed at short extraction times. When the extraction time is longer, the displacement of analytes with lower affinities occurs. In this case, lysozyme (having a stronger affinity for the coating) replaces the other 2 compounds during extraction. This effect is associated with the fact that there is only limited surface area available for adsorption. If this area is substantially occupied, then the displacement effects occur (7,8) and the equilibrium amount extracted may vary with concentrations of both the target and other analytes. However, in the extraction of analytes with liquid coatings, partitioning between the sample matrix and extraction phase occurs. In this case, equilibrium extraction amounts vary only if the coating property is modified by the extracted components, which only occurs when the amount extracted is a substantial portion (a few percent) of the extraction phase. This is very rarely observed because SPME is typically used to determine trace contamination samples.

The only way to overcome this fundamental limitation of the porous coatings is to use an extraction time much less than the equilibrium time so that the total amount of analytes accumulated onto the fiber is substantially below the saturation value (suggested by Figure 3). When performing such experiments, not only is it critical to precisely control extraction times, but it is also important to monitor convection conditions in order to ensure that they are constant or can be compensated. One way of eliminating the need for compensation of convection is to normalize or use the same agitation conditions. For example, the stirring means used at well-defined rotation rates in the laboratory or fans used for field air monitoring ensure consistent convection.

The short-time-exposure SPME measurement described has an advantage associated with the fact that the rate of extraction is defined by the diffusivity of analytes through the boundary layer of the sample matrix and the corresponding diffusion coefficients

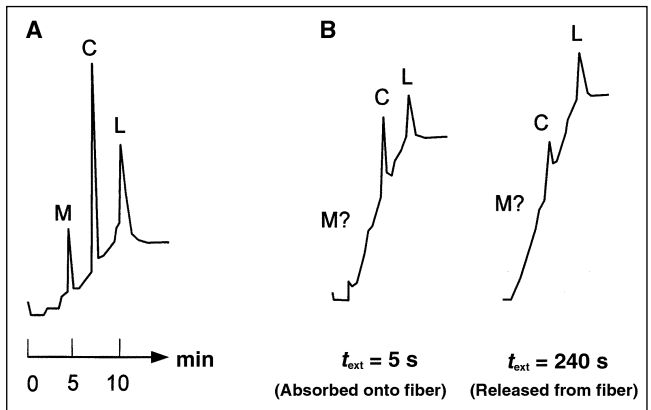

Figure 3. Demonstration of myoglobin (M) and cytochrome (C) displacement by lysozyme (L) with time (A) and protein extraction using a polyacrylic acid-coated fiber (B).

rather than the distribution constants (Figure 4). The mass of extracted analyte can be estimated from the following equation (9):

$$n(t) = \frac{2\pi D_g L}{\ln\left(\frac{b+\delta}{b}\right)} C_g t \quad (3)$$

where n is the mass of extracted analyte over sampling time (t), D_g is the gas-phase molecular diffusion coefficient (cm^2/s), b is the outside radius of the fiber coating (cm), L is the length of the coated rod (cm), δ is the thickness of the boundary layer surrounding the fiber coating (cm), and C_g is the analyte concentration in the bulk air (ng/mL). The linear uptake of the analytes with respect to time for short exposures is demonstrated in Figure 5.

A precise understanding of the definition and thickness of the boundary layer in this sense is useful. The thickness of the boundary layer is determined by both the rate of convection (agitation) in the sample and analyte diffusion (10). Thus, in a single sample, the boundary layer thickness will be different for various analytes. Strictly speaking, the boundary layer is a region in which analyte flux is progressively more dependent on analyte diffusion and less on convection when the extraction phase is approached. However, 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. The thickness of the boundary layer is defined as the position in which this transition occurs, or the point at which convection into the boundary layer is equal to diffusion away from the boundary layer. At this point, diffusion-controlled analyte flux from the thickness of the boundary layer towards the extraction phase is equal to the analyte flux from the bulk of the sample towards the thickness of the boundary layer, which is controlled by convection. The differences in the diffusion coefficients between compounds are small compared to the differences in the distribution constants. This makes it easier to calibrate the system. Because of the large differences in the distribution constants between analytes, the resulting chromatograms are characterized by small peak areas for compounds with small distribution constants and large peak areas for those with large constants. With the uptake dependent on diffusion coefficients, all compounds with similar molecular masses in a chromatogram will have similar peak areas (given they have similar detector responses). Also, it is relatively simple to calculate the diffusion coefficient for a particular analyte and also to correct for the small differences in it. It must be understood that this system is only suitable for trace analysis. When sample concentrations become too high, saturation of the active sites occurs and uptake rates are no longer linear. Shorter exposure times in which smaller amounts are extracted can solve this problem. Also, at these higher concentrations, samples are easily extracted and analyzed with a PDMS fiber using conventional SPME extraction methods. The results of extraction by the diffusion-type approach are shown in Figure 6. The accumulation of volatile components on the solid coating in 10 s is much larger compared with the 10-min equilibrium extraction on PDMS. This approach to extraction is not limited to devices using the fiber geometry, but is generally applicable.

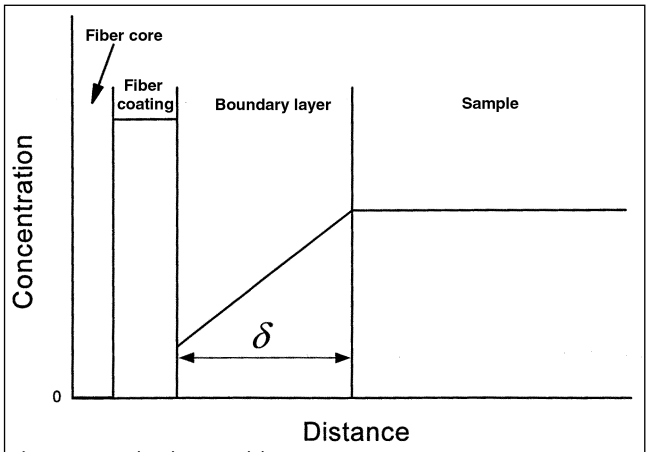

Figure 4. Boundary layer model.

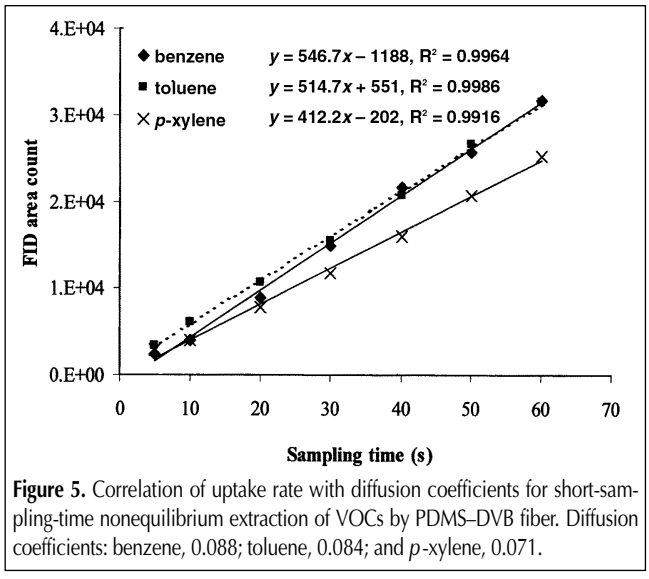

Figure 5. Correlation of uptake rate with diffusion coefficients for short-sampling-time nonequilibrium extraction of VOCs by PDMS–DVB fiber. Diffusion coefficients: benzene, 0.088; toluene, 0.084; and *p*-xylene, 0.071.

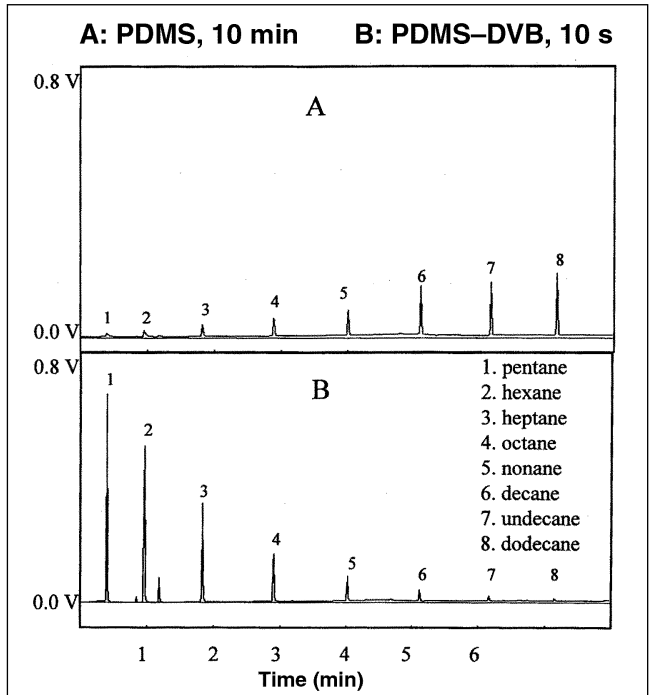

Figure 6. Comparison of VOC mass loading between PDMS and PDMS–DVB.

Extraction modes with coated fiber SPME

SPME sampling can be performed in 3 basic modes: (a) direct extraction, (b) headspace extraction, and (c) extraction with membrane protection. Figure 7 illustrates the differences between these modes. In direct extraction mode (Figure 7A), the coated fiber is inserted into the sample and the analytes are transported directly from the sample matrix to the extracting phase. To facilitate rapid extraction, some level of agitation is required to transport the analytes from the bulk of the sample to the vicinity of the fiber. For gaseous samples, the natural flow of air (e.g., convection) is frequently sufficient to facilitate rapid equilibration for volatile analytes, but for aqueous matrices, more efficient agitation techniques such as fast sample flow, rapid fiber or vial movement, stirring, or sonication are required to reduce the effect of the depletion zone produced close to the fiber as a result of slow diffusional analyte transport through the stationary layer of liquid surrounding the fiber.

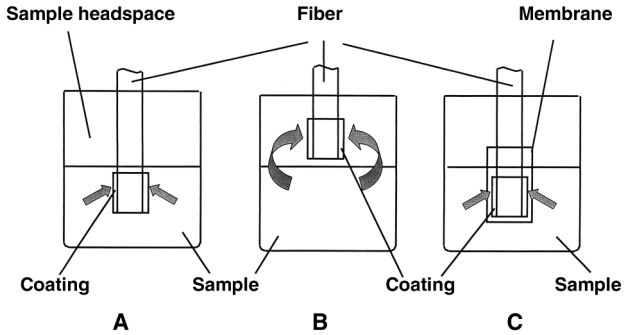

Figure 7. Modes of SPME operation: direct extraction (A), headspace SPME (B), and membrane-protected SPME (C).

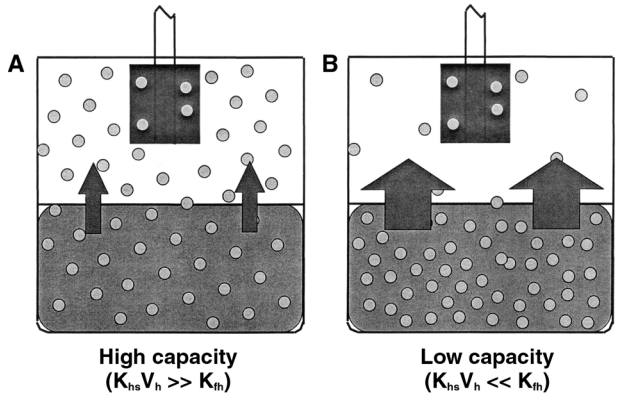

Figure 8. Headspace SPME of compounds characterized by a high (A) and low (B) Henry's constant.

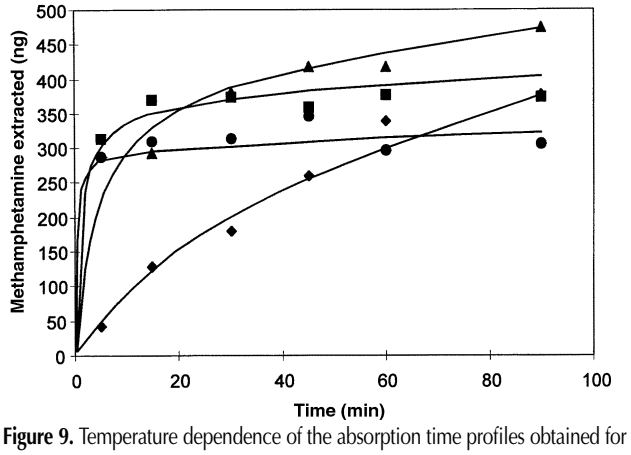

Figure 9. Temperature dependence of the absorption time profiles obtained for methamphetamine.

In the headspace mode (Figure 7B), the analytes are extracted from the gas phase equilibrated with the sample. The primary reason for this modification is to protect the fiber from adverse effects caused by nonvolatile, high-molecular-weight substances present in the sample matrix (e.g., humic acids or proteins). The headspace mode also allows matrix modifications (including pH adjustment) without affecting the fiber. In a system consisting of a liquid sample and its headspace, the amount of an analyte extracted by the fiber coating does not depend on the location of the fiber (in the liquid or gas phase); therefore, the sensitivity of headspace sampling is the same as the sensitivity of direct sampling as long as the volumes of the 2 phases are the same in both sampling modes. Even when no headspace is used in direct extraction, a significant sensitivity difference between direct and headspace sampling may occur only for very volatile analytes. However, the choice of sampling mode has a very significant impact on the extraction kinetics. When the fiber is in the headspace, the analytes are removed from the headspace first, followed by indirect extraction from the matrix. If the Henry's Constant of a given compound is high, then the concentration of analytes in the headspace is high, resulting in very rapid extraction because the extracted analytes originate primarily from the gaseous headspace (Figure 8A). However, if the Henry's Constants are low, then the extraction is long because the analytes need to diffuse from the condensed phase before they reach the fiber (Figure 8B). Therefore, in the case of the extraction of aqueous samples, volatile and nonpolar analytes are extracted much faster than semivolatiles or polar volatiles. Temperature has a significant effect on the kinetics of the process, because it determines the vapor pressure of analytes above the condensed phase. In general, the equilibration times for volatile compounds are shorter for headspace SPME than for direct extraction under similar agitation conditions because of the following 3 reasons: (a) a substantial portion of the analytes is present in the headspace prior to the beginning of the extraction process, (b) there is typically a large interface between the sample matrix and headspace, and (c) the diffusion coefficients in the gas phase are typically higher by 4 orders of magnitude than in liquids. The concentration of semivolatile compounds in the gaseous phase at room temperature is small, and headspace extraction rates for those compounds are substantially lower. These compounds can be improved by using very efficient agitation or by increasing the extraction temperature. Figure 9 illustrates the equilibration time profiles obtained for the extraction of methamphetamine from a urine sample at various temperatures. At 22°C and 40°C, the equilibration is very long—exceeding 100 min as indicated in this graph. It drops to approximately 20 min when the extraction temperature is 60°C and to only a few minutes when the temperature is 73°C. The dramatic change with the equilibration time is associated with the fact that an increase in temperate results in an increase of the analyte's Henry's Constant, an increase in the diffusion coefficient, and a decrease of the amount extracted at equi-

librium. This decrease is associated with the fact that the distribution constant decreases when the temperature increases. Therefore, it is important to carefully optimize the extraction temperature for the shortest equilibration times and for acceptable sensitivities. In most SPME applications, equilibrium extraction is performed. However, often when the equilibration times are long, pre-equilibrium quantification can be considered. It is important in such experiments to ensure constant agitation conditions and acceptable extraction times in order to obtain good precision.

In the third mode (SPME with membrane protection, Figure 7C), the fiber is separated from the sample with a selective membrane, which lets the analytes through while blocking the interferences. The main purpose for the use of the membrane barrier is to protect the fiber against adverse effects caused by high-molecular-weight compounds when very dirty samples are analyzed. Although, extraction from headspace serves the same purpose, membrane protection enables the analysis of less-volatile compounds. The extraction process is substantially slower than direct extraction because the analytes need to diffuse through the membrane before they can reach the coating. The use of thin membranes and an increase in extraction temperature result in shorter extraction times.

Extraction modes with in-tube SPME

There are 2 fundamental approaches to in-tube SPME: (a) active or dynamic (when the analytes are passed through the tube) and (b) passive or static (when the analytes are transferred into the sorbent by diffusion). In either of these approaches, the coating may be supported on a fused-silica rod or coated on the inside of a tube or capillary. The theoretical aspects of the extraction processes that use these geometric arrangements will be briefly discussed.

Dynamic in-tube SPME

In this system, we assume (a) a piece of fused-silica capillary internally coated with a thin film of extracting phase (a piece of open tubular capillary GC column) is used or (b) the capillary is packed with an extracting phase dispersed on an inert supporting material (a piece of micro-liquid-chromatography capillary column). During the introduction of the sample, the front of the analyte migrates through the capillary with a speed proportional to the linear velocity of the sample and inversely related to the partition ratio (11,12). For short capillaries with a small dispersion, the extraction time can be assumed to be similar to the time required for the center of the band to reach the end of the capillary. The extraction time is proportional to the length of the capillary and inversely proportional to the linear flow rate of the fluid. Extraction time also increases with an increase in the coating–sample distribution constant and an increase in the thickness of the extracting phase, but decreases with an increase in the void volume of the capillary. An increase in the coating–sample distribution constant produces an increase in the absolute amount extracted. It has been observed that increases in the amounts extracted can be achieved in many cases by preconditioning the capillary with methanol or some other appropriate solvent prior to extraction. Enhancement has even been observed when a plug of methanol was aspirated into the capillary before the sample was drawn in and the sample followed the plug while in the capillary during the extraction aspirate–dispense steps. This is similar to the solvent preconditioning used in SPE to enhance extraction.

In practice, in-tube SPME is implemented by replacing a section of the tubing in a commercially available autosampler and then programming the autosampler to pass the sample in and out of the extraction capillary until equilibrium or a suitable extraction level has been reached.

It should be emphasized that this is valid only for direct extraction when the sample matrix passes through the capillary. This approach is limited to particulate-free gas and clean water samples. The headspace SPME approach can broaden the application of in-tube SPME. In such cases, careful consideration of the mass transfer between the sample and headspace should be given in order to describe the process properly. Also, if the flow rate is very rapidly producing turbulent behavior and the coating–sample distribution constant is not very high, then perfect agitation conditions are met and equation 4 can be used to estimate equilibration times. In this case, equilibration time (t_e) is assumed to be achieved when 95% of the equilibrium amount of the analyte is extracted from the sample:

$$t_e = t_{95\%} = \frac{(b-a)^2}{2D_f} \tag{4}$$

In this equation, $b - a$ refers to the thickness of the sorbent material and D_f refers to analyte diffusion in the sorbent.

The removal of analytes from a tube is an elution problem similar to frontal chromatography and has been discussed in detail (12). In general, if the desorption temperature of a GC is high and thin coatings are used, then all the analytes are in the gas phase as soon as the coating is placed in the injector and the desorbtion time corresponds to the elution of 2 void volumes of the capillary. For liquid desorption, the desorbtion volume can be even smaller because the analytes can be focused at the front of the desorption solvent (13).

Static in-tube SPME time-weighted average sampling

In addition to the analyte concentration measurement found at a well-defined place in space and time (obtained by using the approaches discussed previously), an integrated sampling is possible with a simple SPME system. This is particularly important in field measurements when changes of analyte concentration over time and place variations must often be taken into account.

When the extracting phase is not exposed directly to the sample but is contained in a protective tubing (needle) without any flow of the sample through it (Figure 10A), the extraction occurs through the static gas phase present in the needle. The integrated system can consist of the extraction phase coating the interior of the tubing, or it can be an externally coated fiber withdrawn into the needle. These geometric arrangements represent a very powerful method able to generate a response proportional to the integral of the analyte concentration over time and space when the needle is moved through the space (14). In these cases, the only mechanism for analyte transport to the extracting phase is diffusion through the gaseous phase contained in the tubing. During this process, a linear concentration profile (shown in Figure 10)

is established in the tubing between the small needle opening—characterized by the 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 the time interval (dt) can be calculated by considering Fick's first law of diffusion (15):

$$dn = AD_g \frac{dc}{dz} dt = AD_g \frac{\Delta C(t)}{Z} dt \quad (5)$$

where $\Delta C(t)/Z$ is a value of the gradient established in the needle between the needle opening and the position of the extracting phase (Z). The function $\Delta C(t)$ is equivalent to $C(t) - C_z$, where $C(t)$ is a time-dependent concentration of the analyte in the sample near the needle opening and C_z is the concentration of the analyte in the gas phase near the coating. If C_z is close to zero for a high coating–gas distribution constant capacity, then $\Delta C(t)$ is equivalent to $C(t)$. The concentration of the analyte at the coating position in the needle (C_z) will increase with the integration time but will be kept low compared to the sample concentration because of the presence of the sorbent coating. Therefore, the accumulated amount over time can be calculated as:

$$n = D_g \frac{A}{Z} \int C(t) d \quad (6)$$

As expected, the extracted amount of analyte is proportional to the integral of the sample concentration over time and the diffusion coefficient of analytes in gaseous phase (D_g) in the area of the needle opening (A), and it is inversely proportional to the distance of the coating position with respect to the needle opening (Z). It should be emphasized that equation 5 is valid only in a situation in which the amount of analyte extracted onto the sorbent is a small fraction (below RSD of the measurement, typically 5%) of the equilibrium amount with respect to the lowest concentration in the sample. To extend integration times, the coating can be placed further into the needle (resulting in a larger Z), the opening of the needle can be reduced by the introduction of an additional orifice (a smaller A), or a higher capacity sorbent can be used. The first 2 solutions will result in a low measurement sensitivity. An increase of sorbent capacity presents a more attractive opportunity. It can be achieved by either increasing the volume of the coating or its affinity towards the analyte. An increase of the coating volume will require an increase of the device size. The optimum approach to an increased integration time is to use sorbents characterized by large coating–gas distribution constants.

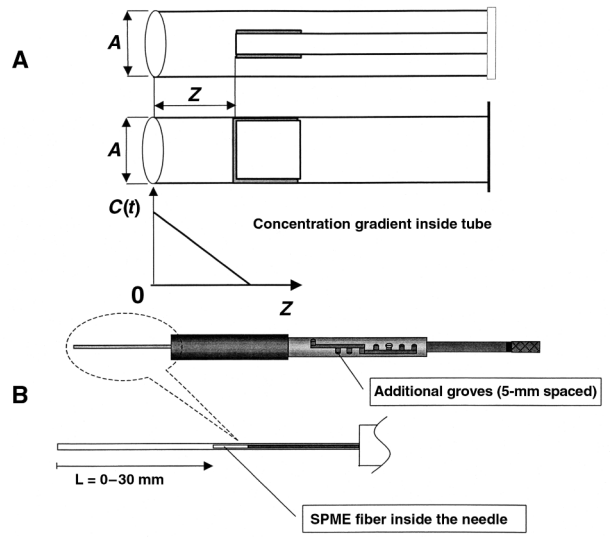

Figure 10. Use of SPME for in-tube TWA sampling: schematic (A) and adaptation of a commercial SPME manual-extraction holder (B).

The exploitation of restricting access to the absorbing medium allows for the implementation of SPME for time-weighted average (TWA) sampling. Whenever diffusion to the sorbent surface is limited, the sorbent can act as a sort of "zero sink" in which extraction is very far from equilibrium under normal sampling conditions. Therefore, in practice, any analytes that reach the sorbent surface are essentially exhaustively absorbed. However, the rate of diffusion is still dependent on the sample concentration; therefore, the total amount absorbed by the coating is proportional to the average of analyte concentrations over time, thus TWA sampling is achieved. This has been implemented to date with the conventional fiber assembly by retracting the fiber to a known distance inside the needle (Figure 10B). The small size of the needle orifice limits diffusion to the sorbent surface, and the ultimate diffusion rate is a function of the distance from the fiber tip to the end of the needle. Depending on the volatility and concentration of the analyte of interest, the fiber may be positioned either closer to the end of the needle or further away from it in order to achieve the desired degree of nonequilibrium extraction and sensitivity. It would also be possible to implement this type of sampling with the sorbent coated onto the interior wall of a capillary. However, to date, the retractable needle implementation has gained the most attention because of its ease of use and adjustability for the analyte and the given sample.

Prediction of distribution constants

In many cases, the distribution constants presented in equations 1 and 2 (that determine the sensitivity of SPME) can be estimated from physicochemical data and chromatographic parameters. This approach eliminates the need for calibration. For example, distribution constants between a fiber-coating and gaseous matrix (e.g., air) can be estimated using isothermal GC retention times on a column with a stationary phase identical to the fiber-coating material (16). This is possible because the partitioning process in GC is similar to the partitioning process in SPME and there is a well-defined relationship between the distribution constant and the retention time. The nature of the gaseous phase does not affect the distribution constant unless the components of the gas (such as moisture) swell the polymer, thus changing its properties. A most useful method for determining coating-to-gas distribution constants uses the linear temperature-programmed retention index (LTPRI) system, which indexes a compound's retention time in relation to the retention times of n-alkanes. This system is applicable for the retention times of temperature-programmed gas–liquid chromatography. The logarithm of the coating-to-air distribution constants of n-alkanes can be expressed as a linear function of their LTPRI values. For PDMS, this relationship is $\log K_{fg} = 0.00415 \times \text{LTPRI} - 0.188$ (17). Thus, the LTPRI system permits interpolation of the K_{fg} values

from the plot of $\log K_{fg}$ versus the retention index. The LTPRI values for many compounds are available in the literature; therefore, this method allows estimation of K_{fg} values without experimentation. If the LTPRI value for a compound is not available from published sources, it can be determined from a GC run. It should be noted that the GC column used to determine the LTPRI value should be coated with the same material as the fiber coating.

Estimation of the coating–water distribution constant can be performed using equation 3. The appropriate coating–gas distribution constant can be found by applying the techniques discussed previously, and the gas–water distribution constant (Henry's constant) can be obtained from physicochemical tables or estimated by the structural unit contribution method (18).

Some correlations can be used to anticipate trends in SPME coating–water distribution constants for analytes. For example, a number of investigators have reported the correlation between the octanol–water distribution constants, K_{ow} and K_{fw}. This is expected because K_{ow} is a very general measure of the affinity of the compounds to the organic phase. However, it should be remembered that the trends are valid only for compounds within a homologous series, such as aliphatic hydrocarbons, aromatic hydrocarbons, or phenols. They should not be used to make comparisons between different classes of compounds because of different analyte activity coefficients in the polymer.

Effect of extraction parameters

Thermodynamic theory predicts the effects of modifying certain extraction conditions on partitioning and indicates the parameters to control for reproducibility. This theory can be used to optimize the extraction conditions with a minimum number of experiments and correct for variations in the extraction conditions without the need to repeat calibration tests under the new conditions. For example, SPME analysis of outdoor air may be done at ambient temperatures that can vary significantly. The relationship that predicts the effect of temperature on the amount of analyte extracted allows for calibration without the need for extensive experimentation (19). Extraction conditions that affect K_{fs} include temperature, salting, pH, and the amount of organic solvent content in water.

An extraction temperature increase causes an increase in the extraction rate and simultaneously a decrease in the distribution constant. In general, if the extraction rate is of major concern, the highest temperature that still provides satisfactory sensitivity should be used.

Adjustment of the pH of the sample can improve the sensitivity of the method for basic and acidic analytes. This is related to the fact that unless ion-exchange coatings are used, SPME can only extract neutral nonionic species from water. By properly adjusting the pH, weak acids and bases can be converted to their neutral forms, in which case they can be extracted by the SPME fiber. To make sure that at least 99% of the acidic compound is in the neutral form, the pH should be at least 2 units lower than the pK_a of the analyte. For the basic analytes, the pH must be larger than pK_a by 2 units.

The volume of the sample should be selected based on the estimated distribution constant K_{fs} (8). The distribution constant can be estimated by using literature values for the target analyte or a related compound with the coating selected. The distribution constant can also be calculated or determined experimentally by equilibrating the sample with the fiber and determining the amount of analyte extracted by the coating. Care must be taken to avoid analyte losses from adsorption, evaporation, or microbial degradation when very long extraction times are required to reach the equilibrium.

The sensitivity of the SPME method is proportional to the number of moles of the analyte (n) extracted from the sample and (for direct extraction) is given by equation 1. When the sample volume (V_s) increases, the amount of analyte extracted also increases until the volume of the sample becomes significantly larger than the product of the distribution constant and volume of the coating (fiber capacity $K_{fs} << V_s$).

Conclusion

A number of parallels can be drawn between the developments and applications of SPME with electrochemical methods. The coulometric technique corresponds to the total extraction method. Although this technique is the most precise, it is not frequently used because of the time required to complete it. SPME is capable of producing exhaustive extraction when the volume of the extraction phase is large enough to combine with high-distribution constants. In fiber geometry, the larger volume translates into thicker coatings, which results in long extraction times. The alternative approach is to disperse the whole volume of the extraction phase onto a larger surface area, resulting in a thinner coating and faster equilibration times. For example, solid support material may include particulate matter, a stirring mechanism, or a vessel's walls (Figure 1). However, in this case, there would be more handling required in order to conveniently introduce the extraction phase into the sample introduction system (GC or HPLC). It might necessitate the use of an organic solvent to desorb the analytes from the extraction phase. Equilibrium potent-ion-metric techniques are more frequently used (pH electrode), particularly when the sample is a simple mixture or selectivity of the membrane is sufficient to quantify the target analyte in complex matrices. The equilibrium SPME method has some advantages in this regard because the technique is typically coupled with separation or MS detection methods or both, which simultaneously allows for the identification and quantitation of many components. The advantage of using electrochemical methods is the response time resulting from the low capacities of electrodes.

Several electrochemical methods, (e.g., amparometry) are based on mass transport through the boundary layers, such as pre-equilibrium SPME. Similarly, in SPME, calibration based on diffusion coefficients can be accomplished when the agitation conditions are constant, the extraction times are short, and the coating has a high affinity towards the analytes. Figure 21 illustrates the results related to the 10-s extraction times using solid coating. In some implementations of the technology, the rate of the mass transfer to the extraction phase can be purposely restricted by placing it in the needle, thus achieving the TWA measurements of concentration in a specific time period.

The potential savings in analysis time, reduced solvent use, and the apparent simplicity of SPME techniques will continue to attract interest among analytical chemists searching for improved analysis methods. As long as analysts have a sound understanding of the theory and principles behind this technique, good accuracy and precision will follow.

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