



Solid-Phase Microextraction

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In this month's "GC Connections," John Hinshaw discusses solid-phase microextraction...

Gas chromatography (GC) is a highly sensitive and selective separation technique all by itself. Capable of resolving hundreds of components in a short time with part-per-million (ppm, one part in 10^6) or better sensitivity, GC has been applied to myriad analytical problems. When applied to samples that originate outside of laboratories, however, GC resolution and sensitivity are often limited by sample matrix effects. Non-volatile constituents, large sample volumes as required for lower detection limits and less-than-ideal chemical activity interfere with separation and detection. Classic liquid-liquid extraction, chemical derivatization and sample preconcentration — as well as headspace, thermal-desorption and large-volume sample injection techniques — increase analyte concentrations and detector response and reduce matrix effects before sample introduction into the column. In-column solvent effects and stationary-phase analyte focusing help remediate sample matrix side effects after injection but are often limited by sample residue build-up.

Solid-phase microextraction (SPME) is a relatively new sample extraction technique that brings some unique capabilities to the chromatographic analysis of dilute solutions in difficult matrices, both liquid and gaseous. Essentially, SPME has two discrete steps: solute absorption from the sample matrix into a thick — relative to conventional capillary GC columns — layer of silicone or related adsorptive material and transfer of the analytes into a chromatography inlet system by gaseous or liquid means. SPME has significant potential to greatly reduce or eliminate solvent consumption and the concomitant issues of used solvent disposal as part of sample preparation. SPME has been used with both GC and liquid chromatography (LC) separations.

Chromatographers should not confuse SPME with solid-phase extraction (SPE), which is a related predecessor with some similar applications. The principal difference is that SPE is performed with a relatively large sorptive surface the size of a small filter paper, and it requires liquid-phase extraction of analytes; SPME, however, is accomplished using a small fibre or tube coated with sorptive material and primarily uses thermal gas-phase desorption for GC analyte extraction and liquid-phase desorption for LC separations. SPME is routinely applied to gas-phase and liquid-phase extraction, and SPE is limited to extraction from liquid-phase samples. In general, SPME is used to extract organic analytes from gaseous or aqueous sample matrices and is not applied to the analysis of organic matrices such as solvent impurities. This "GC Connections" column discusses absorptive SPME primarily, although the principles also apply to adsorptive SPME onto active solid layers.

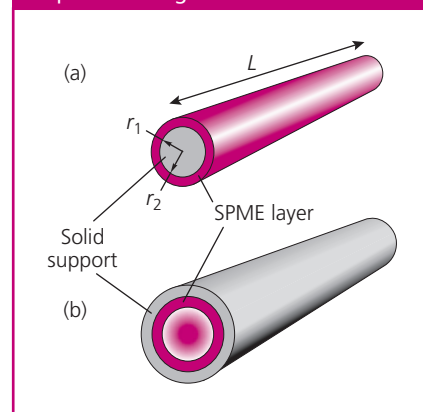
Some of the SPME applications reported within the past few months in the *Journal of Separation Science* and the *Journal of Chromatography* include flower scents,¹ chemical warfare agents,² pharmaceutical process impurities,³ organochlorine pesticides in Chinese teas,⁴ volatile compounds in acidic media⁵ and in cheese,⁶ volatile phenols in wine,⁷ environmental pollutants in water samples,⁸ chloroanisoles in cork stoppers,⁹ volatile aliphatic amines in air¹⁰ and phenylurea herbicides in aqueous samples.¹¹ This list delineates the breadth of applications to which SPME can be applied.

SPME Principles

SPME relies upon the extraction of solutes from a sample into the SPME absorptive layer. After a sampling period — during

which extraction has ideally reached equilibrium — the absorbed solutes are transferred with the SPME layer into an inlet system that desorbs the solutes into a gas (for GC) or liquid (for LC) mobile phase. Success relies upon choosing conditions such that solutes favour the SPME absorptive layer as much as possible in the presence of bulk sample and then are subsequently released as quickly and completely as possible for chromatographic analysis by changing the conditions to favour solute release from the absorptive layer. Secondary trapping and release of desorbed solutes after SPME is sometimes necessary when desorption is too slow to permit full use of column resolving power. This trapping and release can be accomplished using a discrete thermal trap or with column stationary-phase trapping by injection onto a cold column and subsequently temperature programming for solute elution.

Figure 1: Cross-sectional diagram of SPME extraction devices. Shown are (a) a fibre device with external sorptive coating and (b) a tube device with sorptive coating on the inside.



For analysing bulk samples contained in vials or otherwise easily accessed samples, SPME can be performed, as shown in Figure 1(a), with a short, absorptive film-coated fibre. A short tube coated on the inside with an absorptive layer [Figure 1(b)] can also be used for samples that are amenable to

The volume of an SPME layer with the same thickness coated inside a tube [Figure 1(b)] with inner radius (r_2) would be the same. The assumption in Equation 1 is valid for sample volumes more than 100-fold the SPME layer volume or more than approximately 0.2 mL for the thickest

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pumping. The choice of the absorptive-layer chemistry and film thickness strongly influences the degree of absorption and the subsequent efficiency of desorption.

Step 1 — extraction: For the extraction step with an externally coated SPME absorptive layer, the layer is exposed to a sample in a liquid [Figure 2(a)] or gas [Figure 2(b)] phase. In the case of SPME within a coated tube, liquid or gaseous sample is simply pumped. Alternatively, stopped-flow sampling is also possible. The amounts of the solutes in the SPME layer gradually reach an equilibrium level with their surroundings, which represents the maximum solute amounts that can be absorbed and withdrawn under a given set of sampling conditions. The amount of solute, i , in the SPME layer at equilibrium ($M_{i,SPME}$) can be approximated by the following equation

$$M_{i,SPME} \approx K_{i,SPME} V_{SPME} C_i \quad [1]$$

where $K_{i,SPME}$ is an aggregate solute distribution constant between the SPME absorptive layer and the sample, V_{SPME} is the volume of the SPME layer, and C_i is the solute concentration in the sample before SPME sampling.

Equation 1 assumes that the sample volume is much greater than the volume of the SPME layer. SPME coatings typically have thicknesses of approximately 10–100 μm — roughly 10-fold the film thickness range normally encountered in capillary GC. The volume (V_{SPME}) of a 1 cm long by 100 μm thick annular coating on a 0.56 mm o.d. fibre (24-gauge) [shown in Figure 1(a)] is approximately 2 μL :

$$\begin{aligned} V_{SPME} &= \pi L (r_2^2 - r_1^2) \\ &= \pi \cdot 1 \left(0.038^2 - 0.028^2 \right) \quad [2] \\ &= 2.07 \mu\text{L} \end{aligned}$$

SPME layer of 100 μm . Thinner SPME layers, with smaller volumes, would have correspondingly smaller minimum sample volumes.

Influence of the headspace: The presence of a gaseous headspace over a liquid sample causes a portion of each solute to partition into the headspace in competition with the extraction process into the SPME layer. This effect results in a reduction of solute mass in the SPME layer — relative to having no headspace present at all — that depends upon both the headspace volume and the partition ratios between the headspace and the liquid sample as well as between the headspace or the liquid sample and the SPME layer. These relationships are somewhat complex, and they produce dependencies of the extracted solute mass on the relative liquid and headspace volumes. From a practical point of view, it becomes important to maintain constant sample and headspace volumes throughout multiple samples to keep such multiple-phase influences consistent.

Time to equilibrium: A finite time span is required to reach solute equilibrium between a sample and the SPME layer, and equilibrium will ideally occur before the extracted solutes are withdrawn for desorption into a chromatograph. As solute molecules are removed from the sample into the SPME layer, additional solute molecules must diffuse into their places at the SPME-sample interface. The process of absorption is limited by the rate at which solute molecules can replenish the transition layer near the SPME interface. Thorough stirring of the liquid phase helps reduce this time considerably by maximizing exposure of the SPME layer to the sample and greatly reducing the influence of solute liquid-diffusion rates upon SPME uptake. However, stirring the liquid sample does nothing to increase the diffusion rate of absorbed solutes inside the SPME layer itself, which then becomes

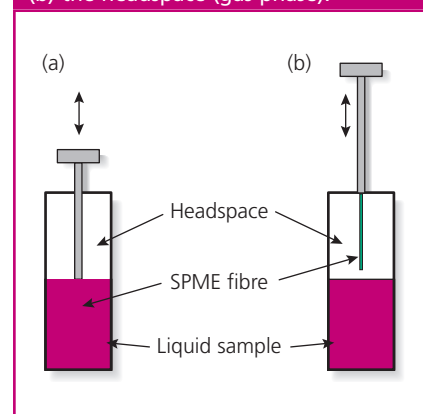
a limiting factor for the rate required to reach equilibrium.

When sampling from the headspace gas instead of the liquid in a two-phase sample system, solute must first cross the liquid-gas interface before encountering the SPME layer. Interestingly, it makes little difference to the ultimate equilibrium solute amounts in the SPME layer whether the sample is obtained from the liquid or the gas. However, the time to reach equilibrium can be greatly influenced by the choice of the sampling phase. Non-polar and volatile solutes that strongly favour the headspace phase will come to equilibrium more rapidly if the SPME layer is exposed to a headspace, and those solutes that favour the liquid phase will equilibrate more rapidly directly from a liquid phase.

Ultimately, chromatographers must characterize the equilibrium times for each solute of interest. If equilibrium is reached in a reasonable time period — perhaps less than 30 min — then they should use a sampling time at least that long. However, if an unreasonably long time is required — in terms of the time available for sampling and analysis — then it is possible to perform SPME without reaching equilibrium. In that instance, operators must ensure that the same SPME sampling time is used for each sample and that amount of time should be as long as possible.

The sample ionic strength, temperature and other factors that influence the partition coefficients — both between the liquid sample and headspace and between the SPME layer and the liquid — must also be kept under careful control for good sample-to-sample consistency. Adding salt to an aqueous sample will often shift the

Figure 2: SPME extraction from a sealed vial. Shown are diagrams representing sampling from (a) the liquid phase and (b) the headspace (gas phase).



partition coefficients for non-polar solutes in favour of the SPME layer and decrease the time required to attain equilibrium.

SPME-layer chemistry: The chemistry of the sorptive SPME layer plays a significant role in enhancing or discriminating against classes of compounds. For the most part, SPME layers for GC absorb solutes in a manner related to their behaviour as GC stationary phases: polar SPME layers such as those that contain polyesters or acrylates will enhance polar constituents and discriminate against non-polar materials. Adsorptive layers with active carbon constituents will retain volatile components more strongly than layers made of non-polar dimethylsilicones. However, chromatographers must give some thought to desorption as well — a very strongly held solute might be too difficult to pry off the SPME layer for analysis.

Step 2 — transfer: The next step after sampling is to transfer the SPME layer and absorbed analytes away from sample exposure and into conditions for desorption in the chromatography mobile phase. Analysts have no need to physically move the SPME layer for SPME tube sampling with a multiple-position valve connected with the tubing, but new conditions must be established that promote solute desorption. An SPME fibre device, however, will be removed from the sample container and then transported to where the solutes are to be desorbed. Removal from the sample environment immediately starts to shift the absorbed solute concentrations away from their in-sample values to lower levels as solutes naturally desorb into their surroundings.

The rate of natural desorption is fairly low for many solutes, but volatile molecules can experience significant losses. In a laboratory situation, the transfer time from sample vial to instrument can be short enough that losses are insignificant. Losses can be minimized during extended transport and storage by sealing the SPME layer into a small enclosure and then ensuring that the contents are included with the rest of the sample after desorption. In addition to volatile sample losses, an SPME layer can easily pick up non-sample components from the ambient air, especially during extended transportation to and from remote sites. Enclosing the SPME layer will also prevent the influx of these contaminants. Commercially available SPME devices incorporate sealing systems such as these.

Step 3 — desorption: Once in place at a chromatograph, the SPME layer must then

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be exposed to conditions that cause the absorbed solutes to desorb with as close to 100% efficiency as possible — and in a time that is short enough to be compatible with the chromatography mode in use. In the instance of an SPME layer coated inside a tube, a simple multiple-position valve arrangement can switch from sample liquid flow to the mobile phase for LC analysis. Stopping the mobile-phase flow in the SPME tube allows time for solute desorption to come to equilibrium between the SPME layer and the liquid mobile phase before the desorbed materials are introduced into the column. Tube heating is necessary for GC analysis of in-tube SPME layers. Chromatographers encounter in-tube SPME sampling infrequently for GC, primarily because of the instrumental complexities.

Desorption from fibre-based external SPME layers is made simpler by inserting the fibres into a standard GC capillary inlet system in much the same way as a syringe. Devices that wash the external SPME layer with mobile phase and pass the desorbed solutes into the LC injection loop are available for LC desorption and analysis.

Several trade-offs arise in the course of thermally desorbing an SPME layer for GC analysis. First, the temperature must be high enough so that the solutes leave the SPME layer rapidly. Too-slow desorption can cause peak broadening and tailing unless additional arrangements are made for trapping solutes at the beginning of a cooled column before temperature programmed elution. Conversely, too-high inlet temperatures can induce thermal decomposition and introduce some contaminants into the column from septum bleed and from the SPME layer itself.

During sample desorption from an SPME fibre into a split-splitless inlet, the inlet split flow should be turned off so that all of the solutes can enter the column without splitting. It is unlikely that enough sample will be absorbed on an SPME layer to necessitate sample splitting. A narrow-bore inlet liner — often called a splitless liner — helps produce better peak shapes by limiting the volume into which the solutes may expand. After the SPME device has been withdrawn from an inlet splitter, the split flow can be turned on to purge the

inlet of any remaining materials and prevent some degree of peak tailing.

Programmed temperature vaporization inlet systems are also well suited to SPME desorption because of their smaller internal volumes. For SPME use, they should be operated at the same elevated constant temperatures as conventional split-splitless inlets because programmed temperature vaporization heat-up rates — on the order of 200–500 °C/min — might be too slow to produce narrow-enough peaks without some form of additional stationary-phase trapping.

Why SPME?

The primary advantages of SPME are its ability to decouple sampling from matrix effects that would distort the apparent sample composition or disturb the chromatographic separation; its simplicity and ease of use; and its reduced or non-existent solvent consumption. These characteristics combine to make SPME an attractive alternative to classic headspace or thermal-desorption sampling, solid-phase extraction and classic liquid-liquid extraction.

As with several related sample preparation and injection techniques such as headspace GC and thermal desorption, SPME lends itself well to handling difficult sample matrices and has the added benefits of low cost and simplicity. SPME doesn't require elaborate and expensive instrument accessories for occasional use, and yet it seems to be capable of delivering very good manual results when in the hands of skilled users, which cannot necessarily be said of manual headspace or thermal-desorption sampling. Autosamplers are also available to perform repetitive unattended SPME sampling.

SPME requires careful optimization and consistent operating conditions for success, but this statement is true of the related techniques as well. Any poorly characterized sampling technique has no valid use in analytical laboratories, and the burden of developing an SPME method is no greater than for developing a method for any of the other techniques. SPME has a significant place in analysts' arrays of sample preparation techniques.

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