# Analytical Microextraction: Current Status and Future Trends

**Janusz Pawliszyn**[1,*] and **Stig Pedersen-Bjergaard**[2]

[1]Department of Chemistry, University of Waterloo, Ontario, Canada N2L 3G1 and [2]School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, 0316 Oslo, Norway

## Abstract

**Analytical microextractions, defined as nonexhaustive sample preparation with a very small volume of extracting phase (microliter range or smaller) relative to the sample volume, represent an important development in the field of analytical chemistry. Analytes are extracted by a small volume of a solid or semi-solid polymeric material, as in solid-phase microextraction (SPME), or alternatively by a small volume of a liquid, as in liquid-phase microextraction (LPME). This paper gives an overview of the SPME and LPME techniques and discusses future trends. This includes a discussion of the different extraction formats available, commercial equipment, method transfer from traditional sample preparation methods to microextraction, and performance as well as robustness for the latter type of systems. In addition, the paper contains a unified approach to the understanding of extraction thermodynamics and kinetics applicable to both SPME and LPME.**

## Introduction

Prior to separation and detection by chromatographic, electrophoretic, and mass spectrometric (MS) techniques, a sample preparation step is normally required to transfer the analytes to a suitable medium, isolate them from the major sample matrix, and enrich them to a concentration level detectable by the separation system. Although substantial efforts have been made to the development of sophisticated chromatographic, electrophoretic, and MS instrumentation in recent years, the area of sample preparation has been characterized by relatively slow progress. In a majority of cases, sample preparation is still carried out by traditional techniques, such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), and purge and trap. Yet the development gap between sample preparation and separation steps still appears to increase in terms of sophistication and performance. More precisely, many sample preparation methods still account for the major inaccuracies and time consumption of the total analytical process, and often the volume of extract exceeds the volumes required for chromatography or electrophoresis by orders of magnitude. Consequently, there is an increasing demand for new, rapid, accurate, and miniaturized sample preparation methods within the field of analytical chemistry, which will open up new opportunities in the monitoring of chemical and biochemical substances.

Microextraction techniques represent an important contribution to the improvement of sample preparation performance, which especially addresses the issues of miniaturization, automation, on-site analysis, and time efficiency. Actually, different types of microextraction techniques were reported in the literature a long time ago (1,2), but the field gained in significance with the invention of solid-phase microextraction (SPME) in 1990 (3), which later became commercially available. In the commercial version of this technique, a small diameter fiber coated with a small volume of stationary phase is placed either in an aqueous or a gaseous sample. The analytes partition into the stationary phase and are subsequently thermally desorbed in the injector of a gas chromatograph (GC). During recent years, SPME has gained substantially in popularity, and from this point, several alternative approaches have been introduced, such as in-tube SPME designed primarily for high-performance liquid chromatography (HPLC) (4). In parallel to the development of SPME, attention has also been directed to the utility of small volumes of liquids for analytical extractions, namely liquid-phase microextraction (LPME). This field was basically initiated in 1996 when the use of small droplets of organic solvents suspended from the tip of a micro-syringe was described for the first time (5,6), and this approach was subsequently refined by implementing the use of porous hollow fibers for protection of the extracting liquids (7).

The current review will focus on the techniques of SPME and LPME, different formats and configurations available, fundamentals, commercial equipment, method transfer, and performance, as well as future trends. In this context, we have limited our discussion and definition of microextraction techniques to nonexhaustive techniques (extracting only a portion of the analyte present in the sample), utilizing very small volumes of extracting phase (sub-microliter range), and with a relatively large volume of sample relative to the volume of the extracting phase.

* Author to whom correspondence should be addressed: email janusz@uwaterloo.ca.

## Discussion

### Overview of the different microextraction formats

*SPME*

SPME was developed to address the need for rapid sample preparation both in the laboratory and on-site, where the investigated system is located. In this technique, a small amount of extracting phase dispersed on a solid support is exposed to the sample for a well-defined period 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 extraction for a longer time does not then accumulate more analytes. The objective of SPME is never the exhaustive extraction, but rather convenience and speed. This substantially simplifies the design of systems. For example, in-tube SPME for analysis of liquids uses 0.25-mm-i.d. tubes and aproximately 0.1 µL of extraction phase because concern about 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 equilibrium extraction has been reached and maximum sensitivity has been accomplished.

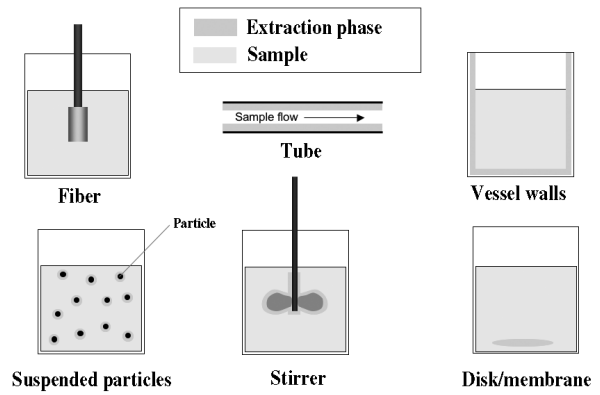


**Figure 1.** Configurations of SPME.

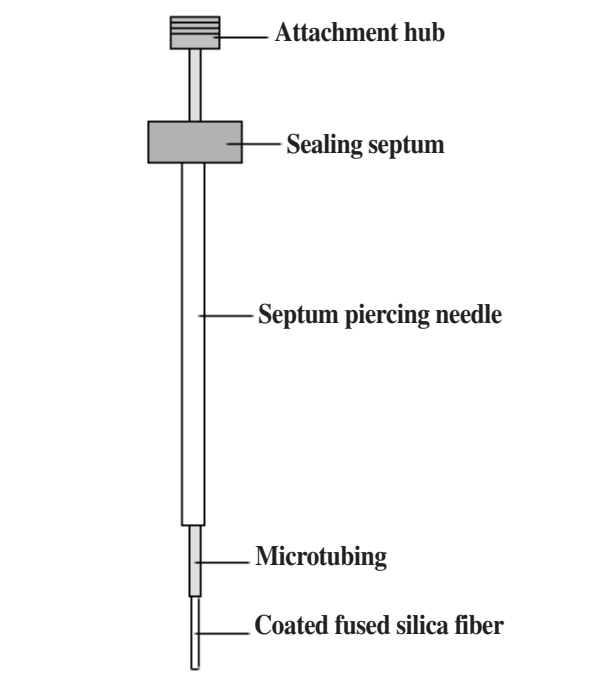


**Figure 2.** Coated-fiber SPME module.

The geometry of the SPME system is optimized to facilitate speed, convenience of use, and sensitivity. Figure 1 illustrates several implementations of SPME that have been considered to date. These include mainly open-bed extraction concepts, such as coated fibers, vessels, agitation mechanism, and disks/membranes, but in-tube approaches are also considered. Some of the implementations better address issues associated with agitation and others ease of implementing sample introduction to the analytical instrument. It should be noted that SPME was originally named after the first experiment using an SPME device, which involved extraction on solid fused-silica fibers and, later, as such, as a reference to the appearance of the extracting phase, relative to a liquid or gaseous donor phase, even though it is recognized that the extraction phase is not always technically a solid.

As Figure 2 illustrates, a useful device can be built from a short piece of stainless steel micro-tubing (to hold the fiber), another piece of larger tubing (to work as a "needle"), and a septum (to seal the connection between the micro-tubing and the needle). The design from Figure 2 is the basic building block of a commercial SPME device described later and illustrated in Figure 3. Another simple SPME construction is based on a piece of internally coated tubing (8). This tubing can be mounted inside a needle or it can constitute the needle of a syringe itself (9). A coated tubing approach is useful in the design of passive sampling devices discussed later because, in this case, the extraction rate is limited by the diffusion of analytes into the needle (10).

The in-tube concept has also been expanded to facilitate automation of sample preparation for HPLC. In that approach, the sample components are extracted by the coating located on the inner surface of the hollow tubing, and, after the extraction is completed, the analytes are washed into the HPLC column using the mobile phase or solvent. Everything is easily automated using a conventional autosampler. This concept is very similar to SPE (some researchers used packed tubes) (11). However, the difference is associated with the principle of the process'—total extrac-

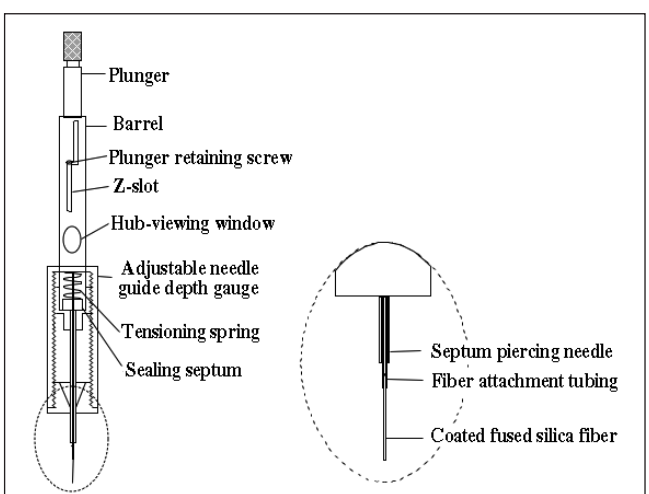


**Figure 3.** Commercial SPME device.

tion versus equilibrium—different selectivities, and taking full advantage of phase capacity.

Alternative geometries (Figure 1) are used to enhance sensitivity by using a larger volume of the extraction phase [polydimethylsiloxane (PDMS)] and improving kinetics of the mass transfer between sample and PDMS by increasing the surface-to-volume ratio of the extraction phase. The main disadvantage of these approaches, however, is the loss of convenience associated with syringe configuration, in particular in introduction to the analytical instrument. This step necessitates the use of high-volume desorption devices and creates difficulties in automation of the extraction process as well as handling volatile compounds, which are lost during transfer of the extraction phase from sample to the injection system. Because high sensitivities are obtained for hydrophobic high-molecular-weight compounds with fiber SPME, the advantages of using larger volume phases are limited, especially for small sample volumes (12).

An alternate way to enhance sensitivity and preserve the convenience of coupling to analytical instrumentation is to use internally cooled SPME. Internally cooled fiber SPME is a very powerful approach to SPME analysis, first demonstrated in 1995 (13). In this technique, a cooled gas or liquid passes through the inside of the coated, hollow fiber during sampling. This cools the extraction phase and, thus, allows an increase in the percentage of analytes that can be extracted from a sample, in some cases enabling quantitative extraction. It can also allow the analyst to heat a sample to much higher temperatures than normally feasible with SPME, without reducing the equilibrium fiber loading. An automated internally cooled fiber SPME has recently been developed (14). The new automated device involved miniaturizing the technology used to first demonstrate the principles of the approach. The fiber was contained in an 18-gauge needle, to accommodate the additional parts of the device. This could perform 30 injections before replacing the GC septum. Fiber failure was not found to be an issue in test experiments. The device could be mounted and used on the CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with only small changes to the GC required, such as enlarging the septum nut and support.

Controlling the fiber coating temperature was achieved by using a temperature controller, solenoid valve, and tubing of different inner diameter. With this system, the temperature of the fiber coating could be controlled to within 5°C of the preset value. Full automation of the process was realized by coupling the external temperature control system with the autosampler through a logic circuit built into the temperature controller. This allowed the controller to be turned on or off as required.

Another promising SPME uses a valve syringe, in which the fiber is withdrawn into the barrel of the syringe along with a sample of the air being analyzed by means of retracting the syringe plunger. This approach combined headspace and fiber extraction, and it is particularly suitable when analysis involves gases in addition to less volatile compounds. The valve is then sealed until analysis (15). Initial data for the new approaches clearly demonstrate advantages of the new designs, compared with commercial devices, to seal the fiber in the needle by eliminating the losses and contamination to the septa material of volatile components.

Although, to date, SPME devices have been used principally in laboratory applications, more current research has been directed toward remote monitoring, particularly for clinical, field environmental, and industrial hygiene applications. In their operating principles, such devices are analogous to the devices described previously, but modifications are made for greater convenience in given applications. For example, adding a tube with a small opening to cover the needle of the SPME syringe results in a useful device for breath analysis in a noninvasive clinical application (16). This design can be further improved by adding a one-way valve mounted on the aperture, but the concept of operation remains the same. An important feature of a field device is the ability to preserve extracted analytes in the coating (17).

Automated SPME flow-through samplers for continuous on-site monitoring have been devised as well (Figure 4). The first such device was reported by Eisert and Levsen (18), in which a flow-through cell was mounted into a slot on the sample carousel of a Varian autosampler (Palo Alto, CA) for the analysis of liquid samples. The device was also modified to allow the sampling of gases (19). Grote et al. (20) developed a stopped-flow device for automated analysis of flowing streams that could treat water samples (pH, internal standard, salt) and perform the SPME extraction and GC analysis. This was used for the analysis of organic compounds in an industrial wastewater stream. The system could be used for a week without any user intervention. The limiting step was the fiber lifetime, which was reduced by soiling the fiber with the wastewater matrix. Memory effects were also an issue. A headspace version of the device was also reported (21), which can be used for significantly longer time periods without user intervention and displayed less memory effects through replacing plastic tubing with glass.

Equations 1 and 2 (see Fundamentals section) indicate that the sensitivity of the extraction process is dependent on the distribution constant, $K_{fs}$. This is a characteristic parameter that describes properties of a coating and its selectivity toward the analyte versus other matrix components. Specific coatings can be developed for a range of applications. Coating volume determines method sensitivity as well (see equation 1), but thicker coatings result in longer extraction times (22). Therefore, it is important to use the appropriate coating for a given application. For example,

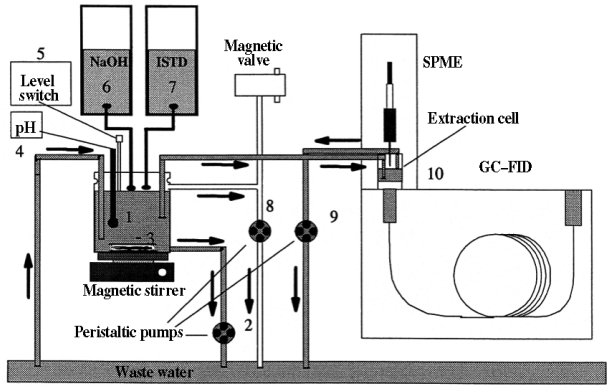


**Figure 4.** Automated analyzer for monitoring organic compounds in a wastewater stream. For the figure, sample preparation vessel, 1; peristaltic pump, 2; magnetic stirrer, 3; pH electrode, 4; level switch, 5; programmable burette (titration), 6; programmable burette (addition of internal standard), 7; peristaltic pumps, 8/9; and extraction vessel, 10.

the distribution constant and the sensitivity of the method drop over two orders of magnitude for *o*-xylene and increase by an order of magnitude for 2,4-dichlorophenol when the film is changed from nonpolar PDMS to polar poly(acrylate) polymer (PA) (23). Coating selection and design can be based on chromatographic experience.

To date, several experimental coatings have been prepared and investigated for a range of applications. In addition to liquid polymeric coatings such as PDMS for general applications, other, more specialized materials have been developed. For example, ion exchange coatings were used to remove metal ions and proteins from aqueous solutions (24,25), liquid crystalline films were used to extract planar molecules and carbowax for polar analytes, metal rods were used to electorodeposit analytes (26), pencil "leads" were used to extract pesticides (27), and Nafion coatings were used to extract polar compounds from nonpolar matrices (28).

Polypyrole coatings have recently been developed to extract polar or even ionic analytes and possibly to explore the conductive polymer properties of the polymer (29). This could involve applying a charge to the polymer during extraction in order to selectively extract analytes of interest and then reversing the charge to facilitate desorption. Polypyrrole coating has excellent biocompatibility and can be used for in vivo applications.

A very pronounced difference in selectivity toward target analytes and interferences can be achieved by using surfaces common to affinity chromatography. Using the method of polymer imprinting (30), antibody mimics can be generated with specificities to an analyte of choice. Briefly, the desired affinity can be introduced by adding an amount of the compound of interest to the polymerization reaction. This "pattern" chemical may be removed after polymerization, leaving vacant sites of a specific size and shape, suitable for binding the same chemical again from an unknown sample. We have observed that it is important when using this approach to control nonspecific binding. It is possible to obtain substantial enhancement in selectivity by this coating technique, particularly at low analyte concentrations.

The flexible titanium–nickel alloy has recently been considered to support the extraction phase instead of more fragile fused silica. Supelco (Bellefonte, PA) has recently introduced SPME fiber assemblies based on this support. Initial results indicate that these fibers are much more reproducible and reliable. They can be used for up to 500 injections, and the interfiber reproducibility is close to 5% relative standard deviation (RSD).

*LPME*

Aside from SPME, LPME has also been carried out in different technical configurations involving different types of extraction chemistry. Early work in the field of LPME involved dispersion of small droplets of organic solvent from fine rods (1,2), but the formats were inconvenient from a practical point of view and received little interest within the analytical community. Nevertheless, this work initiated a more practical approach, introduced in 1996 (5,6) and further refined in 1997 (31), which involved the use of a single drop of organic solvent suspended from the end of a micro-syringe needle (single-drop LPME). This drop was placed in an aqueous sample, and the analytes present in the aqueous sample were extracted into the drop based on their distribution constants (two-phase extraction). Subsequently, the drop was withdrawn into the syringe, and the syringe was transferred to a GC. In single-drop LPME, the high sample-to-drop volume ratios provided excellent enrichment of analytes and with a major saving of organic solvent. In addition to two-phase extractions, single-drop LPME has also been performed in a three-phase system in which analytes in their neutral form are extracted from aqueous samples through a thin layer of an organic solvent on the top of the sample into an aqueous drop placed at the tip of a micro-syringe (32). In the latter, the analytes were transferred to their ionized state (different pH from the sample) to promote trapping within the drop. Subsequently, the aqueous drop was transferred to an HPLC system for the final analysis. In addition to two- and three-phase extractions, headspace extractions have also been reported with single-drop LPME (33). Recently, single-drop LPME has been reviewed (34).

In spite of its simplicity and direct compatibility with chromatographic systems, single-drop LPME, as described previously, has gained limited popularity within the analytical community. A major reason for this is related to the stability of the drop during extraction. In order to speed up extractions, stirring or agitation is required, but the mechanical stability of a single drop suspended from the tip of a micro-syringe is relatively poor, and the drop is easily lost into the sample solution. In addition, biological samples like plasma may emulsify substantial amounts of organic solvents, and this may even increase the stability problem. In order to solve this stability and robustness issue, an alternative concept for LPME was introduced in 1999 based on the use of single, low-cost, disposable, and porous hollow fibers made of polypropylene (7). With this hollow-fiber LPME device, the extracting phase (acceptor solution) is contained within the lumen of a porous hollow fiber, either as a loop or a rod sealed at the bottom, and the extracting phase is not in direct contact with the sample solution (Figure 5). Mass transfer is easily accomplished across the highly porous wall of the hollow fiber, and samples may be stirred or vibrated vigorously without any loss of the acceptor solution. Thus, hollow-fiber-based LPME is a more robust and reliable approach to LPME. In addition, the equipment needed is very simple and inexpensive. Hollow-fiber LPME has recently been reviewed (35).

The chemistry of hollow-fiber-based LPME is similar to the chemistry used for extraction with supported liquid membranes (SLM) (36), but the techniques differ significantly in terms of instrumentation and operation. SLM is a flowing system with a pump that continuously feeds the membrane with fresh sample.

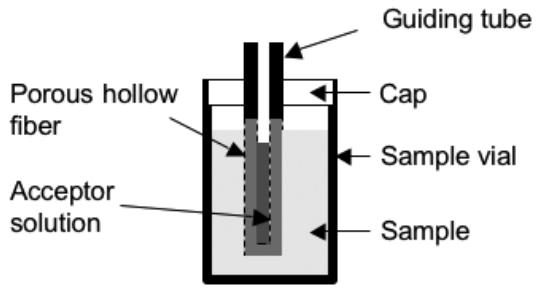


**Figure 5.** Schematic illustration of set-up for hollow fiber LPME.

Thus, SLM is an instrumental sample preparation technique, and each membrane is normally used for a large number of extractions. On the other hand, in hollow-fiber-based LPME, both the sample and the extraction phase are stagnant, the membrane (hollow fiber) is used only for a single extraction, and no instrumentation such as pumps are required for the sample processing.

Hollow-fiber LPME has been operated and reported in several different configurations (37–41). Basically, LPME may carried out in either a two- or three-phase system, and in addition to this, the extractions may be performed either in a static or a dynamic way. In the two-phase mode, an organic solvent suitable for GC introduction is both immobilized in the pores of the hollow fiber (in the wall) and filled inside the lumen of the hollow fiber, with the latter working as the acceptor solution (37). Mass transfer is based on the distribution constant into the organic solvent, and the acceptor solution is typically directly injected into the GC in a subsequent step. This mode of LPME is favorable for GC-amenable analytes with relatively large distribution constants (nonpolar substances). Alternatively, the acceptor solution may be another aqueous phase providing a three-phase system. Again, an organic solvent is immobilized in the pores in the wall of the hollow fiber, and this makes up a thin organic membrane between the sample solution and the acceptor solution. Thus, in three-phase LPME, mass transfer occurs from an aqueous sample through a thin film of organic solvent immobilized in the pores of the hollow fiber and into an aqueous acceptor solution inside the lumen. This extraction method is limited to basic or acidic analytes with ionizable functionalities. For extraction of basic compounds, the pH in the sample has to be adjusted into the alkaline region, whereas pH in the acceptor solution should be low to ensure high distribution constants for the two continuous extraction steps. In a similar way, acidic analytes may be extracted by reversal of the pH gradient. Following extraction, the aqueous acceptor solution is typically directly injected in the HPLC or capillary electrophoresis (CE). In the three-phase mode, LPME is particularly suited for basic or acidic analytes with a certain degree of hydrophobicity (38). On the other hand, as compounds become more polar, the distribution constant between the sample and the organic membrane is reduced, which in turn sacrifice mass transfer. To overcome this, the addition of ion pair reagents to the sample may be accomplished (39). This concept both relies on partitioning of the analyte/ion-pair complex into the organic membrane and on the counter-transport of protons from the acceptor solution and to the sample solution. This active type of transport is well known from industrial membrane applications and has been implemented in the hollow-fiber LPME format under the name "carrier-mediated LPME".

As mentioned previously, hollow-fiber LPME may be performed in either a static or a dynamic mode (this also essentially applies to single-drop LPME). In the static mode, both the sample and the acceptor solution are stagnant, in spite of the fact that the whole assembly is vigorously agitated to ensure efficient mass transfer to the acceptor solution. The major advantage of the static system is its simplicity; only a vibrator (or a magnetic stirrer) is required in addition to the LPME device in order to perform the extractions. The acceptor solution is filled into the lumen of the fiber before extraction and collected afterwards by a micro-syringe, and the fiber is either in the loop configuration or a rod sealed in the bottom. This, in turn, enables simultaneous extraction of a large number of samples in parallel, such as in a 96-well system (illustrated in Figure 6). On the other hand, the static system may involve a slight sacrifice in terms of kinetics. Several papers have

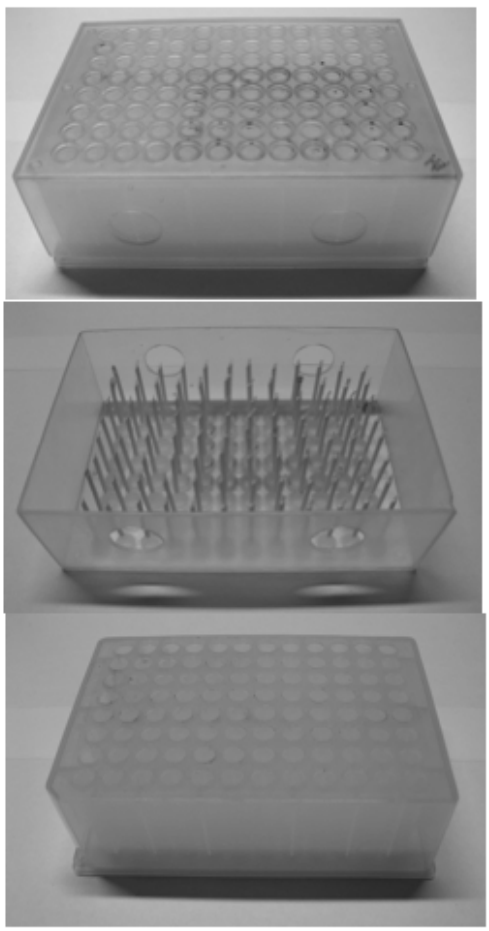

**Figure 6.** Photo of prototype 96-well LPME plate. Upper photo: equipment assembled for extraction. Middle photo: top plate with hollow fibers. Lower photo: well plate. *Courtesy of Varian.*

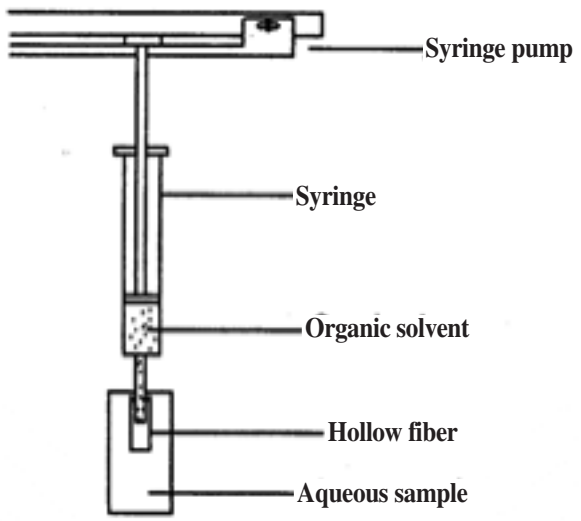


**Figure 7.** Schematic illustration of set-up for dynamic two-phase LPME.

discussed improvements in kinetics utilizing a dynamic system, reported both in two- and three-phase configurations (40,41). An example of the set-up for dynamic two-phase LPME is illustrated in Figure 7. A micro-syringe is filled with a few microliters of organic solvent immiscible with water. A small piece of porous hollow fiber (1–2 cm) is soaked in the same organic solvent to fill the pores, and, subsequently, the piece of hollow fiber is connected to the needle of the micro-syringe. The syringe needle and the piece of hollow fiber are placed in an aqueous sample, and during extraction, small volumes of the aqueous sample are repeatedly pulled in and out of the hollow fiber using the syringe plunger. During withdrawal of aqueous sample, a thin film of organic solvent is built up in the hollow fiber and vigorously extracts the analyte from the sample segment; whereas during sample expulsion, this thin film recombines with the bulk organic phase in the syringe. During this recombination, the portion of analyte extracted in the current cycle is trapped in the bulk organic solvent. After extraction, which includes many repeated cycles, a portion of the bulk organic solvent is subjected to further chromatographic analysis. Dynamic LPME is carried out with hollow fibers in the rod configuration without sealing the end. The major disadvantage of dynamic LPME is the demand for instrumentation to perform the plunger movements, which may be difficult to perform in a 96-well format.

## Fundamentals

### *SPME*

In SPME, a small amount of extracting phase associated with a solid support is placed in contact with the sample matrix for a predetermined amount of time. The theoretical discussion here is focused on the more traditional approach to SPME, which 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. Typically, SPME is considered to be complete when the analyte concentraton has reached distribution equilibrium between the sample matrix and the fiber coating. In practice, this means that once equilibrium is reached, the extracted amount is constant within the limits of experimental error and it is independent of further increase of extraction time. The equilibrium conditions can be described as (42):

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s} \qquad \text{Eq. 1}$$

where $n$ is the number of moles 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.

Strictly speaking, this discussion is limited to partitioning equilibrium involving liquid polymeric phases such as PDMS. The method of analysis for solid sorbent coatings is analogous for low analyte concentrations because the total surface area available for adsorption is proportional to the coating volume, if we assume constant porosity of the sorbent. For high analyte concentrations, saturation of the surface can occur, resulting in nonlinear isotherms, as discussed later. Similarly, 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 that 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. To obtain reproducible data, however, 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 sample concentration and the amount of analyte extracted. This is the basis for analyte quantitation. The most visible advantages of SPME exist at the extremities 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 sub-micrometer-diameter fibers permits the investigation of single cells. Because SPME does not extract target analytes exhaustively, its presence in a living system should not result in significant disturbance (43). In addition, the technique facilitates speciation in natural systems because the presence of a minute fiber, which removes small amounts of analyte, is not likely to disturb chemical equilibrium in the system. It should be noted, however, that the fraction of the analyte extracted increases as the ratio of coating to sample volume increases. Complete extraction can be achieved for small sample volumes when distribution constants are reasonably high. This observation can be used to 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 rapid extraction and transfer to an analytical instrument. These features result in an additional advantage when investigating intermediates in the system. For example, SPME was used to study biodegradation pathways of industrial contaminants (44). The other advantage is that this technique can be used for studies of the distribution of analytes in a complex, multiphase system (45) and speciate different forms of analytes in a sample (46).

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

$$n = K_{fs} V_f C_0 \qquad \text{Eq. 2}$$

which points to the usefulness of the technique for on-site 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, as the fiber can be exposed directly to the ambient air, water, production stream, etc. The amount of extracted analyte will correspond 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, and errors associated with analyte losses through decomposition or adsorption on the sampling container walls will be prevented. This advantage of SPME could be enhanced, practically, by developing portable field devices on a commercial scale.

Quantitation based on the distribution constant discussed previously assumes that the system reached equilibrium. The equilibration time is inversely proportional to the sample matrix/coating material distribution constant, coating thickness, and agitation conditions (22,47). Therefore, increased capacity of the fiber results in increased sensitivity of determination and longer extraction times. One way to overcome this fundamental limitation is to use short exposure times. When performing such experiments, not only is it critical to precisely control extraction times, but also convection conditions must be monitored to ensure that they are constant or can be compensated for. One way of eliminating the need for compensation of convection is to normalize (use the same) agitation conditions. For example, the use of stirring means at well-defined rotation rates in the laboratory or fans for field air monitoring will ensure consistent convection.

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

The amount of analytes accumulated on the fiber as a function of time and fiber geometry can be estimated as (48):

$$n = \frac{2\pi D_s L C_s t}{\ln((b + \delta)/b)} \qquad \text{Eq. 3}$$

where $n$ is the mass of extracted analyte over sampling time ($t$) in nanograms; $D_s$ is the analyte molecular diffusion coefficient in sample matrix ($cm^2/s$); $b$ is the outside radius of the fiber coating (cm); $L$ is the length of the coated rod (cm); $d$ is the thickness of the boundary layer surrounding the fiber coating (cm); and $C_s$ is analyte concentration in sample matrix (ng/mL).

The differences in diffusion coefficients between compounds are small compared with the differences in distribution constants. This makes it easier to calibrate the system. Because of the large differences in distribution constants between analytes, the resulting chromatograms are characterized by small peak areas for compounds with small distribution constants and large areas for those with large constants. With uptake dependent on diffusion coefficients, all compounds in a chromatogram with similar molecular masses will have similar peak areas, given similar detector responses. Also, it is relatively simple to calculate the diffusion coefficients for given analyte and therefore 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, where smaller amounts are extracted, can solve this problem. Also, at these higher concentrations, samples are easily extracted and analyzed with PDMS fiber, using conventional SPME methods. 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 it is generally applicable.

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, the extraction occurs through the static gas phase present in the needle. The integrated system can consist of 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) (10). In these cases, the only mechanism of analyte transport to the extracting phase is diffusion through the gaseous phase contained in the tubing. During this process, a linear concentration profile is established in the tubing between the small needle opening, characterized by 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 (49):

$$n = AD_g \frac{dc}{dz}\, dt = AD_g \frac{\Delta C(t)}{Z}\, dt \qquad \text{Eq. 4}$$

where $DC(t)/Z$ is a value of the gradient established in the needle between the needle opening and the position of the extracting phase, $Z$; $DC(t) = C(t) - C_z$, where $C(t)$ is a 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 gas phase in the vicinity of the coating. $C_Z$ is close to zero for a high coating/gas distribution constant capacity, then: $DC(t) = C(t)$. The concentration of analyte at the coating position in the needle ($C_Z$) will increase with integration time, but it will be kept low compared with the sample concentration because of the presence of the sorbing coating. Therefore, the accumulated amount over time can be calculated as:

$$n = D_g \frac{A}{Z} \int C(t)dt \qquad \text{Eq. 5}$$

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 ($A$). It is inversely proportional to the distance of the coating position in respect of the needle opening ($Z$). It should be emphasized that equation 5 is valid only in a situation where the amount of analyte extracted onto the sorbent is a small fraction (below RSD of the measurement, typically 5%) of the equilibrium amount in respect to 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 (smaller $A$), or a higher capacity sorbent can be used. The first two solutions will result in 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 increased integration time is to use sorbents characterized by large coating/gas distribution constants.

The calibration of diffusion-based sampling, in which the time weighed average (TWA) sample is an example, is simple because it can be based on fundamental parameters (50). For complex samples or field analysis, internal standard and standard addition are important for calibration. They compensate for additional capacity or activity of the matrix. However, such approaches require delivery of the standard to the matrix. This adds an addi-

tional step to the sample preparation, which makes the process longer and is sometimes prohibitive (e.g., in the case of on-site or in vivo determinations). Recently, an alternative approach has been introduced in which the standard is delivered together with the introduction of the extraction phase (51–53). This approach is not possible to implement practically for the exhaustive extraction techniques because a large volume of the extraction phase, having a high affinity for the target analytes, is used in these approaches to facilitate as complete a removal of the analytes from the matrix as possible. In microextraction, however, the substantial portion of the analytes is present in the matrix during the extraction and after equilibrium is reached. This fact presents the opportunity of adding the standard to the investigated system together with the extraction phase. For example, when performing small-volume (few milliliters) sample analysis (as frequently encountered in automated analysis) involving a microextraction step, placing the extraction phase/standard mixture in the vial with the sample can be combined with the addition of the standard, thereby eliminating the step of standard spike. Because the desorption and re-equilibration of the standard originally present in the matrix occurs simultaneously with the mass transfer and equilibration of the target analytes from the matrix to the extraction phase, the standard delivery process is not expected to add substantially to the extraction time. For example, in automated-fiber SPME analysis, the standard can be introduced onto the coating during the automated analysis process by exposing the fiber to the standard containing vial.

It is expected that the most significant impact of the standard in the extraction phase approach for calibration would be for on-site, in situ, or in vivo investigations. In this case, however, we want to minimize the amount of foreign substances added to the investigated system. Therefore, a direct standard spiked into the matrix is typically not possible. Full re-equilibration of standards present on the fiber is frequently not feasible because of the contamination issues and large dilution, which might occur in on-site investigations. However, the successful calibration can be accomplished by investigating kinetics of the desorbtion/sorption process. Because in most of the practical extractions the rate of extraction is controlled by the mass transfer through the boundary layer, the desorbtion rate or the standard can be used to give an indication of the extent of the boundary layer (existing either in the matrix or the extraction phase or both), and this information can then be used for calibration of the target analytes. In the most advanced approach, the standards can be added to balance the analyte loss from the matrix during extraction, similar as it is performed sometimes in dialysis, to minimize the impact of standard on the investigated system. This objective is accomplished by adding the same amount of the standard as the amount of analyte being removed from the matrix. The standard can be chosen to be the isotopically labeled analog of the target analyte to minimize impact on the investigated system. In addition, this approach can allow for studying of the physicochemical partitioning and adsorption phenomenon among sample matrix components. The standard in the extraction phase calibration can be applied in any microextraction or steady-state approach, including SPME and LPME.

*LPME*

Like SPME, LPME is basically a nonexhaustive extraction technique. In other words, the technique does not extract the total amount of analyte present in the sample. For equilibrium extractions, the recovery of analyte ($R$), defined as the percentage of analyte present in the acceptor phase at equilibrium relative to the amount initially present in the sample, may be calculated with the following equations for two- and three-phase LPME, respectively (54):

$$R = [K_{a/s} \cdot V_a / (K_{a/s} \cdot V_a + V_s)] \cdot 100\% \quad \text{Eq. 6}$$

$$R = [K_{a/s} \cdot V_a / (K_{a/s} \cdot V_a + K_{org/s} \cdot V_{org} + V_s)] \cdot 100\% \quad \text{Eq. 7}$$

where $K_{a/s}$ is the distribution constant for the analyte between the acceptor solution and the sample, $K_{org/s}$ is the distribution constant for the analyte between the organic phase and the sample, $V_a$ is the volume of acceptor solution, $V_{org}$ is the volume of the organic phase, and $V_s$ is the volume of the sample. The equations basically apply both to single-drop LPME and to hollow-fiber LPME, but the equations do not account for possible interactions between analytes and the hollow fiber material in the latter.

In addition to the mentioned equilibrium equations, equations are available for the description of LPME kinetics in the two-phase mode (single-drop LPME) (6,34). The concentration of analyte in the acceptor solution as a function of time [$C_a(t)$] may be described by the following:

$$C_a(t) = C_{a,eq} \cdot [1 - e^{-kt}] \quad \text{Eq. 8}$$

where $C_{a,eq}$ is the analyte concentration in the acceptor phase at equilibrium, and $k$ is the rate constant. The rate constant is given by the following:

$$k = [k_{tot} \cdot A_i / V_a] \cdot [1 + (K_{a/s} / V_s)] \quad \text{Eq. 9}$$

where $k_{tot}$ is the overall mass transfer coefficient of the analyte

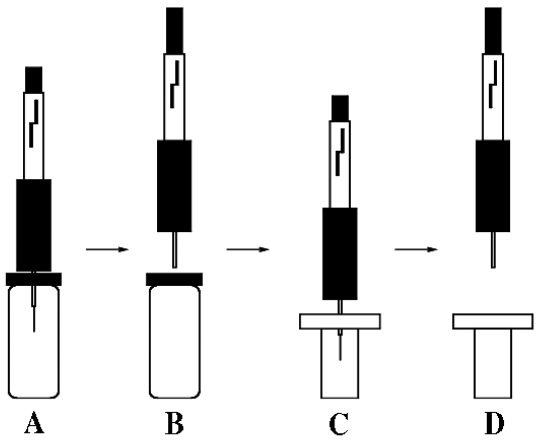


**Figure 8.** Manual fiber SPME sampling and analysis. The user is required to (A) pierce the sample with the needle and expose the fiber, (B) retract the fiber and remove the device after the sampling time is complete, (C) inject the loaded fiber into the injection port and expose for the required desorption time, and (D) remove the fiber for the next sample.

with respect to the acceptor solution, and $A_i$ is the interfacial area.

**Commercial equipment**

The initially developed "fiber" SPME device continues to be the most widely used form of the technique. In this configuration, the extraction phase, usually some kind of polymer, is coated onto a fiber material that, in turn, is attached to a plunger (illustrated in Figure 2). This is all contained inside a syringe that protects the extraction phase when transporting the device and when inserting it into a sample vial or an injection port. Once inside a sample vial, the extraction coating is exposed and analytes concentrate in the coating. The extraction phase containing analytes is then directly transferred to the injection port of the chromatographic (or other) analytical instrument in use. The syringe-like shape means it is easily coupled to techniques such as GC and HPLC. Manual use of the fiber–SPME device is illustrated in Figure 8. The main attractive feature of SPME is that it is in the form of a syringe that can be conveniently automated using an autosampling system that is capable of handling syringes.

*Fiber–SPME approach*

The fiber arrangement of SPME is most suited for automation with GC with its similarity to the traditional GC syringe for liquid injection. The first reference in the literature to automated SPME analysis was published even before the first commercial SPME fibers were released (55). This work involved adapting a Varian model 8100 syringe autosampler so that it could accept an SPME device. Magnetic stirring was used for agitation, which was achieved by setting up a micro-stirrer so that it would be in close proximity to the vial being sampled. The first commercial GC autosampler with a capability for SPME was the model 8200, released by Varian in 1998. Initially this could only perform static sampling and was not temperature controlled, but it was able to start the extraction of the next sample while the previous one underwent GC analysis (56). During 1996, a modified device was launched that allowed vibration of the fiber to agitate the sample. In 1998, temperature control in the form of a thermostated sample carousel was added. One limitation of the system was the vial sizes that it could accept (57). In principle, any autosampler that is able to perform syringe injection can be modified to be capable of automated SPME–GC.


**Figure 9.** Commercial SPME–GC autosampler (CTC Analytics CombiPAL): sample preparation/injection arm (A), sample trays (B), needle heater (C), and heater/agitator (D).

CTC Analytics introduced an SPME option on their CombiPAL autosampler at the beginning of 1999 (illustrated in Figure 9). This allows additional capabilities such as full temperature control of individual samples (sample trays that can be heated or cooled), stirring provided by rotation of an agitator tray, and a fiber conditioning device that allows "bake-out" of the SPME fiber outside of the injection port. The CombiPAL is also sold by a number of other instrument distributors and in some cases under different names. Recently, another robotic arm has been described (58) for analyzing samples with an awkward shape or size, such as living plants (59). However, this device is only able to perform static sampling.

In any fiber–SPME–GC application, septum coring can be an issue when traditional GC septa are used because of their wider 23- or 24-gauge needle, compared with the traditional 26-gauge for standard liquid GC injection. Such problems are exacerbated using automated methods because of the higher throughput possible and that the system is likely to be unattended for significant periods of time. This problem can be minimized by using septa predrilled with an SPME fiber needle prior to installation in the injection port, or solved by use of a septum-less injection device. The Merlin Microseal (Agilent Technologies, Palo Alto, CA) septum replacement is an example of the septum-free injector. It is equipped with two seals to provide (*i*) sealing of the syringe/SPME needle during the injection and (*ii*) closure of the inlet while the separation procedure takes place. The septum replacement kits are commercially available for most of the commonly used types of injectors. The main advantages of the septum replacement are the noncoring nature, wide range of operation temperatures and pressures applicable, and its durability. The lifetime of the Merlin Microseal can range from 1,000 to over 10,000 injections, depending on the sample type and operating conditions during the analysis.

For automated fiber–SPME, the two main methods that have been used for sample agitation are fiber vibration (Varian autosampler) and sample tray rotation (CombiPAL). This is in contrast to manual fiber SPME in which magnetic stirring is the most common technique. Both the vibration and rotation techniques have an advantage over magnetic stirring in that a foreign object is not added to the sample. This saves sample preparation time because the stirrers do not have to be manually added to the sample vials and also prevent any chance of sample contamination if the stirrer bars are cleaned and reused. The fiber vibration technique, though more efficient than magnetic stirring in small (2 mL) vials, was not particularly effective for larger sample volumes. The method also puts stress on the fiber needle. The rotating tray agitation mechanism similarly causes stress on the fiber needle through the rotating action, and agitation is restricted to between 250 and 750 rpm with the PAL system. Using the 24-gauge needles, stirring often must be less than 750 rpm or breakage of the needle can occur because of the stress applied. This problem appears to have been overcome with the availability of 23-gauge needles. These restrictions can result in slower equilibration times than would otherwise be possible (60). It has also been noted in one paper that the rotating tray agitation technique can result in splashing of the fiber in headspace sampling, which decreased the fiber lifetime.

Since 2002, a commercial magnetic stirrer module has been an

available option for the CombiPAL autosampler. Magnetic stirrers not controlled by the software can also be used in conjunction with this unit (61). However, the use of a magnetic stirrer bar may introduce contaminants to the sample.

One way to improve the robustness and sample throughput of an automated SPME method is to increase the number of analyses possible with a single SPME assembly. Regarding the fiber–SPME approach, Supelco recently developed a new generation of super-elastic metal fiber assemblies. The fiber needle, plunger, and fiber core are made of a special type of inert, flexible alloy that makes the assemblies more robust to be able to perform several hundreds of analyses in a sequence. In order to pierce the sample vial septum more effectively, the fiber tip is of a bevelled design. The alloy used in the manufacturing of these fiber assemblies has excellent shape-memory properties and tensile strength. Therefore, the fiber is straight even after several hundreds of injections. The PDMS 100-, 30-, and 7-µm fiber assemblies are already commercially available. In the future, all of the present types of commercial coatings attached to the fused-silica cores will also be available in this super-elastic arrangement. Because the fiber thin-wall needle size is 23 gauge, septum coring may occur during the injection procedure. Therefore, the septum-free injectors such as the aforementioned Merlin Microseal or similar septum-less sealing systems are strongly recommended to be used in a combination with these new assemblies.

Splitless direct injection (DI) glass liners (also called SPI liners or uniliners) significantly help to improve the transfer of sample molecules onto the chromatographic column (62). The DI liners are, to a certain extent, able to simulate on-column injection. Although there are several types of DI liners of various internal diameters, the internal diameter of the DI liners used for SPME applications should be similar to that of the SPME injection sleeve (typically, 0.75–1-mm i.d.). The new, super-elastic fiber assemblies used in conjunction with a septum-less injection port and low-volume DI liners seems to be a very powerful combination that is capable of significantly increasing the number of SPME applications in various scientific fields of interest. The long-lasting fiber assembly is obviously of great importance (e.g., in various classification studies requiring analysis of hundreds of individual samples to be able to perform statistical evaluation of the data or "fingerprint" chromatographic comparison). A further improvement to the mentioned system would be the development of an automated micro-programmable temperature-vaporizing injector configuration modified according to the literature (63). This technique is able to desorb the analytes from the fiber in a more focused band than traditional methods.

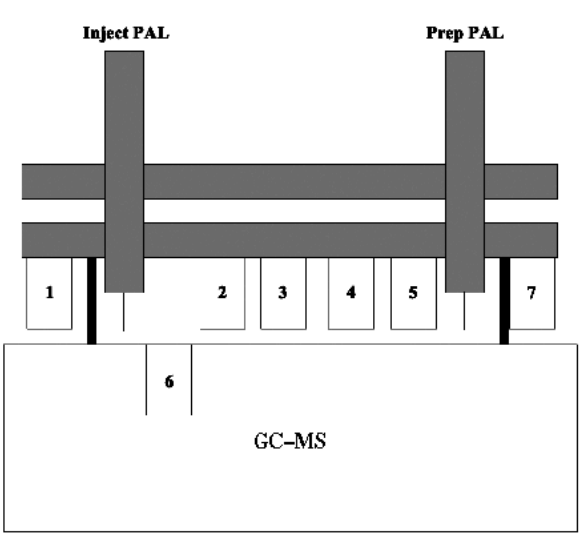


**Figure 10.** Diagram of the dual-arm PAL used for the analysis of organometallics. For the figure, sample heater/agitator, 1; fiber heater, 2; cooled sample tray, 3; sample tray, 4; fast water station, 5; GC injector port, 6; and sample heater/agitator 2, 7. The prep PAL contains a syringe, and the inject PAL contains an SPME fiber.

*Automated in-tube and other SPME configurations coupled with GC*

The fiber configuration is not the only form of SPME that has been successfully automated for use with GC. An in-tube device in which the extraction phase is coated onto the inside of a needle has also been automated using the CTC autosampler (64). This has been marketed commercially since 2000 (65) as "solid-phase dynamic extraction". The device is assembled onto a gastight syringe and, by repeated pulling and depression of the plunger, sample is drawn past the extraction phase. For effective desorption of analytes into the GC, some means of passing the gas past the extraction phase into the injection port is required. This has been achieved using nitrogen either drawn into the syringe immediately prior to the injection or added through an inlet in the side of the syringe. Greater robustness of the device, a larger extraction phase, and a greater surface-to-volume ratio compared with fused-silica fiber SPME have been given as advantages of the technique. In terms of robustness, the use of one needle for 200 or more analyses have been reported (66). In contrast, the traditional (fused silica) SPME fibers are typically anticipated to last for 100 analyses (67), regardless of whether manual or automated techniques are used. This has relevance to automation because a longer lifetime will allow the instrument to run unattended for longer periods of time. One limitation of the technique is that it requires magnetic stirring for agitation because the needles cannot be bent, as may occur when using the rotating tray device of the CombiPAL sampler (68) or the fiber vibration technique. Method development is similar to that used for fiber–SPME techniques. However, the different configuration requires that the

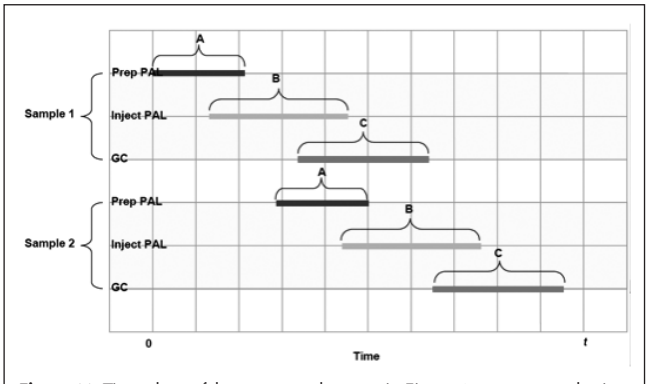


**Figure 11.** Time chart of the automated system in Figure 6, programmed using "Cruise Control" software that maximizes sample throughput by allowing simultaneous use of both arms.

number of plunger strokes, plunger speed, aspiration volume and desorption flow rate conditions need to be optimized. Additionally, by withdrawing or adding air into the vial, pressure in the vial can increase or decrease, creating the potential for leaks. In addition, because of the fact that the technique is very complex and requires a large number of precise plunger strokes, it is much better suited to automated methodology and could not be performed as easily in manual mode.

*Dual-arm sample preparation*

A further dimension to automated SPME analysis has been facilitated by the development of a dual arm PAL autosampler (69) that can perform sample preparation steps prior to SPME extraction. The device consists of two CombiPAL autosamplers stacked on top of one another (Figure 10). One robotic arm is fitted with a syringe and the other has an SPME fiber installed. This approach has been used for in-vial derivatization of organometallic compounds using sodium tetraethyl borate in aqueous samples followed by SPME of the derivatives (70). The system was able to fully automate the entire procedure, which involved addition/mixing of buffer and derivatization reagent, the derivatization, and finally the SPME. The availability of a cooler tray with the autosampler was beneficial because this increased the lifespan of the derivatization reagent solution. The system was also able to prepare diluted standards from a stock solution. With "cruise control" software (Key Pad Terminal Leap Technologies, Carrboro, NC) that was specifically designed for use with this dual arm system, it was possible to use the arms simultaneously, and the program ensured that the arms could never collide. This enabled the maximum sample throughput, as there was no time wasted waiting for one arm to finish its tasks prior to the second starting (as highlighted in Figure 11). For this application, the reduction in analysis time was 24% compared with when the arms could only be moved one at a time, as was the case when the original CombiPAL software was used. A comparison of the precision of the automated and manual methods showed a clear improvement with the automated technique.

The dual arm system has also been used to automate the derivatization of 2-chlorovinylarsonous acid in urine prior to SPME analysis, but in this method the buffer and internal standard were added manually. A method for the determination of ethyl carbamate in alcoholic beverages used one arm of the dual system to deliver a salt solution to the samples prior to SPME, which was then performed by the second. In this study, the salt solution was added to the sample by two procedures. In the first procedure, addition to each sample occurred immediately prior to extraction. In the second procedure, the solution was added to all samples and then sequential extraction was performed. A higher peak area was obtained using procedure 1.

Complete automation of SPME formats other than fiber or in-tube type configurations has not yet been achieved. Semi-automated methods have been reported for the configuration, known as stir bar sorptive extraction (71), in which the extractive phase is coated onto a stirrer bar. To maximize sample throughput and minimize the analyst time required using this technique, a number of extractions can be performed concurrently using a multiposition magnetic stirrer. For GC analysis, the loaded stirrer bars are inserted into thermal desorption tubes/liners, which can be automatically loaded and removed from a GC injection port fitted with a thermal desorption unit and a temperature-programmable injector (72). However, the user needs to manually transfer the stirrer bar into the sample vial and from the sample vial to the liner tray. The latter step creates the possibility of losses or contamination during transfer, and also makes the technique, in its current form, unsuitable for the analysis of volatiles.

*LPME*

Unlike the situation for SPME, the availability of commercial equipment for LPME is currently somewhat limited. Gerstel in Germany recently launched a membrane extraction device for two-phase extractions called "automated membrane extraction" or "membrane-assisted solvent extraction (MASE)", in which analytes are extracted from aqueous samples into a small polymeric bag containing an organic solvent (73). The bag is made of nonporous polypropylene with a very thin wall, and mass transfer

**Table I. Regression Line Parameters for SPME Versus Purge and Trap for Clean Water Analysis**

| | Regression parameters (P&T on *x*-axis, SPME on *y*-axis) | | |
|---|---|---|---|
| Compound | Slope | *y*-Intercept | Correlation |
| Benzene | 1.07 ± 0.087 | –5.9 ± 6.6 | 0.9951 |
| Toluene | 1.06 ± 0.060 | –4.6 ± 4.6 | 0.9976 |
| Ethylbenzene | 1.06 ± 0.046 | –3.8 ± 3.7 | 0.9986 |
| *m*/*p*-Xylene | 1.05 ± 0.073 | –8.4 ± 11 | 0.9964 |
| *o*-Xylene | 1.07 ± 0.060 | –4.0 ± 4.6 | 0.9977 |

**Table II. Statistical Characteristics of the Results Obtained in the Round Robin Test on Pesticide Analysis by SPME*,†**

| Compound | $ss_r$ | $ss_L$ | $ss_R$ | rr | R | rGA | CI | TV |
|---|---|---|---|---|---|---|---|---|
| Dichlorvos | 2.06 | 5.04 | 5.44 | 5.83 | 15.4 | 227.3 | 27 ± 5.8 | 25 ± 1.35 |
| EPTC | 0.56 | 1.56 | 1.66 | 1.57 | 4.7 | 9.9 | 10 ± 1.6 | 10 ± 0.54 |
| Ethoprofos | 0.82 | 4.79 | 4.86 | 2.32 | 13.74 | 15.5 | 16 ± 2.3 | 17 ± 0.92 |
| Trifluralin | 0.27 | 0.57 | 0.63 | 0.76 | 1.79 | 1.6 | 1.6 ± 0.76 | 2 ± 0.11 |
| Simazine | 2.34 | 3.45 | 4.17 | 6.61 | 11.79 | 23.6 | 24 ± 6.6 | 25 ± 1.35 |
| Propazine | 1.21 | 2.04 | 2.37 | 3.42 | 6.71 | 9.5 | 10 ± 3.4 | 10 ± 0.54 |
| Diazinon | 0.63 | 2.13 | 2.22 | 1.79 | 6.29 | 8.2 | 8 ± 1.8 | 10 ± 0.54 |
| M. chlorpyriphos | 0.12 | 0.32 | 0.34 | 0.35 | 0.97 | 1.6 | 1.6 ± 0.35 | 2 ± 0.11 |
| Heptachlor | 2.03 | 2.89 | 3.53 | 5.75 | 10 | 8.9 | 9 ± 5.8 | 10 ± 0.54 |
| Aldrin | 0.54 | 0.73 | 0.91 | 1.53 | 2.58 | 2.0 | 2 ± 1.5 | 2 ± 0.11 |
| Metolachlor | 0.73 | 2.83 | 2.92 | 2.07 | 8.28 | 15.7 | 16 ± 2.1 | 17 ± 0.92 |
| Endrin | 0.87 | 3 | 3.13 | 2.47 | 8.85 | 8.8 | 9 ± 2.5 | 10 ± 0.54 |

* Abbreviations: sr, repeatability standard deviation; $s_L$, interlaboratory standard deviation; $s_R$, reproducibility standard deviation; r, repeatability; R, reproducibility; GA, gross average; CI, confidence interval of the gross average; and TV, confidence interval of the "true" value.
† All values are expressed in µg/L.

across this solid membrane is accomplished as the membrane is swelled with the organic solvent present inside. The volumes of organic solvent used in MASE are currently in the range of 0.5 to 1 mL, which exceeds the volumes used in most LPME work by a factor of 10 to 100. The membrane device of Gerstel is highly interesting, and this in combination with current efforts to commercialize hollow fiber LPME for three-phase extractions will hopefully provide a strong platform of LPME equipment in the near future.

### Robustness, performance, and comparison with traditional extraction methods

*SPME*

SPME has been extensively evaluated with respect to robustness and performance in comparison to alternative techniques. Validation of the method might include comparison of quantitation results with certified values obtained for standard reference materials that have similar matrix and target analytes. Another approach is to validate the method against officially accepted techniques for the analysis of target samples and analytes. Table I summarizes the results of multilevel validation of SPME against the standard purge-and-trap technique for the analysis of benzene, toluene, ethylbenzene, and xylene compounds in water. The regression line has a slope close to one, with a very small intercept value and linear correlation coefficients better than 0.99 for all species. The very good agreement between both methods indicates suitability of headspace SPME for the analysis of volatile organic compounds in aqueous samples (74).

Interlaboratory studies are frequently performed to validate robustness and performance of new technology when it is deployed in different laboratories. Table II summarizes the results obtained in a round robin test on pesticide analysis by SPME, which involved a number of laboratories in Europe and North America (75). In general, the results are characterized by good repeatability, which proves that SPME is a valid method for the determination of pesticides at trace levels. As expected, the interlaboratory and reproducibility standard deviations are higher because they include differences between laboratories. However, they are still satisfactory. The results also indicate that SPME is an accurate method. In all cases, the confidence intervals of the gross average and the "true" value overlap, which indicates that any differences between the two respective values are attributable to random factors. Interestingly, for 10 out of 12 compounds, the values of the gross average are slightly lower than the "true" values. This might be attributable, in part, to losses of analytes through adsorption (as described previously) in cases in which the aqueous pesticide solutions were not prepared directly before the analysis, as required by the test protocol. Results described previously demonstrate that SPME methods can be transferred successfully from one laboratory to another.

*LPME*

Unlike SPME, most LPME work to date has been accomplished with home-built equipment, which naturally has implications on the results presented on performance and robustness. Nevertheless, the literature contains substantial information for LPME on important issues such as extraction times, recovery, enrichment, selectivity/cleanup, and validation. In terms of extraction time, LPME behaves in a similar manner as SPME. Thus, LPME comes to equilibrium after a certain time, and prolonged extraction times provide no additional gain in total mass transfer. Equilibrium times for LPME differs substantially from application to application depending on parameters like the geometrical configuration of the equipment, the distribution constant, and the level of sample agitation. However, extractions for 15 to 45 min are typically required in static LPME to reach equilibrium (35). Compared with traditional LLE, in which exhaustive extractions are performed with a large excess of solvent, extraction times in LPME are relatively long. The principal reason for this is the small contact area between the extracting phase and the sample, surrounded by a boundary layer with no convection. On the other hand, the unfavorable time aspect may be compensated by the possibility of simultaneously extracting a large number of samples as in a 96-well format (Figure 6). In addition, extraction times may be reduced, and LPME may be accomplished in the nonequilibrium mode with no loss of accuracy or precision, provided there is precise timing of the experiments (34,35). Furthermore, some improvements in extraction speed may also be expected by the application of dynamic LPME.

For two-phase LPME, equation 6 in the Fundamentals section predicts that extraction recoveries depend on the distribution constant and volumes of sample and acceptor solution. For relatively hydrophobic substances, the $K_{a/s}$ value is high, and the capacity of the two-phase LPME system, expressed by the term $K_{a/s} \cdot V_a$, is significant. In other words, from relatively small sample volumes, high recoveries may be expected, and this has also been supported by several experimental studies. Thus, recovery values in the range up to approximately 50–90% have been reported for both environmental contaminants (76,77) and for drug substances (37). For three-phase LPME, the situation is slightly more complicated; again, the distribution constant as well as the volumes of sample and acceptor solution affects the recovery, but for three-phase extraction the capacity of the organic membrane ($K_{org/s} \cdot V_{org}$) also has a significant impact on the recovery. In other words, the analyte may be partially trapped in the organic phase, especially for compounds with $K_{org/s}$ close to $K_{a/s}$. Nevertheless, experimental studies on both drug substances (7,38,54,78) and environmental contaminants (41,79) have demonstrated recovery values in the range of up to 40–95%. Thus, also in the three-phase mode, LPME may be tuned to nearly exhaustive extraction for analytes with high distribution constants recovered from relatively small sample volumes (typically below 5–10 mL). In terms of extraction recovery, the difference between LLE and a properly tuned LPME system may actually be relatively small, but, as mentioned previously, extraction times are prolonged when moving from LLE to LPME.

Besides high extraction recoveries, the enrichment of analyte is also an important feature of LPME. For both two- and three-phase LPME, the enrichment factor (E), defined as the concentration ratio of analyte in the acceptor solution at equilibrium relative to the initial concentration in the sample, may be calculated with the following equation (54):

$$E = V_s \cdot R/100 \cdot V_a \qquad \text{Eq. 10}$$

Thus, with properly tuned extraction chemistry to ensure a high

value for $R$, and with a small volume of acceptor solution ($V_a$) relative to the sample volume ($V_s$), very high enrichments may be obtained. This has been demonstrated by several papers in the literature, with values ranging from 10 to 1000 (37,38,40, 41,54,77,80). In a particular case, a two-step LPME process transferred analytes almost quantitatively from 100 mL of water samples to only 2 µL of acceptor solution, providing enrichment values exceeding 15.000 (80). The enrichments obtained by LPME are difficult to achieve in traditional LLE, even if evaporation of the solvent and reconstitution is applied.

Selectivity plays an important role during extractions, in general, because selectivity controls the degree of sample cleanup. A closer examination of equation 6 in the Fundamentals section, which essentially also applies to traditional LLE, reveals that as the volume of acceptor solution ($V_a$) is reduced relative to the volume of sample ($V_s$), which is the case when moving from LLE to LPME, the extraction becomes more sensitive to the distribution constant. One important consequence of this is that selectivity increases from LLE to LPME. In other words, LPME provides a higher degree of sample clean-up than LLE. This applies to two-phase LPME and, especially, three-phase LPME involving a back-extraction step from the organic phase to a second aqueous solution (acceptor solution). An example of the excellent sample cleanup is illustrated in Figure 12, in which methamphetamine and citalopram were extracted from whole blood without any matrix components visible in the electropherograms from a CE analysis. A comprehensive comparison of LLE and LPME was recently published (54).

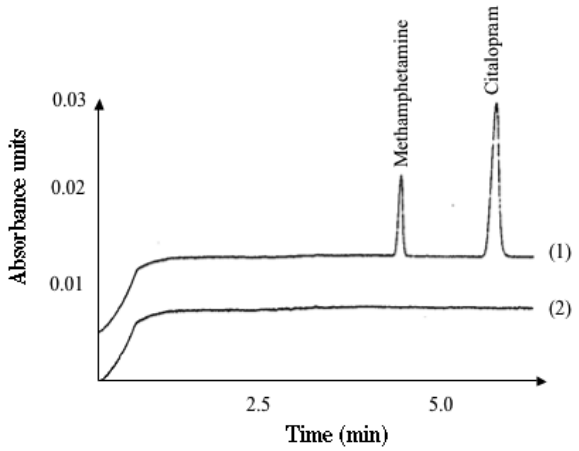


**Figure 12.** Three-phase LPME and CE of 100 ng/mL methamphetamine and citalopram in human whole blood. For the figure, spiked sample (1) and drug-free sample (2).

**Table III. Validation Results for Hollow Fiber LPME and HPLC of Aromatic Amines Present in Water Samples**

| Analyte | RSD (%) ($n$ = 6)* Interday | Intraday | Linearity range (µg/L) | Correlation coefficient ($r^2$) | Detection limit (µg/L) |
|---|---|---|---|---|---|
| 3-Nitroaniline | 4.28 | 5.63 | 1–500 | 0.9988 | 0.10 |
| 4-Chloroaniline | 4.83 | 7.26 | 1–500 | 0.9998 | 0.08 |
| 4-Bromoaniline | 3.92 | 5.12 | 0.5–500 | 0.9988 | 0.05 |
| 3,4-Dichloroaniline | 3.84 | 4.89 | 1–500 | 0.9917 | 0.10 |

* Repeatability was investigated at a concentration of 50 µg/L for each analyte.

An important concern when moving from traditional extraction techniques to LPME is related to validation, in general, and, especially, to the method precision. Several papers in the literature have addressed the issue of validation of LPME, with a major focus on drugs and environmental analysis. One example of data is reported in Table III for different aromatic amines extracted by two-phase hollow fiber LPME followed by HPLC (79). In general, the validation results of the various LPME publications mostly comply with the requirements demanded by authorities such as the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA). However, as mentioned previously, all validation data for LPME has been accomplished with home-built equipment, and it is expected that the figures of merit will be improved with the emerging commercial equipment. Thus, the down-scaling of equipment and the extracting phase is expected to not have a negative impact on method precision and accuracy.

A final question concerning performance is the comparison of figures of merit for SPME and LPME. Unfortunately, until now, only limited information is available on this subject, and exact studies based on identical or comparable experimental conditions and analytes are missing. It is, however, an important issue, which should be investigated within the near future.

## Transfer from traditional extraction methods to microextraction

### *SPME*

As Tables I and II illustrate, SPME is able to provide accurate data equivalent to traditional techniques. SPME is fundamentally equivalent to static headspace techniques and can directly replace these techniques with additional benefits of increased sensitivity for less volatile components, broader polarity, and volatility range of analytes being extracted. The method development principles in headspace and SPME are analogues with additional steps in SPME application involving determination of optimum coating and extraction time.

The transfer from exhaustive technique methods, such as liquid–liquid extraction to sorbent trapping or SPE to SPME, is not as straightforward, especially for complex samples. In principle, the amount of analytes extracted in SPME is proportional to the free concentration of analyte in a sample, not the total concentration as in exhaustive techniques. On one hand, this presents a significant advantage when the bioavailable portion of the analyte is of interest or binding needs to be characterized (82). However, on the other hand, it requires external calibration in equivalent matrix to the sample matrix or, alternatively, internal standard calibration or standard addition with a closely related compound to obtain an accurate measure of the total concentration. Therefore, the SPME optimization procedures are more involved and have more steps compared with exhaustive techniques (83). It should be emphasized, however, that when the method has been developed, the cost of analysis per sample is very low considering simplicity and automation capability.

SPME is often mistakenly considered to be another form of SPE or micro-SPE. However, there are significant differences between

the methods. SPE is, essentially, a three-step process. A sample is initially passed through the sorbent bed, and analytes present in the sample are exhaustively extracted from the sample matrix to the solid sorbent. In a second step, unwanted analytes are selectively desorbed from the solid sorbent by washing with a solution capable of desorbing unwanted analytes but leaving desired analytes retained on the sorbent. In the final step, the wash solution is changed for one able to desorb analytes of interest. The resulting eluent may then be concentrated by evaporation, to the desired volume. However, SPME takes advantage of equilibrium extraction and selective sorption from the matrix onto the coating. In the first step, the coating is exposed to the sample, and analytes with a high affinity for the sorbent are selectively extracted. In the second step, everything extracted by the fiber is desorbed into the analytical instrument. No intermediate clean-up step is normally implemented. Micro-SPE is more related to SPE as it is a total extraction method, but it utilizes a reduced sample and sorbent volume. A comparison with SPME is, therefore, inappropriate.

A degree of selectivity is required for any sample preparation method. It is impractical to introduce all compounds present in a sample to an analytical instrument. The method developed must eliminate compounds incompatible with the instrument, including matrix components. It is also desirable to remove as many of the unwanted compounds as possible in order to make the resulting data interpretation as clean and simple as possible. Thus, with selective extraction, sample preparation is simplified and typically results in a significant savings in time and precision.

Selectivity is, therefore, quite important when choosing an SPME coating. High capacity, even for a range of analytes, is more important for SPE, in which prevention of break-through is a significant concern. Because break-through is not an issue to be addressed in an equilibrium extraction method such as SPME, more emphasis may be placed on sorbent selectivity.

SPME differs from SPE in another significant way, in that SPE sorbent, because of the large volume of sorbent required relative to SPME, has the potential to retain nonadsorbed components in the void volume. It is difficult to design a wash regimen that removes unwanted compounds completely without impacting retention of the analytes of interest. In this way, there is the potential that unwanted compounds may remain, either adsorbed or present as nonadsorbed analytes in the bulk of the sorbent. Because of the geometry of the SPME device and the modes of extraction used, unwanted analytes are not normally present in the sorbent at the time of desorption. For example, headspace SPME can be used to separate volatile from nonvolatile analytes. Also, use of restricted access materials as a coating eliminates macromolecules from being extracted by the extraction phase located within the coating's porous structure.

*LPME*

Basically, LPME is based on the same chemistry as LLE; two-phase LPME is similar to conventional LLE, and three-phase LPME resembles LLE with back extraction. Nevertheless, because of downscaling, several points have to be considered when moving from LLE to LPME. Generally, many of the points discussed previously for SPME also apply to transfer of methods to LPME. The nature of the analyte determines the mode of extraction. If the final analysis is performed by GC, LPME in the two-phase mode should be selected, whereas three-phase LPME is recommended for HPLC and CE for compatibility reasons (discussed earlier). The volume of the acceptor solution is normally in the range of 10 to 25 µL, whereas the volume of sample should be optimized based on the actual application. The volume of sample should be selected to give sufficient sensitivity for the assay, but this has to be balanced by the fact that extraction time increases with increasing sample volume. The acceptor phase, as well as the organic phase (in three-phase LPME), largely determines the extraction recovery as discussed in the previous section and may require some optimization. Several papers in the literature describe how this may be accomplished (35,78). The solvents used in LPME often differ from the solvents used in LLE. Both in the single-drop format and for hollow fiber LPME, solvents of low volatility are used to avoid partial evaporation during the extraction process because this will have a major impact on the performance and precision in the microextraction format. In addition, in hollow-fiber LPME, the solvent used should also be selected to ensure strong immobilization in the pores of the hollow fiber. Frequently used solvents in LPME include *n*-octanol, dihexyl ether, dodecyl acetate, and different silicon oils. Extraction time, agitation, pH, and eventually salt addition are important parameters to optimize and control during LPME, but these factors have already been discussed for SPME (22) and apply to LPME as well. Finally, like in SPME, calibration becomes highly important because extractions in most cases are nonexhaustive.

## Future Trends

Automated SPME–GC is now a firmly established technique with the original fiber-type SPME device and, to a lesser extent, in-tube approaches. Future developments in this area are likely to focus on the automation of more complicated procedures that use devices such as the dual-arm sampler or cold fiber approach. It is also expected that other autosampler modules will be developed that will increase the range of tasks that are amenable to automation. The recently developed, automated cold-fiber SPME device is one example that would add significantly to the capabilities of automated SPME. Finally, it is anticipated that with further improvements in the robustness of SPME extraction phases, such as the super-elastic fiber assemblies currently being introduced, automation of the technique will further benefit in robustness of the technique. This will allow longer periods of analysis without user intervention. This would be particularly beneficial for situations such as automated field analysis.

SPME–LC automation has been made possible with the use of in-tube-based extraction devices. These do have some limitations, and the challenge is to develop a sampler that can successfully automate the fiber approach or another SPME configuration with LC that will allow a broader range of applications. The use of SPME fibers in conjunction with multiwell plates will allow the high-throughput screening suitable for drug discovery or in vivo monitoring purposes. The construction of a suitable "brush-like" array of SPME fibers lends itself towards robotic automation for the extraction, agitation, and liquid desorption steps involved in

the SPME technique (84).

Automated coupling with other analytical instrumentation, such as CE and spectroscopic techniques, would also be anticipated to receive attention in the future. Finally, the full automation of other SPME configurations, such as stir-bar based extractions, would also be of benefit because of the their greater sensitivity. Overall, the percentage of SPME analyses conducted in an automated fashion is certain to increase in the coming years and, with this trend, the use of this sample preparation method will continue to grow.

Unlike SPME, LPME has until recently been accomplished with home-built equipment within a relatively few laboratories around the world. Yet the availability of commercial equipment is still limited. In light of this, the most important issue for the future of LPME is the development of more commercial equipment with a special focus on three-phase extractions. This is in progress based on hollow-fiber technology, but it is somewhat unclear when a commercial product will be launched. The opportunities of three-phase LPME should particularly stimulate more commercial development, and the technique should be of high interest for the analytical community because (*i*) it is highly compatible with HPLC and LC–MS, (*ii*) it may easily be fully automated in a 96-well format, and (*iii*) it is complementary to SPME. Microextraction approaches can provide more information about the investigated systems compared with exhaustive techniques not only because of on-site analysis capabilities, as mentioned previously, but also because the amount of analyte extracted is proportional to free concentration of analytes in the matrix. Therefore, bioavailability (85) of different compounds could be investigated, as well as binding constants to matrix components (82). These measurements can be performed directly on-site if the microextraction technique is applied in combination with a portable exhaustive technique, such as the needle-trap approach (86), in which amount extracted corresponds to the total concentration of analytes in the sample. Such measurements would allow better characterization of the investigated system.

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Manuscript received September 14, 2005;
revision received December 1, 2005.