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**ARTICLE** *in* CHEMICAL REVIEWS · APRIL 2011

Impact Factor: 46.57 · DOI: 10.1021/cr100203t · Source: PubMed



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# Nondestructive Sampling of Living Systems Using *in Vivo* Solid-Phase Microextraction

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## 1. INTRODUCTION

The development of techniques to monitor levels of biologically active compounds in living systems in natural environments is currently an important research topic of interest.[1−5] The main reason behind this trend is that *in vitro* analysis may not be able to accurately indicate or predict the processes occurring in a complex living system. For example, the composition of the volatile extracts obtained from detached or damaged plants can differ significantly from those emitted by living undamaged specimen.[6−8] During metabolism or toxicology studies, an *in vitro* assay may not accurately predict the fate of a xenobiotic, thus necessitating verification using an *in vivo* model. An *in vivo* sampling approach can also eliminate errors, reduce the time associated with sample transport and storage, result in more accurate, precise, and faster analytical data,[4] and therefore give a better indication of what will happen in the real world.[5] Furthermore, *in vivo* sampling permits repeated temporal and longitudinal studies, for example, to monitor scent emission from a developing flower over time or to monitor progression of disease in an individual with respect to time without having to sacrifice the individual at each sampling point. In other words, the amount and high quality of information obtainable using *in vivo* approaches are the main driving forces behind interest in the development of sampling approaches that cause minimal perturbations to the system under study.

Chemical analysis of complex samples, such as live biological samples, requires state-of-the-art techniques for sampling and sample preparation, analyte separation, detection, and quantitation.[5] Current techniques that are applicable for *in vivo* analysis include microdialysis, arrays, sensors, microfluidics, nanomaterials, and solid-phase microextraction.[1]

An ideal *in vivo* sampling technique should be miniature and solvent-free and should offer integration of the sampling, sample preparation, and sample analysis steps.[9] The reliability and accuracy of *in vivo* research have been significantly improved with the development of highly specific and sensitive instruments, such as gas chromatography (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). The simplification of sample preparation and its integration with sampling and convenient introduction of extracted components to analytical instruments is a significant challenge for the contemporary analytical chemist.[10] To address this challenge, microextraction methods represent an important development in the field of analytical chemistry.[11] Microextraction is defined as nonexhaustive sample preparation where a very small volume of extraction phase (microliter range or smaller) relative to the sample volume is used. Although different types of microextraction techniques were reported in the literature much earlier,[12,13] the field gained in significance with the invention of solid-phase microextraction (SPME) in 1990.[14]

**Received:** July 1, 2010
**Published:** January 27, 2011

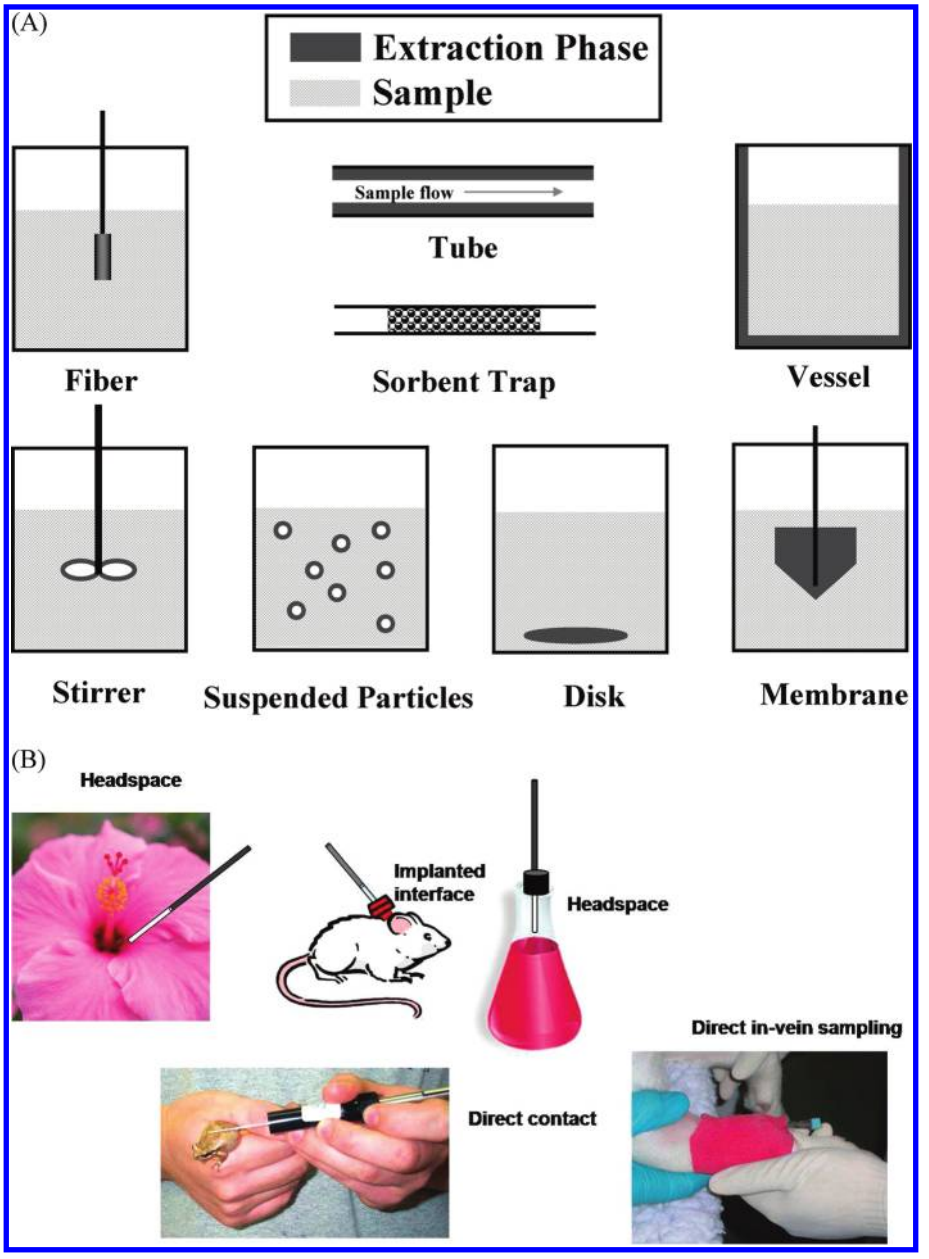


**Figure 1.** (A) Various configurations of solid-phase microextraction. (B) Example illustrations of various modes of sampling using *in vivo* SPME.

SPME is a solvent-free sample preparation technique and combines sampling, analyte isolation, and enrichment into one step. In this approach, microquantities of the solid sorbent or liquid polymer in appropriate format are exposed to the sample. Quantification is based on the amount of analyte extracted at appropriate conditions. It should be noted that solid-phase microextraction was originally named after the first experiment using a SPME device that 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 phase, even though it is recognized that the extraction phase is not always technically a solid. The geometry of the SPME system is optimized to facilitate speed, convenience of use, and sensitivity.[15] Figure 1A illustrates several implementations of SPME that have been considered to date. They include mainly open bed extraction concepts such as coated fibers, vessels, stirrers, and membranes, which are ideally suited for *in vivo* sampling, as well as in-tube approaches (coating inside or packed with sorbent), which are more suitable for *in vitro* research.

SPME was considered one of the great ideas in Analytical Chemistry during the decade from 1989 to 1999,[16] along with electrospray, capillary electrophoresis (CE), matrix-assisted laser desorption/ionization (MALDI), DNA analysis by CE, and micrototal analysis system ($\mu$TAS or lab on a chip). Since its conception, SPME has been widely applied to the sampling and analysis of environmental,[17−23] food,[24−30] aromatic,[31−33] metallic,[34−36] forensic,[37−42] biological,[43−46] and pharmaceutical samples.[47−49] To date, thousands of SPME papers have been published, and the number of the papers increases every year. In 2009, almost 1000 papers related to SPME have been published, and the number of citations has increased to more than 18 000 per year, as shown in Figure 2, indicating that the technique has matured and is capable of addressing a multitude of analytical problems.


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

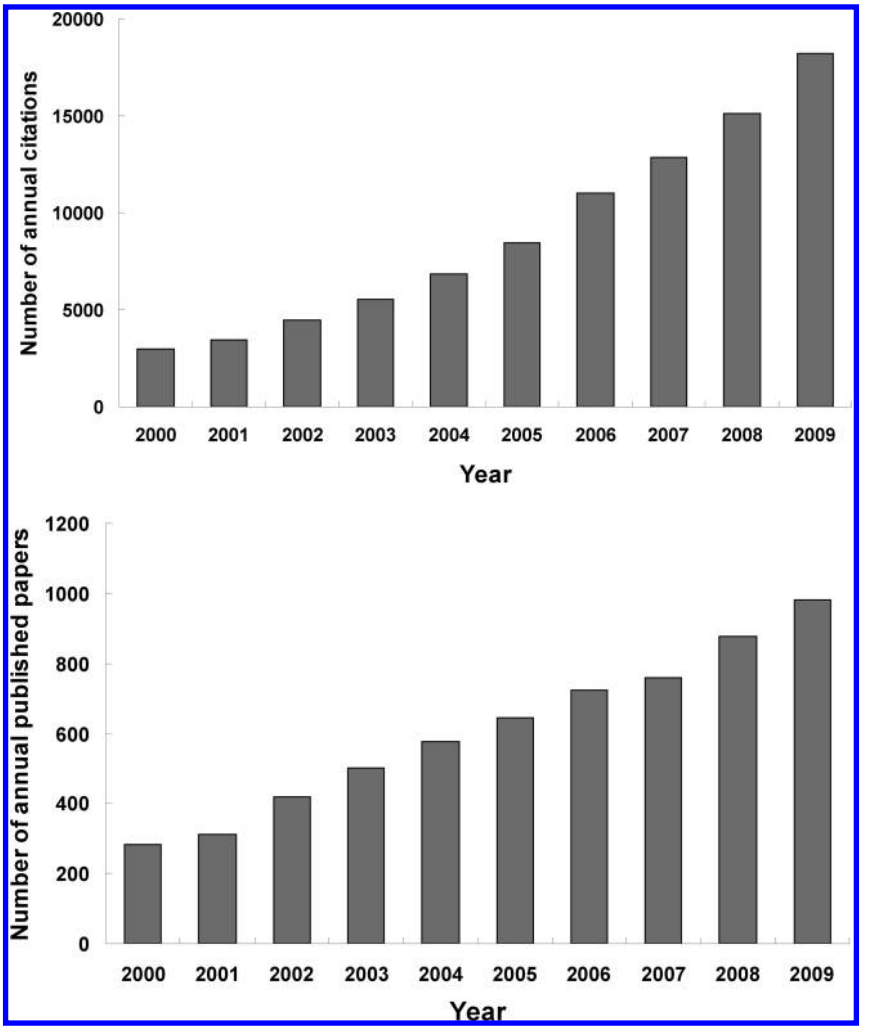


**Figure 2.** Summary of the published SPME papers and the citations during the past decade (data from ISI Web of Knowledge).

Unlike traditional sample preparation methods, such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE), the objective of SPME is never the exhaustive extraction of all the analyte from the sample, but rather convenience and speed. The nonexhaustive microextraction techniques, such as SPME, possess unique advantages, because typically only a small portion of the target analyte is removed from the sample matrix. This feature allows for the monitoring of chemical changes, partitioning equilibria, and speciation in the investigated system since sampling causes minimal perturbation to the system.[50,51] Therefore, the use of SPME results in better characterization and more accurate information about the investigated system or process compared with exhaustive techniques. SPME also provides signal magnitudes that are proportional to the free concentration of target analyte, thus defining the fraction of the analyte that is bioavailable. This unique feature of microextraction methods allows for the measurement of binding constants in complex matrices, providing additional information about the investigated system.[47,51,52]

*In vivo* analysis is a special application area where SPME is gaining ground because of its unique format and convenient device design. In its most common configurations, a syringe-like (for headspace sampling) or a needle-like device (for direct sampling) is exposed directly to the living system under study. After a short sampling time, the device is directly introduced to GC for thermal desorption or desorbed using solvents for injection into LC-MS. Example *in vivo* SPME implementations are shown in Figure 1B. In state-of-the-art methods, this simple workflow can now be as rapid as several seconds or minutes due to great improvements in the analytical sensitivity of the instrumentation used for detection over the past decade.

This review aims to describe the fundamentals of *in vivo* SPME from both theoretical and experimental viewpoints. The advances of SPME techniques in the past decade for *in vivo* analysis of compounds produced by microorganisms, insects, plants, animals, and humans will be presented and discussed. These studies clearly show the potential of *in vivo* SPME as a new tool in life science. In fact, some of the presented applications could not be carried out using any other sampling and sample preparation methods, because they would cause severe damage to the living system or would demand its sacrifice.[5,6] Finally, the opportunities and challenges associated with the use of SPME for *in vivo* research will be highlighted.

## 2. FUNDAMENTALS OF *IN VIVO* SPME

SPME can be performed using three basic extraction modes: direct extraction, headspace extraction, and membrane-protected

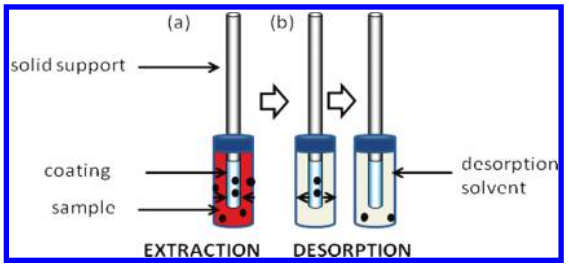


**Figure 3.** Schematic of SPME procedure for nonvolatile or low-volatility compounds using direct extraction mode: (a) fiber is exposed directly to the sample solution, and analyte of interest is extracted into the coating; (b) fiber is now exposed to desorption solvent, and the analyte is desorbed from the coating into the solvent solution. Small arrows indicate the direction of mass transfer.

extraction. Among these modes, direct extraction and headspace extraction are most frequently used for *in vivo* sampling.

In the direct extraction mode, the coated fiber is inserted into the sample and the analytes are transported directly from the sample matrix to the extraction phase. For sampling nonvolatile or low volatility compounds, such as sampling of pharmaceuticals in animal tissue or blood, this mode is required. The analytes are then desorbed from the coating using solvent (Figure 3) and injected directly into the LC-MS/MS instrument.

In headspace sampling, the fiber is inserted into the headspace above the sample matrix. Only relatively volatile analytes are effectively extracted using this mode making it suitable for breath sampling and sampling of volatile emissions from plants, animals, microorganisms, or insects.

SPME eliminates or minimizes the use of organic solvents, integrates sampling and sample preparation, and therefore substantially reduces the total time and cost of analysis. On the other hand, microextraction methods require careful calibration and optimization. The development of robust quantitative analytical methods based on SPME requires more time, but when the procedures are optimized, they are more convenient and cost-effective compared with conventional methods.[53] An understanding of SPME theory provides insight and direction when developing methods and identifies parameters for rigorous control and optimization. The effective use of SPME theory minimizes the number of experiments that need to be performed and facilitates appropriate choice of calibration procedures. Fundamental understanding of SPME principles has advanced in parallel with the development of new technologies. For proper optimization of *in vivo* sampling with SPME techniques, it is important to understand the distribution and mass transfer processes of the extraction, while the type of application dictates the selection of the most appropriate extraction phase and calibration method in order to address a given analytical problem. The following section will briefly discuss each of these parameters and how they relate to *in vivo* SPME method development.

### 2.1. Thermodynamics−Distribution

The most widely used technique of sampling with SPME consists of exposing a small amount of extraction phase (coating), immobilized on a fiber or wire, to the sample for a well-defined period of time. In a second step, the fiber is removed from the sample, and the analyte is desorbed and analyzed. Typically, the microextraction process is considered complete when the analyte concentrations in the sample matrix and the coating reach equilibrium. The amount of analyte extracted at

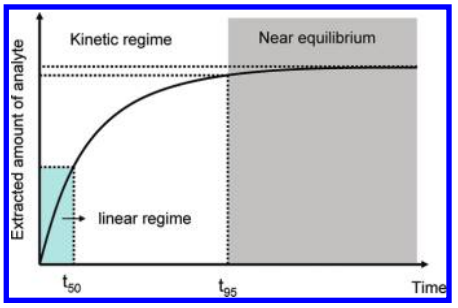


**Figure 4.** Typical extraction time profile of SPME. Reprinted with permission from ref 55. Copyright 2007 Elsevier.

equilibrium ($n_e$) can be described by eq 1 according to the law of mass conservation and thermodynamics of partition equilibrium, if only two phases, the sample matrix and the coating, are considered:

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

where $C_0$ is the initial concentration of the analyte in the sample, $V_s$ and $V_f$ are the volume of the sample and the coating, respectively, and $K_{fs}$ is the distribution coefficient of the analyte between the fiber coating and sample matrix. Equation 1 indicates that the amount of analyte extracted onto the coating ($n_e$) is linearly proportional to the analyte concentration in the sample ($C_0$), which is the analytical basis for quantitative analysis using SPME.

When the sample volume is very large, that is, $V_s \gg K_{fs} V_f$, eq 1 can be simplified to

$$n_e = K_{fs} V_f C_0 \quad (2)$$

which points to the usefulness of the SPME techniques when the volume of the sample is unknown and is the basis of the use of SPME for *in vivo* sampling. In practice, this means that there is no need to collect a defined sample prior to analysis, because the fiber can be exposed directly to the sample matrix, such as flowing blood or exhaled air, while the amount of extracted analyte will correspond directly to its concentration in the matrix without depending on the sample volume.

Fundamentally, the distribution coefficient ($K_{fs}$) describes the distribution of analyte between the sample matrix and the extraction phase. When a liquid coating is used as the extraction phase, the distribution constant, $K_{fs}$, can be described by

$$K_{fs} = a_f / a_s \approx C_f / C_s \quad (3)$$

where $a_f$ and $a_s$ are the activities of analyte in the extraction phase (fiber coating) and the sample matrix and can be approximated by the appropriate concentrations. $K_{fs}$ defines the equilibrium conditions, and more importantly, it determines the ultimate enrichment factors achievable by using an absorption extraction medium.

For a solid extraction phase, adsorption equilibrium can be explained by

$$K^s_{fs} = S_f / C_s \quad (4)$$

where $S_f$ is surface concentration of adsorbed analyte on the solid extraction phase. The relationship is similar to eq 3, except for the replacement of the extraction phase concentration with the surface concentration. The $S_f$ term in the numerator indicates that the sorbent surface area available for the adsorption must


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

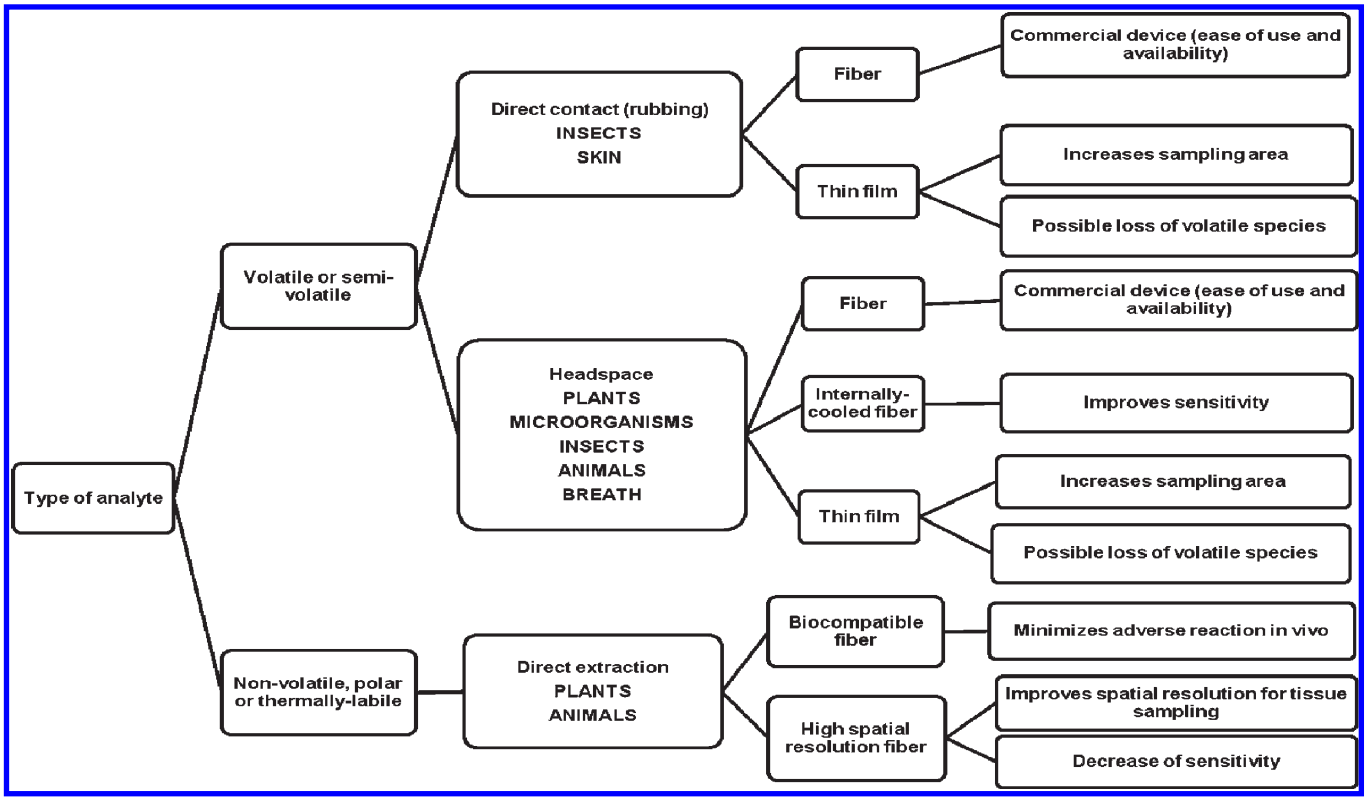


**Figure 5.** Summary of *in vivo* SPME sampling modes and device selection.

also be considered. This will complicate calibration under equilibrium conditions, because of the displacement effects and the nonlinear adsorption isotherm.

The extraction phase/sample-matrix distribution constants depend on a variety of conditions including temperature, pressure, and exact matrix composition. Temperature effects must be considered when temperature variations occur while sampling outdoors or when heating is used to increase extraction rate, stop metabolic activity, or enhance the release of analytes. If both sample and fiber temperature change from $T_0$ to $T$, the distribution constant changes according to the following equation:

$$K_{fs} = K_0 \exp\left[-\frac{\Delta H}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)\right] \quad (5)$$

where $K_0$ is the distribution constant at temperature $T_0$, $\Delta H$ is the molar change in enthalpy of the analyte when it moves from sample to fiber coating, and $R$ is the gas constant.[54] When the $K_{fs}$ value is greater than 1, the analyte has a lower chemical potential energy in the fiber coating than in the sample at the beginning of extraction, so the analyte partitioning into the fiber coating is an exothermic process, which means $\Delta H$ is greater than 0 and therefore raising the temperature will decrease the $K_{fs}$ (i.e., lower the ultimate enrichment factor), according to eq 5.

## 2.2. Kinetics−Mass Transfer

The kinetics of the extraction process determines the speed of extraction. Kinetic theory identifies extraction rate "bottlenecks" of SPME and therefore indicates strategies to increase speed of extraction. The extraction process of SPME generally follows the profile shown in Figure 4.[55] The graph shows that immediately after the contact of the fiber with the sample, there is a rapid increase in the mass absorbed by the fiber. The rate of increase then slows and eventually reaches equilibrium. If the sampling time is less than $t_{95}$, the extraction is a kinetic process, and there is almost a linear mass uptake when the sampling time is less than $t_{50}$. Since the time required to reach equilibrium is infinitely long, in practice, the equilibrium time is assumed to be achieved when 95% of the equilibrium amount of an analyte is extracted from the sample.

To understand the kinetics of SPME process, Prandtl boundary layer model can be used for simplification of corresponding equations. In this model, a thin boundary layer of unstirred fluid exists around the fiber. Fluid movement gradually increases as the distance from the fiber surface increases until the fluid movement corresponds to the bulk flow in the sample. The thickness of the boundary layer is determined by the viscosity of the fluid and the agitation conditions. When the extraction rate is determined by the diffusion in the boundary layer, equilibration time can be estimated from the equation below:

$$t_e \approx t_{95} = 3\frac{\delta K_{fs}(b-a)}{D_s} \quad (6)$$

where $\delta$ is the thickness of the boundary layer surrounding the fiber coating, $(b-a)$ is the thickness of the fiber coating, and $D_s$ is the diffusion coefficient of the analyte in the sample matrix. According to eq 6, equilibration time is proportional to the coating and boundary layer thickness. The sensitivity of the technique can be improved by increasing the coating thickness. However, a significant increase in the extraction time will occur. Decreasing the boundary layer thickness will accelerate the extraction process and result in shorter equilibrium time. Equation 6 also indicates that an analyte with a high $K_{fs}$ value will have a long equilibrium time.

The use of the headspace above the sample accelerates the extraction of analytes characterized by high Henry's law

constants. In the headspace mode, the analytes need to be transported through the barrier of air before they can reach the coating. Of course, headspace sampling mode has an advantage that the fiber is not directly in contact with the sample matrix, so the fiber coating is protected from damage by high molecular mass and other nonvolatile interferences present in the sample matrix, such as humic materials or proteins. For headspace sampling, the overall mass transfer to the fiber is typically limited by mass transfer rates from the sample to the headspace. Therefore, volatile analytes are extracted faster than semivolatiles, since they are at a higher concentration in the headspace, which contributes to faster mass transport rates through the headspace. Temperature has a significant effect on the kinetics of the process by determining the vapor pressure of analytes. In fact, the equilibration times for volatiles are shorter in the headspace SPME mode than for direct extraction under similar agitation conditions. This outcome is the result of two factors: a substantial portion of the analyte is in the headspace prior to extraction, and diffusion coefficients in the gaseous phase are typically 4−5 orders of magnitude larger than in liquid media.

The absorption kinetics of analyte from the sample matrix into a SPME liquid coating can be described by[56,57]

$$n = [1 - \exp(-at)]n_e = [1 - \exp(-at)]\frac{K_{fs}V_fV_s}{K_{fs} + V_s}C_0 \quad (7)$$

where $n$ is the amount of the extracted analyte at time $t$, $n_e$ is the amount of analyte extracted at equilibrium, and $a$ is a rate constant that is dependent on the volumes of the extraction phase and sample, the mass transfer coefficients, the distribution coefficients, and the surface area of the extraction phase. When the sampling time is long enough for the extraction to reach equilibrium, eq 7 simplifies to eq 1, which proves that this dynamic model can be used during the entire process of SPME, including both kinetic and equilibrium regimes (Figure 4).

### 2.3. Design of SPME Devices

A better understanding of SPME theory allows more rational design of SPME devices, which can offer improved extraction efficiency or convenience of use. Main types of SPME devices useful for *in vivo* analysis, including fiber SPME and thin-film microextraction, are presented below. Figure 5 shows a flowchart to facilitate the selection of the most appropriate device depending on the application of interest.

**2.3.1. Fiber SPME.** The most widely used SPME technique is fiber SPME. Figure 6A illustrates the commercial SPME device by Supelco. The assembly contains a piercing needle and an inner needle or tubing that has a piece of coated fiber attached to it. The key to the fiber is the sealing septum that seals the outer needle to keep it from leaking when inserted into a pressurized GC injection port. The length of the coated fiber is usually 1 cm, although 2-cm lengths are also available for selected coatings.

The initial SPME device design utilized fiber cores made of fused silica or quartz. However, because of the fragility of such devices, metal or other alloy wires were introduced in order to improve durability and thermal stability.[58,59] From an *in vivo* sampling perspective, these commercial SPME fibers, housed in syringe-like devices, are the most suitable for headspace sampling of volatile and semivolatile compounds, followed by direct introduction into GC instrument for thermal desorption and analysis.

At elevated temperature, native analytes can effectively dissociate from the matrix and move into the headspace for rapid extraction by the fiber coating. However, the coating/sample distribution coefficient also decreases with an increase in temperature, resulting in a decrease in the amount of analytes extracted at the equilibrium. To simultaneously increase both the sensitivity and extraction speed of SPME, an internally cooled fiber approach was developed.[60] In this device, a fused silica tube is sealed and coated at one end. Liquid carbon dioxide is delivered via the inner capillary to the coated end of the outer capillary, resulting in a coating temperature lower than that of the sample. Further modifications of this device included miniaturization and automation.[61] More recently, a new cold fiber SPME device was designed and constructed based on thermoelectric cooling.[62] A three-stage thermoelectric cooler (TEC) was used for cooling a copper rod coated with a polydimethylsiloxane (PDMS) hollow fiber, which served as the SPME fiber. A heat sink and a fan were used to dissipate the generated heat at the hot side of the TEC, as shown in Figure 6B. The new device was shown to be relatively rapid, precise, and sensitive for headspace sampling of off-flavor compounds. The extraction recoveries obtained with this device were higher than those obtained with commercially available fiber. The main advantage of the new cold fiber SPME device compared with the previously designed cooling system is that the cooling source and the SPME device are integrated into a single device, which can be operated from a low-voltage power supply (i.e., a car battery) and can be used for on-site field sampling of volatile compounds from living plants.

The current commercial SPME fibers have some limitations for *in vivo* sampling using direct extraction mode where the device is exposed directly to the complex biological matrix. First, the application of SPME to sample directly inside animals or plants requires greater robustness in both the coating and the supporting fiber core. Furthermore, fouling of the fiber coating surface with large biomolecules such as proteins is undesirable because it both impacts analyte uptake and may cause adverse reactions such as clotting *in vivo*. In addition, the commercial fiber assemblies are costly for *in vivo* applications, because multiple fibers may be required (for example, in a multianimal pharmacokinetic study) or the reusability of fibers may be limited. To address the above requirements, new and simplified SPME fibers were developed for *in vivo* studies. These fibers typically use metal or metal alloy wires as the supporting core, and coatings are made of biocompatible polymers including single polymer[63−67] or polymers impregnated with sorbent particles in order to increase extraction efficiency.[46,64,68,69] For blood sampling, these fibers can be inserted within a commercial hypodermic needle,[70] and this design was adopted by Supelco for the commercial device as shown in Figure 7A.[69] The main advantage of such hypodermic-needle-based devices is that coating is protected within the needle when not in use and that the needle is useful to pierce skin or septum of the sampling interface. For blood sampling, the length of the coating is typically 10−15 mm (Figure 7B), and this is compatible with both direct insertion in the blood vessel through a catheter[63,68] and rodent interface sampling methodologies.[70,71] However, for *in vivo* sampling of tissues (for example, muscle or brain), further reduction of dimensions is desirable in order to improve spatial resolution of sampling. For this purpose, small sections (1−2 mm) of PDMS hollow fiber membrane tubing can be fixed on the wire with customized spacing between the two coatings,[46] as shown in Figure 7C. The device shown in Figure 7C is a space-resolved SPME fiber, which is designed to facilitate rapid *in situ*

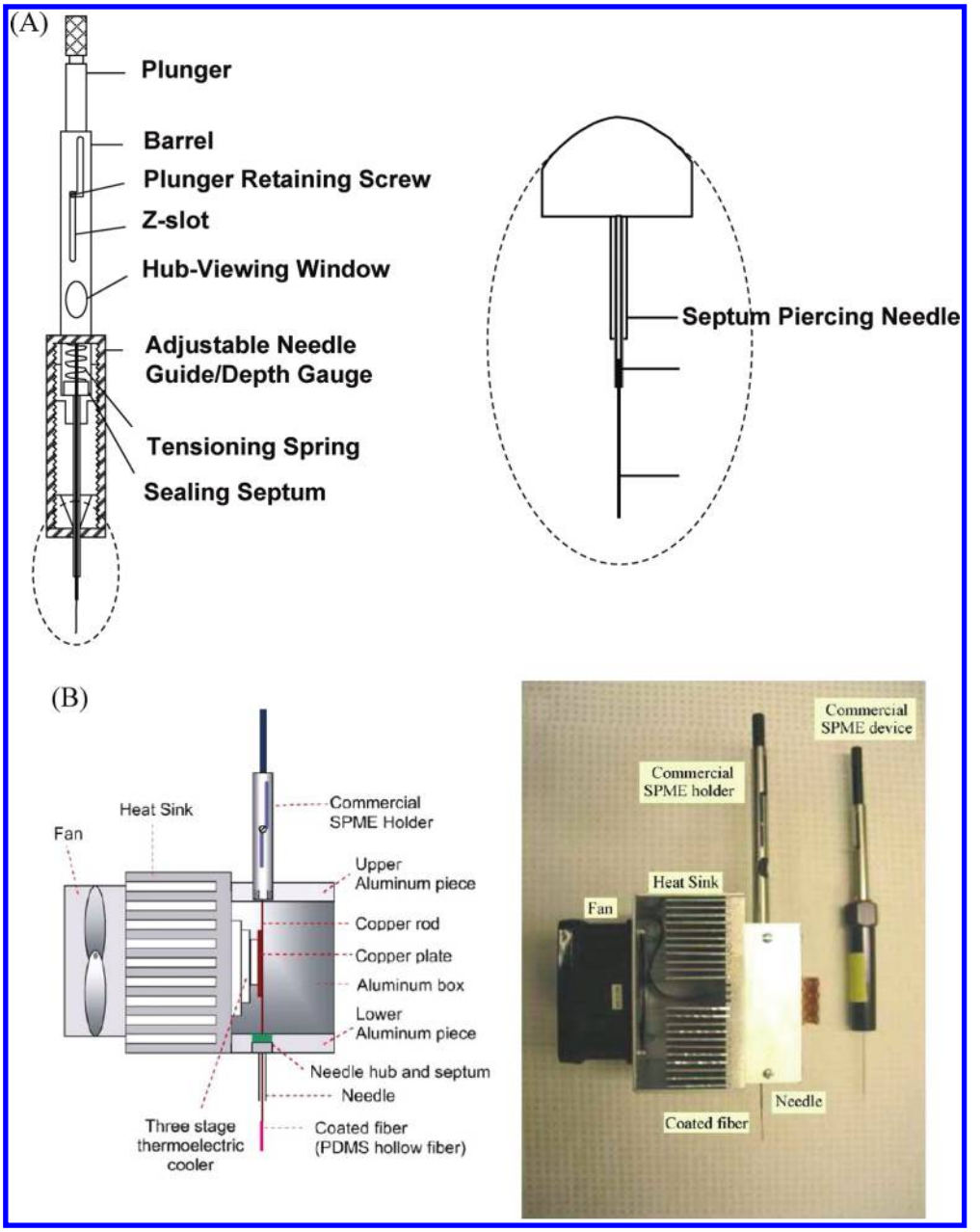


**Figure 6.** (A) Commercial SPME device and (B) schematics and picture of the cold fiber SPME device based on TEC. Reprinted with permission from ref 62. Copyright 2009 Elsevier.

analyte monitoring within heterogeneous samples such as tissue. The segmented design of the fibers and stepwise desorption procedure offers high spatial resolution and increases capability for high-throughput parallel *in vivo*/*in vitro* sampling with a single probe because it permits simultaneous sampling of adjacent tissues. The simplified SPME fibers and fiber assembly significantly lower the cost of analysis and ease of use for most *in vivo* applications. In the future, improved commercial availability of a range of devices with appropriate properties, coatings, and dimensions is crucial to facilitate the implementation of SPME technology in laboratories lacking in-house capability to produce their own devices.

Another fiber SPME configuration uses the silanized tip of an optical fiber for extraction of target analytes from the sample. The treated optical fiber was coupled to matrix laser desorption/ionization (MALDI) for the detection of large biomolecules.[72,73] The fiber served as the sample extraction surface, the support of the sample plus matrix, and the optical pipe to transfer laser energy from the laser to the sample. Both an ion mobility spectrometer and a quadrupole time-of-flight (Q-TOF) mass spectrometer were used for the detection of the SPME-MALDI signal. The combination of SPME-MALDI with a Q-TOF system offers simple sample handling paired with the specificity and sensitivity of high-performance mass spectrometry. Even more importantly, it extends the usefulness of SPME method to polar high molecular weight biopolymers. The application of this technique holds promise, especially in biochemical analysis, pharmaceutical research, clinical diagnostics, and screening, but as of yet has not been explored for *in vivo* sampling purposes.

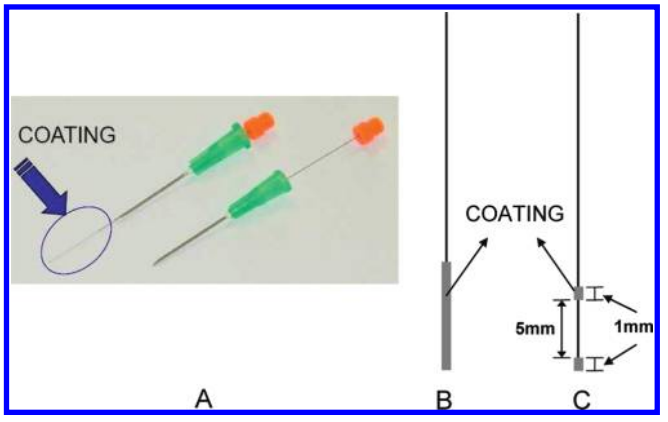


**Figure 7.** Commercial prototype SPME fiber assembly based on hypodermic needle for *in vivo* applications (A) and schematic of conventional SPME fiber with typical coating length of 10−15 mm (B) and high-spatial-resolution fiber with discontinuous coating (C).

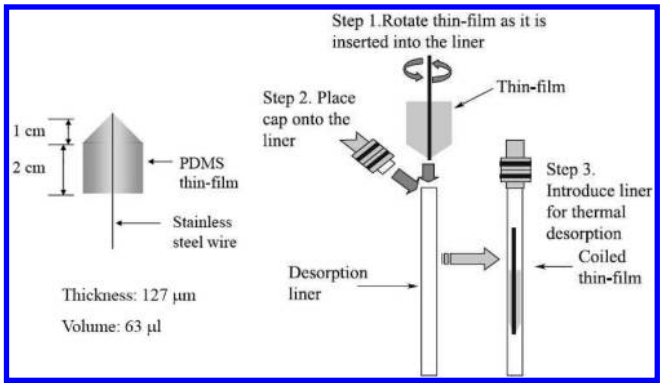


**Figure 8.** Thin-film microextraction and its introduction into a GC injector.

**2.3.2. Thin-Film Microextraction.** Although fiber SPME techniques are widely used, in some cases better sampling rates and sensitivities are required. According to eq 1, the amount of analyte extracted by SPME is proportional to the volume of the extraction phase. The extraction rate after exposure of the SPME device to the sample is proportional to the contact surface area between the extraction phase and the sample. An increase in the volume of the extraction phase can be achieved by increasing the thickness of the extraction phase, as is accomplished in stir-bar sorptive extraction (SBSE).[74,75] However, this configuration is characterized by longer equilibrium time, which obviously leads to lower sample throughput.[76] In order to increase the mass uptake rates and therefore sensitivities, large surface area sorbent geometries can be used. For example, the PDMS extraction phase can be a thin film (Figure 8). In this case, a high surface area to volume ratio is obtained, resulting in very high accumulation rates and much better sensitivities.[76−80] To facilitate convenient introduction to the analytical instrument, the membrane can be attached to the holding rod, and after extraction, the membrane can be rolled around the rod and introduced to the injection system for the desorption automatically (Figure 8).[76,81] The thin-film microextraction approach has been successfully used for the study of biogenic volatile organic compounds (VOCs) from human skin.[82] It is also a potential tool for the analysis of VOCs in human breath, due to the high sampling rate and sensitivity.

## 2.4. Extraction Phases

The performance of SPME is critically dependent on the properties of the extraction phase, which determine the selectivity and the reliability of the method. These properties include both bulk physicochemical properties (e.g., polarity) and physical properties (e.g., thermal stability and chemical inertness).

The development of selective extraction materials for SPME often parallels that of the corresponding selective chemical sensors.[83] Similar manufacturing approaches and structures similar to those sensor surfaces have been implemented as extraction phases. For example, specific phases such as molecularly imprinted polymers (MIP)[84−90] and immobilized antibodies[91−94] have recently been developed for SPME. Demands on the specificity of extraction phases for SPME are typically less stringent than those for sensor surfaces, because a powerful separation and quantification technique (e.g., GC-MS or LC-MS) is typically used after extraction, facilitating accurate identification of the analyte. More demand is, however, placed on the thermal stability and chemical inertness of the extraction phase, because the extraction materials are frequently exposed to high temperatures and different solvents during extraction, desorption, or introduction to the analytical separation instruments.

It is important to know that there is a substantial difference between the performance of liquid and solid extraction phases.[10] With liquid coatings, the analytes partition into the extraction phase, in which the molecules are solvated by the coating molecules. With solid coating, sorption occurs only on the porous surface of the coating. Competitive displacement might occur during extraction with porous coating after long extraction time. Compounds with poor affinity toward the extraction phase are frequently displaced by analytes with good affinity toward the extraction phase or those compounds present in the sample at high concentrations. The substantial difference between the performance of liquid and solid extraction phases is indicated by the smaller linear range of porous coating, compared with liquid coating.

**2.4.1. Preparation Methods.** Current commercially available extraction phases for SPME fibers are limited and restrict the wide application of SPME.[95] To address this limitation, more specialized materials and coating methods have been developed for SPME coatings. There are several methods of depositing coatings onto fibers,[10] including direct use of hollow fiber membrane/adhesive tape, dipping, electrodeposition, adhesion of coatings to solid support using chemical adhesive, sol-gel, etc.

The simplest way to prepare a coating is to use a piece of hollow fiber membrane (small i.d. tubing, commercially available),[65−67,96] made from the desired extraction material, as mentioned in section 2.3.1. Preparation consists of swelling the membrane by means of an appropriate volatile solvent, placing the enlarged membrane onto the tip of the metal wire, and evaporating the solvent. Membrane thickness determines the thickness of the coating. Therefore, the volume of the coating can be large, reaching up to 3 $\mu$L for a 300 $\mu$m thick PDMS hollow fiber membrane. A porous hollow-fiber membrane can also be used for adsorption of target analytes, or its pores can be filled with organic solvent to allow for solvent microextraction.[97] More recently, carbon tape (commercially available, traditionally used to immobilize samples prior to microscopy) was found to have good extraction properties for some nonvolatile analytes amenable to LC.[98] This type of coating is adhesive, so it is easily immobilized on a piece of stainless steel wire of desired diameter.

Dipping can also be used as a simple fiber coating preparation method. The dipping technique typically consists of placing a

fiber or metal wire in a concentrated organic solvent solution of the material to be deposited for a short time. After removal of the fiber from the solution, the solvent is evaporated by drying and the deposited material can be cross-linked.[99]

An extension of the dipping method is electrodeposition, which can be used to deposit thin coatings on the surface of metallic rods.[100−102] Typically, the device for electrodeposition is a three-electrode system. The metal wire is used as the working electrode to deposit the coating material.[103,104]

The use of a strong adhesive to immobilize sorbent particles was originally proposed in 1997 to prepare SPME fibers suitable for GC use.[105,106] The main idea in this approach is to immobilize a thin layer of sorbent particles (for example, porous coated silica particles) on a metal wire using an appropriate adhesive. Depending on the choice of adhesive, coatings suitable for LC can also be prepared.[49,107] The main advantages in this approach are (i) flexibility in the choice of glue, (ii) wide availability of different commercial sorbents so that the coating can be tailor-made for particular application, and (iii) low cost. For GC applications, the adhesive used should have good thermal stability, while for LC applications good chemical stability is crucial so that the desorption step does not result in loss of coating. Depending on the diameter of metal support used as well as proper optimization of coating procedure, a coating with excellent interfiber reproducibility (<15% RSD) can be made using this approach.[49]

Another method of preparation of SPME fibers is sol-gel chemistry.[59,108−115] The main advantages of this type of coating is low cost and strong adhesion of the coating to the substrate, which can translate into improved thermal and chemical stability. For example, sol-gel PDMS coating was able to withstand GC injector temperatures of 320 °C in contrast to commercial PDMS coating where bleeding may be observed for injector temperatures above 200 °C.[116] In addition, sol-gel procedures can be used to prepare very thin coatings (as thin as 1 $\mu$m, but typically around 5−10 $\mu$m), which improves the extraction kinetics and results in shorter extraction times. The main steps of the sol-gel coating procedure typically include (i) pretreatment of the substrate surface, (ii) preparation of the sol-gel solution, (iii) coating of the substrate with sol-gel solution using dipping method, and (iv) conditioning of the coating.[59,117] The most commonly employed sol-gel precursors are methyltrimethoxysilane and tetraethoxysilane, while the extractive properties of the coating can be adjusted by incorporation of various modifiers such as crown ether,[118] PDMS,[116] PEG,[119] etc. For example, to prepare sol−gel coating suitable for LC use, Gbatu et al. incorporated *n*-octyltriethoxysilane in order to increase hydrophobicity of the coating.[117]

**2.4.2. Extraction Phases for Headspace or Gaseous *in Vivo* Sampling.** For headspace or gaseous *in vivo* sampling, any extraction phase is suitable as long as it is robust and innocuous. This includes both commercial fibers and the custom-made fibers based on the preparation methods mentioned above.

Several coatings are commercially available for SPME analysis, including polydimethylsiloxane (PDMS), polyacrylate (PA), divinylbenzene (DVB), Carboxen (CAR), and poly(ethylene glycol) (PEG), and fibers are available in different thicknesses with single coatings, mixtures, or copolymers.[10] These fibers are suitable for application of SPME to the analysis of polar and nonpolar organic compounds amenable to GC analysis. Solvents and liquid polymeric phases, for example, PDMS, are very popular because they have wide linear dynamic ranges associated with linear absorption isotherms. They also facilitate "gentle" sample preparation, because chemisorption and catalytic properties, frequently associated with solid surfaces, are absent. No loss or modification of the analyte occurs during extraction or desorption. Despite these attractive properties of liquid extraction media, solid phases are frequently used because of their superior selectivity and sensitivity for some groups of compounds. For example, carbon-based sorbents are effective for extraction of volatile analytes[120−124] and nanostructured coatings showed higher extraction rate and shorter desorption time than microstructured coatings.[125]

**2.4.3. Biocompatible Coating for Direct *in Vivo* Sampling.** For direct *in vivo* sampling, for example, monitoring of selected analytes in flowing blood or animal tissue, the extraction material must be biocompatible. The biocompatibility of an artificial device introduced into the body can be defined as the compatibility with the living tissue with which it is brought into contract.[126] Bioincompatibility leads to toxic reactions or immunological rejection.[10] According to one definition of biocompatibility, a material can be considered biocompatible if the sum of adverse humoral and cellular reactions occurring during exposure is lower than for a reference material.[126,127] Typical exposure time of the SPME device to the biological system is on the order of minutes or seconds, so stringent biocompatibility requirements such as needed for implantable devices are not required. From a SPME perspective, a biocompatible coating is coating that (i) does not cause toxic reactions to the system under study and (ii) does not permit adhesion of large biomolecules such as proteins to the surface of the coating. Furthermore, all the materials should be sterilizable, preferably by autoclaving, which is a widely available and accepted sterilization procedure.

Biocompatible materials for extraction that have been developed and used so far include PDMS,[128] polypyrrole (PPY),[63] poly(ethylene glycol) (PEG),[129−131] and restricted access materials (RAM).[132−134] Biocompatible membranes have been prepared from polyurethane,[135] chitosan,[136] cellulose,[137,138] and polyacrylonitrile (PAN).[139−141]

As biocompatible materials, PPY and its derivatives have been intensively used and studied in recent years, due to additional advantages: (i) they can be easily polymerized from organic or aqueous media at neutral pH by electrochemical or chemical methods, (ii) they are relatively stable in air and solution, (iii) pyrrole monomer and some of its derivatives are available commercially, and (iv) they are conducting polymers. PPY coatings have been used in SPME for *in vivo* pharmacokinetic studies[63,142] and the extraction of polar or even ionic analytes.[143,144] Since polypyrrole is a porous coating, it extracts analytes mainly by adsorption processes. Consequently, the linear range of the probe is low and depends on the concentration of other compounds. This problem is significant in complicated matrices such as whole blood or plasma where many endogenous compounds exist. Other disadvantages of polypyrrole coatings are the use of hazardous chemicals[68] and the relatively poor interfiber reproducibility (∼30% RSD).[145]

To simplify the fiber preparation procedure and eliminate the use of hazardous chemicals, a PEG/C18-bonded silica SPME fiber was prepared for *in vivo* analysis.[68] C18-bonded silica particles were immobilized on the metal fiber using PEG as glue. The loading capacity of the PEG/C18-bonded silica coating was significantly increased when compared with PEG-coated fibers, mainly because of the stronger extraction capability of C18. The fiber also showed enhanced sensitivity compared with PPY fiber;

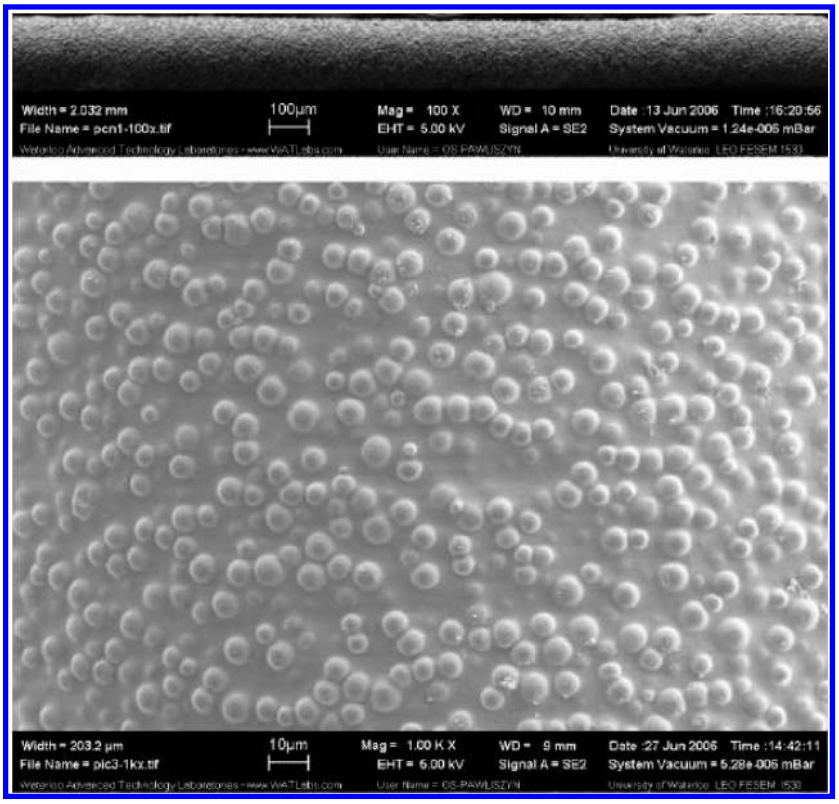


**Figure 9.** SEM images of PAN/C18-silica coating. Reprinted with permission from ref 64. Copyright 2007 American Chemical Society.

therefore it can be employed for monitoring drugs with higher affinity to proteins and lower circulating concentrations.[68]

New types of biocompatible fiber coatings, prepared by covering flexible stainless steel wires with a mixture of PAN and different extraction particles (C18-silica, RP-amide-silica, HS-F5-silica, 5 $\mu$m), were developed for direct extraction of drugs from biological fluids.[64] The particles are completely covered with PAN and are homogeneously distributed within the coating as shown in Figure 9. The coatings showed on average much higher extraction efficiency toward the test drugs: ~90 times more than PPY, ~50 times more than PDMS or RAM, and ~20 times more than commercially available CW/TPR.[64] These coatings can be sterilized with alcohol when maximum extraction capacity is needed.

A new SPME sorption system for the isolation and enrichment of polar organic analytes from aqueous samples was reported recently.[146] Unlike the frequently used cross-linking technique, in this system, polar sorbent (PEG) is separated from the sample by a hydrophobic membrane (PDMS, thickness 5 $\mu$m), which restricts the access of water. The method provides an opportunity for the use of new classes of polymers, which have been rejected from these applications due to their solubility in water, as well as some bioincompatible materials, through the isolation of the material from living tissues with biocompatible polymers.

Restricted access materials (RAM), such as alkyl-diol-silica (ADS) and ion exchange diol silica (XDS), constitute a class of promising biocompatible sample preparation materials. These materials consist of silica particles with a diameter of 5, 10, or 25 $\mu$m and with pores of about 3 nm in radius; the small pores yield a molecular mass cutoff of ~15 kDa that allows direct fractionation of a sample into the protein matrix and the analyte fraction. In addition to a defined pore size, one specific feature of diol silica particles is the topochemically bifunctional surface of the particles: the outer particle surface is modified with hydrophilic diol groups, whereas the inner pore surface is covered with hydrophobic alkyl chains or ion exchange groups. RAMs were successfully used as extraction phase in SPME for the determination of angiotensin-I in whole blood[133] and benzodiazepines in urine[134] but should also be suitable for *in vivo* sampling types of applications.

## 2.5. Calibration

SPME is a nonexhaustive extraction technique in which only a small portion of the target analyte is removed from the sample matrix. Therefore, an appropriate calibration method for SPME is required for quantitative analysis. The development of calibration methods facilitates the evolution of SPME sampling techniques and extends the applications of SPME. The existing calibration methods of SPME have been summarized and discussed in a review paper.[147] Among these, equilibrium extraction, external standard calibration, and kinetic calibration are the most suitable for *in vivo* SPME use and are briefly described below. Figure 10 provides an overview of calibration methods suitable for *in vivo* SPME and aids in the selection of the most appropriate approach depending on the application requirements.

The equilibrium extraction method is a widely used quantification method for SPME, especially for on-site and *in vivo* sampling. As shown in eq 2, in this special situation, the amount of extracted analyte is directly proportional to its concentration in the matrix, without depending on the sample volume. Therefore, the concentrations of target analytes can be easily determined from the amount of analytes detected on the fiber under extraction equilibrium by knowing the distribution coefficients of the analytes between the fiber coating and the sample matrix. Several methods have been developed to measure the distribution

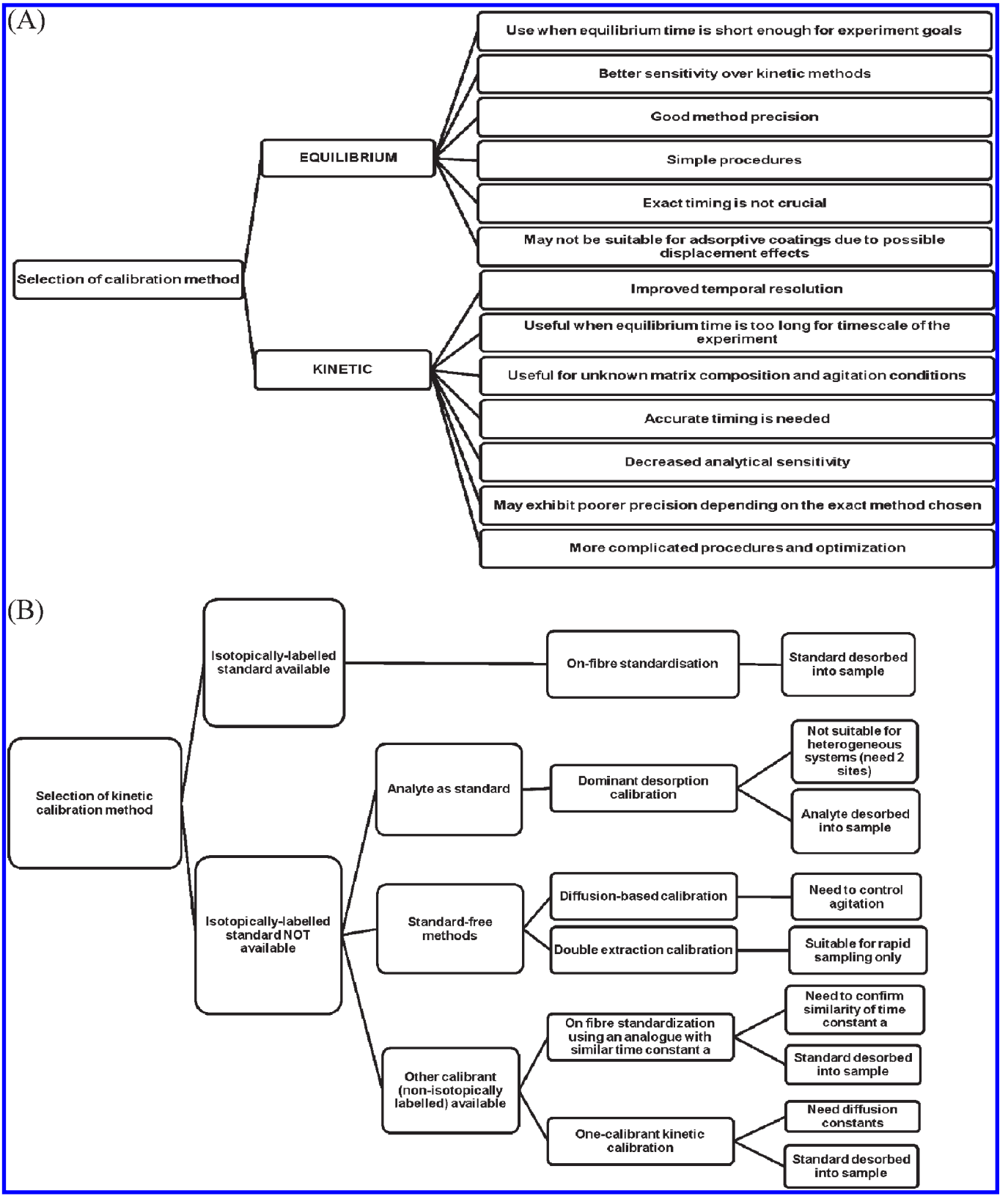


**Figure 10.** Flowchart for the selection of the most appropriate calibration method for SPME: (A) selection of equilibrium versus kinetic calibration method; (B) summary and selection of the most appropriate kinetic calibration method.

coefficients of different compounds between fiber coating[52,148−151] and the sample matrix experimentally. Extensive literature data is also available.[152−166] In addition to direct partition measurements, distribution constants can also be estimated from physicochemical data and chromatographic parameters. For example, distribution constants between a fiber coating and a gaseous matrix


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

(e.g., air) can be estimated with retention indexes from a linear temperature-programmed capillary GC[167,168] on a column with stationary phase identical to the fiber coating material. For headspace or gaseous *in vivo* sampling, the extraction can be performed in static or dynamic mode. The movement of sample matrix or sampler can decrease the thickness of the boundary layer surrounding the fiber coating and therefore significantly shortening the time required to reach equilibrium.[78,169−171]

SPME external standard calibration involves the preparation of several standard solutions in the sample matrix to obtain the relationship between the peak responses and the known standard concentrations. The samples are subsequently analyzed using the same extraction conditions. Then, the concentrations of the target analyte in the samples can be calculated using the equation of the calibration curve. When this method is used for on-site sampling, the calibration is normally performed in the laboratory with standard gas mixture.[172−176] To shorten long equilibrium extraction time or address the displacement effects of adsorptive coatings, extraction can be interrupted before equilibrium. Even though the extraction equilibrium is not reached, there is still a linear relationship between the amount of analyte extracted onto the fiber and the concentration of analyte in the sample matrix, if the convection conditions, the extraction time, and the temperature remain constant.[10] Because the convection conditions are difficult to keep the same for both on-site and laboratory, equilibrium extraction is preferable. Therefore, from an *in vivo* sampling perspective, the external calibration method is most suitable for on-site sampling of gaseous samples, because the equilibrium time for gaseous sampling is short.

In some situations, it may not be feasible to use extraction times sufficient to establish equilibrium. For such cases, recently developed kinetic calibration methods are useful. In 1997, Ai proposed a theoretical model based on a diffusion-controlled mass transfer process to describe the entire kinetic process of SPME as described in eq 7.[56,57] On the basis of this model, Chen et al. demonstrated the symmetry of absorption and desorption in the SPME liquid coating fiber, and a new concept, standard in the extraction phase or kinetic in-fiber standardization technique, was proposed.[177−180] The method uses the desorption of the standards, which are preloaded in the extraction phase, to calibrate the extraction of the analytes. The concentration of the analyte in the sample matrix can be calculated with[180]

$$C_s = \frac{n}{K_{fs} V_f (1 - Q/q_0)} \quad (8)$$

where $n$ is the amount of the extracted analyte after sampling time $t$, $q_0$ is amount of the preloaded standard, $Q$ is the standard remaining in the SPME fiber coating after sampling time $t$, $V_f$ is the volume of the fiber coating, and $K_{fs}$ is the distribution coefficient of the analyte between the fiber coating and the sample matrix.

The concept of standard in the extraction phase is especially important for the calibration of on-site, *in situ*, or *in vivo* analysis, since it is difficult to measure or control the agitation conditions in these cases and direct spiking of standards into the matrix is not possible. This technique has been successfully used for liquid-phase microextraction (LPME)[179,180] and fast *in vivo* drug analysis by SPME.[52,66,142,181,182]

However, the technique requires that the physicochemical properties of the standard should be similar to those of the analyte, which is normally achieved by using isotopically labeled standards. For multianalyte applications, the methodology required preloading of an isotopically labeled compound for each analyte, which limited the application of the technique. Furthermore, isotopically labeled analogues may not be readily available for all analytes of interest. To overcome this problem, a dominant desorption method was proposed.[65] In this method, the desorption of the target analytes in high concentrations serves as calibration procedure for the extraction of the analytes into the coating and completely eliminates the use of isotopically labeled standards. However, the dominant desorption method still requires preloading numbers of standards and a new problem is presented: the sampling of analytes and the desorption of standards need to be performed separately with two fibers. If the sampling and the desorption are performed simultaneously at very close positions, the desorbed analytes may pollute the sampling site and result in inaccurate report, because the concentrations of the analytes in the desorption fiber are very high.

Recently, a standard-free kinetic calibration method was proposed for rapid on-site sampling by SPME,[183] in which all extracted analytes can be calibrated with two samplings. This method eliminates the need to preload any standard to determine the desorption rate constant. However, this methodology requires that the conditions of two samplings should be kept constant, so it is only suitable for rapid sampling. The method has been successfully used for *in vivo* pharmacokinetic study in rats.[70]

Lastly, a new method, termed one-calibrant kinetic calibration technique, which uses the desorption of a single standard to calibrate all extracted analytes, was proposed.[184] The one-calibrant technique eliminates the requirement of preloading multiple isotopically labeled compounds or high concentration standards and simplifies the standard loading and quantitation procedures, which is extremely important for future applications of the kinetic calibration technique. However, the technique requires the knowledge of the diffusion coefficients of the target analytes and the calibrant in the sample matrix. Therefore, it is feasible for air or water sampling, since molecular diffusion coefficients in air or water can be easily obtained in literature or calculated with empirical equations, but it is not practical for direct sampling of analytes in blood or animal tissues.

Another calibration method for *in vivo* SPME is quantitation using the predetermined sampling rates of the analytes. Within a linear model, it is assumed that the rate of mass transfer or sampling rate remains constant throughout the duration of sampling, and the relationship between the concentration of target analytes in the sample matrix ($C_s$) and the extracted amount of analytes at time $t$ ($n$) can be expressed with

$$C_s = \frac{n}{R_s t} \quad (9)$$

where $R_s$ is the sampling rate for the target analyte and $t$ is the sampling time. For rapid *in vivo* sampling in animal tissue, the short sampling time suggests that passive sampling will follow linear response patterns. The intersample matrix differences in semisolid tissues (such as fish muscle) are slight between individuals of the same species. Consequently, the sampling rate of the SPME fiber can be predetermined under laboratory conditions and directly used for the applications. For the analysis of contaminants in fish muscle, experimental results showed that the calculated analyte concentrations for wild fish quantitated with the sampling rates determined in separate laboratory fish are very close to those obtained by traditional liquid extraction method, demonstrating the efficacy of the quantitation method of sampling rate.[185] With this method, the use of SPME as a rapid

and nonlethal sampling technique is further simplified, because it is no longer necessary to determine $K$ values. This methodology also completely eliminates preloading any standards onto each fiber prior to sampling. Additionally, potential contamination of the biological system with dissociated deuterated analogue (or compound serving as a calibrant with similar physicochemical properties to the target analyte) from preloaded fibers is avoided, allowing tissues to be used for additional analyses. The same type of approach is applicable to blood sampling as long as blood flow rate can be carefully controlled during experiment. This was recently achieved by semiautomating blood sampling of rats using Culex unit.[186]

Kinetic calibration methods described above play an important role for thicker coatings and for applications requiring good temporal resolution. For example, for commercial Supelco prototypes with C18 or mixed-mode coatings of 45 $\mu$m thickness, the time required to reach equilibrium was typically $\leq$5 min for polar compounds and several hours or longer for more hydrophobic species.[71] Figure 11A illustrates compounds for which equilibrium was reached within 5 min (represented by squares) versus compounds requiring longer times to reach equilibrium (represented by triangles) in a typical global metabolite profiling method. These results show that for applications where polar compounds are of interest (amino acids, organic acids, etc.), equilibrium calibration is a feasible option. For more hydrophobic compounds such as hormones, lipids, and most drugs, the time required to reach equilibrium is too long with these types of probes, necessitating the use of kinetic calibration methods. Therefore, the knowledge of log $P$ values of analytes of interest (or retention behavior on reverse phase column such as illustrated in Figure 11A) provides good starting point for estimating what type of calibration method is the most appropriate for a given application. From a metabolomics viewpoint, where the majority of species in discovery studies are unknown *a priori*, the above finding has important consequences in terms of absolute quantitation. Namely, for polar compounds for which equilibrium was reached *in vivo*, absolute quantitation can be performed *a posteriori*, once the compound has been positively identified and an authentic standard is available thus permitting the determination of $K_{fs}$ value. For species where equilibrium is not reached within the sampling time of the experiment, the incorporation of one calibrant kinetic calibration within the study design can be useful for subsequent absolute quantitation but requires knowledge of diffusion coefficient of identified compounds.

A recent study compared above-described calibration methods in terms of accuracy, precision, and experimental ease-of-use using an artificial vein system in order to facilitate the selection of the most appropriate strategy for *in vivo* sampling.[187] The results of this study indicate that all methods provide good accuracy (93−119%). In terms of precision, diffusion-based methods performed better (9−15% RSD) than the dominant desorption calibration method (20−30% RSD) because of experimental simplicity since it uses only one fiber per sampling and one experimental determination per fiber. The performance of two calibration strategies, external and kinetic calibration, was also compared during an *in vivo* pharmacokinetic study on beagles for the determination of benzodiazepines in circulating blood.[142] The probes were exposed to the flowing blood for 2 min for equilibrium extraction and 30 s for kinetic calibration. Both methods yielded results in good agreement with conventional methodology based on blood withdrawal followed by plasma protein precipitation and LC-MS/MS analysis. Another study compared the performance of double extraction kinetic calibration with external calibration and standard on the fiber calibration methods using pharmacokinetic studies on rats as the model system and found satisfactory performance of both methods.[70]

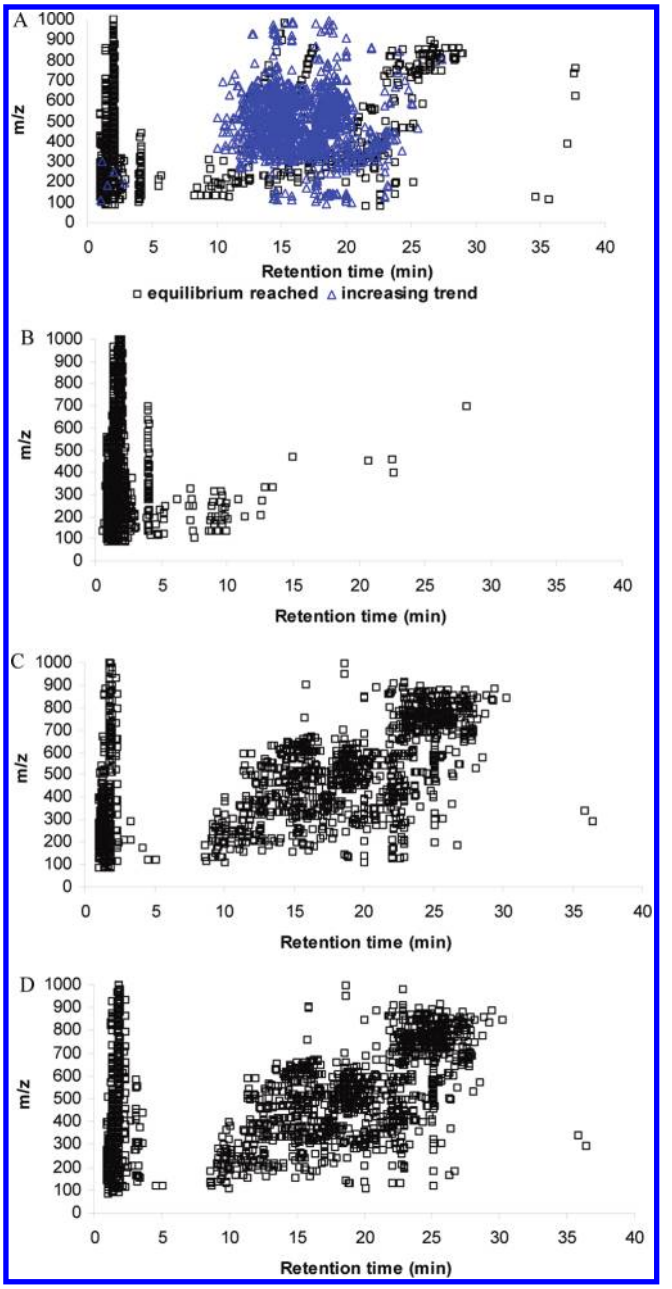


**Figure 11.** Ion maps obtained after analysis of human plasma sample using (A) SPME, (B) ultrafiltration (UF), (C) solvent precipitation using acetonitrile (PP), and (D) solvent precipitation using methanol/ethanol (PM) in negative ESI mode with pentafluorophenyl reverse phase column and high-resolution MS using Orbitrap instrument. The results for SPME (A) also show the influence of increasing extraction time on the observed number of metabolites detected. Metabolites shown in triangles increase with increase in extraction time, while for metabolites shown with squares, equilibrium is reached within 5 min and no further increase in the amount extracted is observed with increasing time.

Briefly, for *in vivo* applications of SPME, if the fiber coating/sample matrix distribution coefficients of the analytes are known,


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

an equilibrium calibration method can be used. An external calibration method does not need the distribution coefficient, but it requires the availability of standard samples with similar matrix composition. The choice of whether to use kinetic or equilibrium calibration will depend on the time required to reach equilibrium and whether this length of time is compatible with the goals of the experiment and the dynamics of the system under study. Among pre-equilibrium calibration methods, the kinetic on-fiber standardization method can compensate the matrix effects and significantly shorten the sampling time, but the technique also requires the knowledge of the distribution coefficient. Furthermore, this approach requires the optimization of preloading procedure for the calibrant in order to ensure good method precision and accuracy and to ensure the appropriate levels of calibrant are used. The sampling rate method is much simpler to implement, because it does not require the knowledge of the distribution coefficient, and eliminates the need to preload any standards onto fiber. However, this method requires the determination of the sampling rate and cannot be used in situations where sampling rate is changing over time (requires controlled agitation conditions over the sampling time, for example, by the use of pumps with controlled flow rate). The selection of the most suitable calibration method for *in vivo* SPME applications requires user familiarity with the available methods and the basic principles of each method in order to ensure proper practical implementation and good fit with the requirements of a given application. In turn, the selection of the most appropriate calibration technique for a given application can simplify the operation and therefore increase the overall efficiency of the *in vivo* SPME procedure while ensuring excellent quantitative results.

### 2.6. Automation

With the increases in capability of *in vivo* SPME, efforts are currently under way to further automate these procedures in order to increase sample throughput and permit unattended analysis. From the SPME−GC perspective, fiber exchanger attachments can facilitate the unattended analyses of multiple SPME fibers (for example, from sampling several individual plants in a given study). From the SPME−LC perspective, the majority of studies are currently performed by desorbing the fibers manually in the small-volume vial inserts. However, efforts are currently underway to desorb multiple fibers using a 96-well plate format for high-throughput sample preparation, which would facilitate large-scale studies in the future.[49,52,188,189] Furthermore, the automation of SPME sampling procedure for rodent sampling can further improve the precision and acceptance of this alternative methodology for animal studies.

## 3. COMPARISON OF *IN VIVO* SPME TO CONVENTIONAL METHODOLOGIES

For the majority of *in vivo* applications of SPME, the sensitivity and precision provided by SPME were comparable to or better than those of traditional techniques.

Microdialysis is a catheter-based sampling method that enables continuous monitoring of tissue chemistry *in vivo*.[190,191] Both *in vivo* SPME and microdialysis provide sampling with minimal perturbation to the system under study and are applicable for *in vivo* monitoring of analyte concentrations in awake and freely moving animals, which makes them suitable for studies such as pharmacokinetics, pharmacodynamics, and behavior studies. Although microdialysis is widely used for *in vivo* chemical collection,[192−194] the drawbacks of this technique are significant, such as the loss of perfusion fluid, the need for a pump, poor performance for hydrophobic species and the complicated calibration.[195,196] However, despite these drawbacks microdialysis is considered the gold standard for *in vivo* research, so the performance of *in vivo* SPME was compared with microdialysis to sample pesticides in the leaf of a jade plant.[67] Both techniques yielded equivalent results, although concentrations determined by microdialysis were consistently slightly lower than those for SPME indicating possible adsorptive losses of analytes to the membrane. Also the molecular size of the analyte is limited by the molecular cut off of the membrane. SPME provided several advantages over microdialysis, including higher sensitivity, better precision and accuracy, improved compatibility with LC-MS analysis due to the reduction of ionization suppression effects, ability to extract higher molecular weight compounds since no molecular cut off membranes are used and wider range of applications because both headspace and direct extraction is possible. Besides these, the SPME technique does not require a syringe or osmotic pump, which simplifies field sampling. The main drawback of *in vivo* SPME in comparison to microdialysis is that it cannot perform continuous sampling as done by microdialysis. This makes microdialysis more suitable for applications where very high temporal resolution is needed. Overall, SPME has the potential to replace microdialysis for some *in vivo* studies and is a particularly attractive alternative for metabolomics studies (where microdialysis may not be able to adequately sample hydrophobic species), studies requiring high spatial resolution (where SPME fibers of very small dimensions such as space-resolved fibers can be used in order to improve resolution over what is achievable by microdialysis), for long-term studies of the same individuals such as monitoring the onset or progress of a disease (where repeated setting up of microdialysis may be cumbersome, and continuous monitoring is not feasible on the time scale of weeks or months), for extraction of macromolecules, and for field studies (where implementation of pumps or syringes may not be practical). In some cases, a combination of SPME and microdialysis can be used in which instead of using perfusate the SPME fiber can be placed in the lumen of the microdialysis membrane (membrane-protected SPME[15]). This approach can address to some extent limitation of each technique in some *in vivo* applications allowing semi-continuous monitoring by SPME while reducing loss of hydrophobic compounds in microdialysis.

For pharmacokinetic studies, the most commonly accepted methodology relies on blood withdrawal followed by appropriate sample cleanup and analysis by LC−MS/MS. The results for *in vivo* SPME were therefore compared against this traditional approach and found to be equivalent as illustrated in Figure 12.[70] Other similar studies also demonstrated the equivalency of SPME to conventional blood withdrawal methods.[52,63,68,142]

The comparison of *in vivo* SPME to tissue extraction is shown in Figure 13.[66] It shows the complementary nature of the two approaches, as the concentration determined by SPME corresponds to free concentration while solvent extraction methodology yields total concentration. However, both techniques show similar trends with respect to monitoring of drug concentrations in time, so either approach is suitable for environmental monitoring, although *in vivo* SPME has the important advantage of being nonlethal.

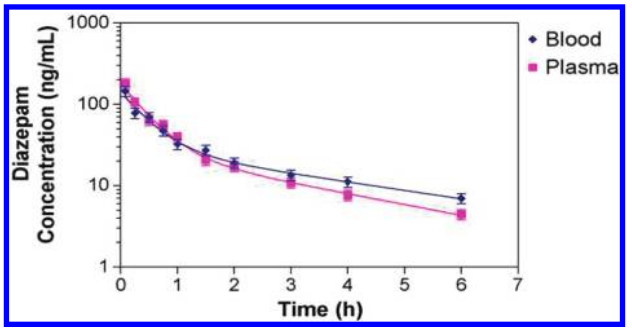


**Figure 12.** Comparison of equilibrium *in vivo* SPME (◆) with conventional plasma analysis (■) following blood withdrawal for the determination of diazepam concentrations in circulating blood of rats: blood = *in vivo* SPME in flowing blood; plasma = conventional blood withdrawal followed by plasma protein precipitation. Reprinted with permission from ref 70. Copyright 2008 Elsevier.

## 4. APPLICATIONS

### 4.1. Overview and Types of Applications

The majority of applications of *in vivo* SPME techniques to date focus on monitoring of volatile and semivolatile emissions from microorganisms, insects, plants, and breath.[5,6] In these cases, the analytes of interest are typically extracted in the headspace. The SPME fibers do not need to be directly embedded into the living system, thus simplifying the experimental setup required for this type of study. In recent years, significant improvements have been made in the new field of direct extraction of various compounds directly from the living system under study. This type of study imposes much more stringent requirements on the properties of the extraction phase as discussed in section 2.4. The types of devices necessary for this type of work were not available commercially up to now, thus limiting the number of reported applications to the laboratories equipped for custom preparation of devices. However, the recent commercial introduction of the new biocompatible *in vivo* SPME devices from Supelco addresses this shortcoming to a certain extent. As a result, this type of application can be expected to grow rapidly in the near future and to open up new possibilities for SPME in life science research.

*In vivo* SPME can be used for both targeted quantitative analysis of selected compounds and more qualitative and screening studies such as metabolomics studies, where the goal is to capture all low molecular weight species present in a given living system. From an experimental viewpoint, the main difference between these two types of studies lies in the selection of the most appropriate coating. For targeted studies, the coating with the highest $K_{fs}$ for a given set of analytes is selected in order to ensure the best analytical sensitivity of the developed method. For global profiling and metabolomics-type studies, the extraction of wide range of species is required (e.g., volatile and semivolatile, polar and nonpolar), so for this type of study more universal coatings are selected or a combination of several complementary coatings can be used. Until very recently, this type of global metabolomics study was largely confined to headspace sampling followed by analysis by GC-MS (Tables 1−3 and Table 5). However, focused efforts to identify the most suitable coatings for use in direct extraction mode yielded very promising results with three types of coatings providing excellent metabolite coverage spanning a vast polarity range.[71] These three types of coatings are mixed-mode coating

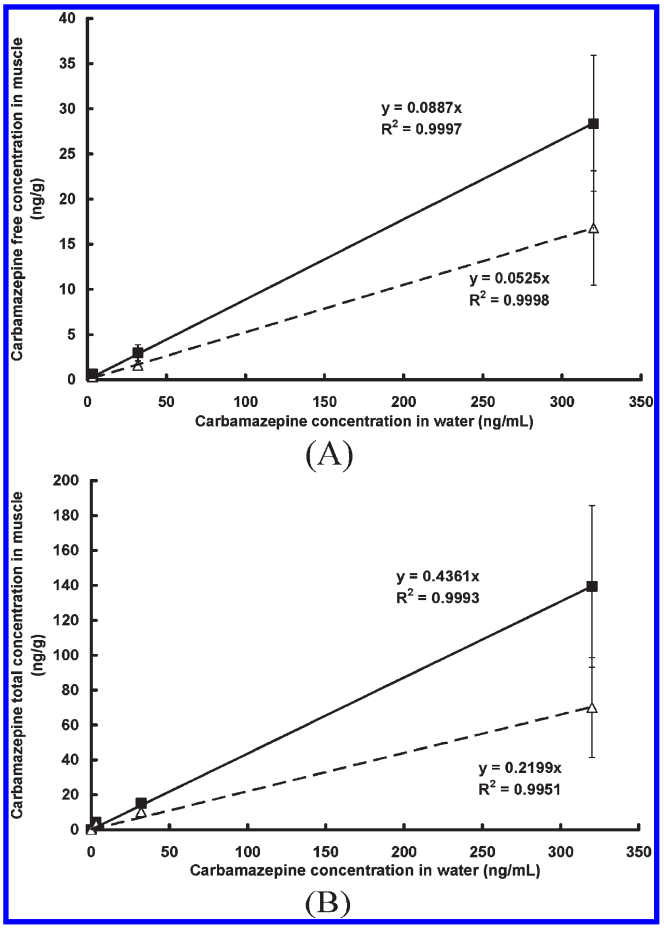


**Figure 13.** Relationships between free or total carbamazepine concentrations in fish muscle and exposure water. Fish were exposed to carbamazepine for 7 (■) or 14 d (△) at nominal concentrations of 0 (control), 3.2, 32.0, or 320.0 ng/mL carbamazepine. *In vivo* SPME sampling (A) and liquid extraction (B) of bioaccumulated analytes were performed for carbamazepine ($n$ = 3). Reprinted with permission from ref 66. Copyright 2008 American Chemical Society.

(C18 or C8 with benzenesulfonic acid), polar-enhanced polystyrene divinylbenzene coatings, and phenylboronic acid coatings. In general, the extraction of compounds with log $P$ values as low as −2 was possible with good extraction efficiencies from complex biological fluid such as blood, although some even more polar species could also be successfully observed if their endogenous concentrations were sufficiently high. For example, glutamic acid and choline with log $P$ values of −3.69 and −5.16[197] could routinely be observed in both human and rodent blood.

### 4.2. Microorganisms

Because of its speed, simplicity, and nondestructive nature, SPME is a very useful method for monitoring the volatile emissions of various microorganisms, such as fungi, yeast, and bacteria,[198] and for the study of biodegradation or biotransformation pathways of various chemicals.[199,200] The samples are typically placed in vials or other containers. By use of commercially available fibers, it is very efficient and convenient to extract the volatile analytes in headspace. Separation and identification is normally performed by GC-MS.

Numerous food products require protection against microbial spoilage during their shelf life. The headspace of the cinnamon

**Table 1. Application of Headspace SPME in Microorganism Studies**

| purpose of the study | fiber used | extraction time/temperature | detection | refs |
|---|---|---|---|---|
| volatile profile of cheese during ripening | 50/30 μm DVB/CAR/PDMS | 30 min/50 °C,[202] 30 min/45 °C[209] | GC−MS | 202,209 |
| volatile organoselenium compounds in headspace of *Bacillus* species grown in selenium solution | 75 μm CAR/PDMS | 15−50 min/37 °C | GC−MS, GC−SCD | 203 |
| antimicrobial activity of the vapor generated by essential oils against the growth of Gram-negative and Gram-positive bacteria | 85 μm PA and 100 μm PDMS | 24 h with fiber-retracted SPME | GC−MS | 201 |
| volatile metabolites released from *Glomerella cingulata* | 100 μm PDMS, 65 μm PA, 75 μm CAR/PDMS, 50/30 μm DVB/CAR/PDMS | 30 min/28 °C | GC−MS | 205 |
| volatile metabolites of cooked ham with outgrowth of lactic acid bacteria at different temperature | CAR/PDMS | 30 min/30 °C | GC−MS | 210 |
| determination of the ability of soybean volatile compounds to inhibit *Aspergillus flavus* growth | 50/30 μm DVB/CAR/PDMS | 60 min/60 °C | GC−MS | 211,212 |
| volatile byproduct of a wild yeast at different fermentation temperature | 50/30 μm DVB/CAR/PDMS | 45 min/60 °C | GC−MS | 213 |
| off-odor compounds produced in cork by isolated bacteria and fungi | 50/30 μm DVB/CAR/PDMS | 30 min/50 °C | GC−olfactometry, GC−MS | 214 |
| volatile compounds released from cheese | 65 μm DVB/CAR/PDMS | 10 min/50 °C | GC−MS | 215 |
| microbial VOCs emitted from mold species | 85 μm CAR/PDMS | 24 h with fiber-retracted SPME | GC−MS | 216 |
| VOCs released by the entomopathogenic fungus *Beauveria bassiana* | 65 μm PA | 20 min/60 °C | GC−MS | 217 |
| aromatic compounds produced by wild yeasts | 75 μm CAR/PDMS | 40 min/45 °C | GC−MS | 218 |
| microbial VOCs emitted from indoor mold | 70 μm CW/DVB | 18 h/45 °C | GC−MS | 219 |
| evaluation of hydrocarbon evaporation from biodegrading bilge waste | 100 μm PDMS | 30 min/25 °C, flow rate 60 mL/min | HRGC | 220 |
| identification of the volatile organic compounds emitted from the wood-rotting fungi | 85 μm PA and 100 μm PDMS | 1.5 and 3 h | GC−MS | 221 |
| determination of off-flavor compounds in apple juice caused by microorganisms | 50/30 μm DVB/CAR/PDMS | 10 and 30 min/60 °C | GC−MS | 222 |
| fungal volatile metabolites from *Penicillium roqueforti* | 75 μm CAR/PDMS 50/30 μm DVB/CAR/PDMS 8 μm PA and 100 μm PDMS | 20 min/20 or 50 °C | GC−MS | 223 |
| VOCs produced during interspecific mycelial interactions between four wood rotting fungi | 100 μm PDMS | 1 h/20 ± 5 °C | GC−MS | 224 |

and clove essential oils and their combination was sampled and identified by SPME-GC-MS, and the antimicrobial activity of the vapor against the growth of four Gram-negative and four Gram-positive bacteria was assessed.[201] The volatile profile of the


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

**Table 2. Applications of *in Vivo* SPME in Insect Studies**

| analytes | samples | fiber used | extraction mode/time/temperature | ref |
|---|---|---|---|---|
| cuticular hydrocarbons | *Harpegnathos saltator* | 7 μm PDMS | directly rubbing/5 min | 45 |
| sex pheromones | *Triatoma brasiliensis* | 50/30 μm DVB/CAR/PDMS | HS/10 min/50 °C | 225 |
| sex pheromone | *Prionus californicus* | 100 μm PDMS | direct contact/1 min | 226 |
| sex pheromone | *Anastrepha serpentina* | 100 μm PDMS | HS/1 h | 227 |
| sex pheromone | *Dasylepida ishigakiensis* | 75 μm PDMS/CAR | HS/10 min/22 °C | 228 |
| sex pheromone | *Phyllonorycter insignitella and Ph. nigrescentella* | 100 μm PDMS | HS/15 min/22 °C | 229 |
| communication pheromone | *Busseola fusca* | 65 μm CW/DVB | gently rubbing/5 min | 230 |
| alarming volatiles | *Tessaratoma papillosa* | 65 μm PDMS/DVB | HS/30 min | 231 |
| defensive chemicals | *Graphosoma lineatum* | 100 μm PDMS | HS/15 min | 232 |
| odorants | *Harmonia axyridis* | 50/30 μm DVB/CAR/PDMS | HS/24 h/30 °C | 233 |
| fertility signaling | *Platythyrea punctata* | 7 μm PDMS | rubbing/5 min | 234 |
| cuticular compounds | *Leptothorax* | 7 μm PDMS 30 μm PDMS | rubbing/15 min | 235 |
| defensive volatiles | *Bolitotherus cornutus* | 50/30 μm DVB/CAR/PDMS | HS/3 min | 236 |
| trail-following pheromone | *Kalotermitidae* | 65 μm PDMS/DVB | gently rubbing/5 min | 237 |
| chemical composition of tarsal liquids and cuticular components | *Gastrophysa viridula* | 100 μm PDMS | gently rubbing/4 min | 238 |
| sex pheromone | *Marmara gulosa* | 100 μm PDMS | HS/16 h | 239 |
| sex pheromone | *Cossus insularis* | 100 μm PDMS | HS/16 h/25 °C | 240 |
| sex pheromone | *Phyllonorycter acerifoliella* and *Ph. heegerella* | 100 μm PDMS | HS/2−4 h/13−15 °C | 241 |
| volatiles | *Ixodes ricinus* | 100 μm PDMS | HS/15 min/25−26 °C | 242 |
| sex pheromone and its precursors | *Diprion pini* | 65 μm PDMS/DVB | HS/10 min-5 h | 243 |
| trail pheromone | four temites | 65 μm PDMS/DVB | gently rubbing | 244 |
| attractant (camphor) | chafer | 75 μm PDMS/CAR | HS/30 min/26 °C | 246 |
| pheromone | *Scyphophorus acupunctatus* | 65 μm PDMS/DVB | HS/15 min/25 °C | 247 |
| source, identity, and potential function of volatiles | *Triatoma infestans* | 75 μm PDMS/CAR | HS/30 min/50 °C | 248 |
| volatile compounds | *Apis mellifera L.* | 65 μm PDMS/DVB | HS/10 min/room temperature | 249 |
| aggregation pheromone | *Scapanes australis* and *Strategus aloeus* | 65 μm PDMS/DVB | HS/20 min | 250 |
| pheromone blend composition | *Nezara viridula* | 65 μm PDMS/DVB | HS/4 min | 251 |
| defensive chemicals | *Papilio glaucus* caterpillar | 100 μm PDMS | rubbing | 252 |

Spanish soft cheese made from raw milk was studied in four different stages of ripening by SPME−GC−MS. A total of 46 compounds were detected.[202] Selenium is considered as a major environmental pollutant. The concentration and toxicity of selenium compounds in the environment can be decreased by the reduction of selenium oxyanions to elemental selenium or methylated volatile selenium products by selenium-resistant microbes. Production of dimethyl triselenide and dimethyl diselenenyl sulfide in the headspace of metalloid-resistant *Bacillus* species grown in the presence of selenium oxyanions was detected and reported by SPME-GC with either fluorine-induced sulfur chemiluminescence detector (SCD) or MS.[203] The interactions between plants and microorganisms are universally mediated by VOCs.[204] The profile of VOCs released from *Glomerella cingulata* at different times in the growth progress was investigated by SPME-GC-MS method.[205]

SPME technique was also shown as a promising alternative for screening the biotransformation of terpenes.[206] It is a very convenient method for identifying the biotransformation products, such as limonene-1,2-diol, *R*-terpineol, and the isomers of rose oxide, for both sporulated surface and submerged fungal cultures.[207,208]

Table 1 presents some applications of HS-SPME in microorganism studies.

### 4.3. Insects

*In vivo* SPME is a well-established technique in the field of insect biology. Most of these applications of SPME are focused on the study of sex pheromones,[225−229] communication pheromones,[230] defensive and alarming volatiles,[231,232] odorants,[233] and other compounds of interest.[45,234,235] Headspace sampling with SPME fiber by coupling with GC-MS is an efficient way to monitor and identify the volatile emissions.[225,236] For semivolatile compounds, direct contact and gently rubbing methods were used in order to obtain richer profiles.[230,237,238]

The cuticular surface of insects presents rich reservoir chemicals, some of which have important informational value. One of the key features of insect societies is the division of labor in reproduction between one or a few fertile individuals and many sterile nestmates that function as helpers. HS-SPME was used to measure cuticular hydrocarbons (CHCs) in live ants, *Harpegnathos saltator*, that were experimentally induced to start producing eggs.[45] A striking correlation of hydrocarbon patterns with the ovarian activity of the individuals was discovered. In many insects, mate finding is mediated by volatile sex pheromones.

**Table 3. Applications of *in Vivo* SPME in Plant Studies**

| analytes | samples | device used | extraction mode/time | refs |
|---|---|---|---|---|
| pesticides | jade plant *C. ovata* | custom-made PDMS fiber | direct/20 min | 67 |
| VOCs | Chinese daffodil flowers | 85 μm CAR/PDMS | HS/30 min | 267 |
| VOCs | stem/branch of *Prunus dulcis* | 100 μm PDMS | HS/30 min | 269 |
| sesquiterpenes, oxygenated terpene | branch of ponderosa pine | 65 μm PDMS/DVB | HS/0.5−50 min/air with $CO^2$ ~4 L/min | 270 |
| volatiles emitted during mite-infestation | spruce clone | 65 μm PDMS/DVB | HS/24 h | 271 |
| sesquiterpenes | *Pinus sabiniana, Pinus ponderosa* | 100 μm PDMS | HS/10 min/air flow velocity of 21 cm/s | 272 |
| VOCs | foliage of *Abies fraseri* | 100 μm PDMS, 85 μm PA, 7 μm PDMS | HS/5 min-4 h | 273 |
| VOCs | flowering *Jasminum polyanthum* | 100 μm PDMS, 85 μm PA | HS/static 30 min; 100 mL/min, 10 min | 274 |
| allelochemical uptake | stem of tomato | 100 μm PDMS | direct/1 h | 275 |
| Se−Hg | *Brassica juncea* | 75 μm CAR/PDMS | HS/10−20 min | 277,278 |
| root-exuded thiophenes | roots of marigold | custom-made 0.8 mm PDMS | HS/24 h | 279 |
| VOCs attracting female grape berry moth | shoots of *Vitis riparia* | 85 μm CAR/PDMS | HS/24 h | 280 |
| wound-activated volatiles released as chemical defense | *Dictyota dichotoma* | 75 μm CAR/PDMS | HS/20 min | 281 |
| triazine herbicides | tomato, reed, onion | commercial fiber-coating type not specified | direct/1 h | 282 |
| VOCs released during abiotic and biotic stress | tomato shoots | 100 μm PDMS | HS/60 min | 283 |

It was shown that metasternal glands (MGs) are involved in producing signal related to sexual communication of some insects. HS-SPME-GC-MS was used to identify the compounds produced by the MGs of *T. brasiliensis* females.[225] The most abundant compounds were 3-pentanone, followed by (4*R*)-methylheptanol, 3-pentanone, and (2*S*)-methyl-1-butanol, and the study showed that the compounds produced by the MGs of *T. brasiliensis* females are involved in the sexual communication of this species. *Anatrepha serpentina* is known as the "Sapote fruit fly". Emissions from sexually active *A. serpentina* males were collected by HS-SPME, and two major components were identified as 2,5-dimethylpyrazine (DMP) and 3,6-dihydro-2,5-dimethylpyrazine (DHDMP).[227] HS-SPME-GC-MS was also used for the collection and identification of volatile sex pheromone emitted by moth,[229,239−241] white grub beetle,[228] tick,[242] and sawfly.[243]

An SPME sampling method has also been employed for fast, easy, and reliable monitoring and recognition of volatile defensive and alarming chemicals of insects. An *in vivo* SPME method using pencil lead fiber coupled to GC analysis was developed for the collection and identification of defensive chemicals of scent gland in *Graphosoma lineatum*.[232] In this study, the extraction capacity of pencil lead fiber was 1000-fold improved versus commercial PDMS fiber for the studied chemicals. In response to the specific threat stimulus of human breath, defensive volatiles of the forked fungus beetle, *Bolitotherus cornutus*, were tested by SPME-GC-MS method.[236] The volatile defensive secretions differ between the beetles living on two species of fungi but did not differ between male and female beetles. In addition, an efficient HS-SPME sampling method was established to study the alarming volatile characteristics and potential alarming volatiles of stinkbugs followed by GC-MS detection.[231] During sampling, the stinkbugs were placed in a glass vial, followed by HS-SPME exposure for 30 min. Then, the stinkbugs were irritated by perforating their hypogastriums with a needle. The alarming volatiles of the stinkbug were sampled by HS-SPME again. The stinkbugs were kept alive during the sampling procedure.

In addition to HS-SPME, direct SPME method has been used to test the trail and communication pheromones of insects.[230,237] Cold anesthetized termites were induced to expose their sternal gland by stretching the abdominal segments with forceps under a stereomicroscope. The sternal glands were gently rubbed with a PDMS/DVB SPME fiber. After GC analysis, (*Z*)-dodec-3-en-1-ol, a common major component of the trail-following pheromone in the termites, was identified.[237,244] A similar method was used for the study of cuticular compounds of workers and queens in two ant species, and the results obtained by solvent extraction, solid sampling, and SPME were compared.[235] SPME results were qualitatively and quantitatively similar to solvent extraction. Moreover, SPME obviously has an important advantage over conventional extraction: ants do not have to be killed for the extraction and can be repeatedly investigated.[235,245] In another study, tarsal and cuticular chemistry in the leaf beetle was studied and compared, and SPME and solvent extraction techniques were evaluated.[238] The cuticular hydrocarbon profile of the elytra was obtained by gently rubbing with a PDMS fiber. For sampling the tarsal liquid, a device was designed to allow micromanipulation of the fiber and the beetle (Figure 14). The beetle was slowly and carefully moved back and forth so that the tarsi constantly rubbed over the PDMS coating of the fiber for 4 min, while making sure that other body parts did not come into contact with the fiber. The study demonstrated that the tarsal liquid is chemically very similar to the cuticular lipids of the beetle. In this study, richer mass spectra were obtained using the solvent extraction method, but the specimens had to be sacrificed. Direct SPME sampling not only significantly reduced the handling time and minimized contamination but also allowed repeated sampling of live beetles.[238]

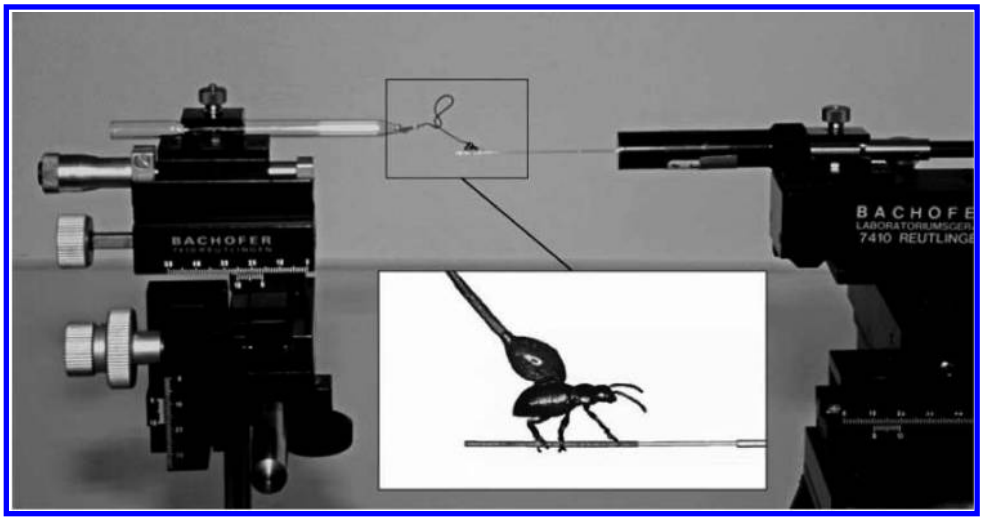


**Figure 14.** Experimental setup for SPME sampling of tarsal liquid. Reprinted with permission from ref 238. Copyright 2009 Springer.

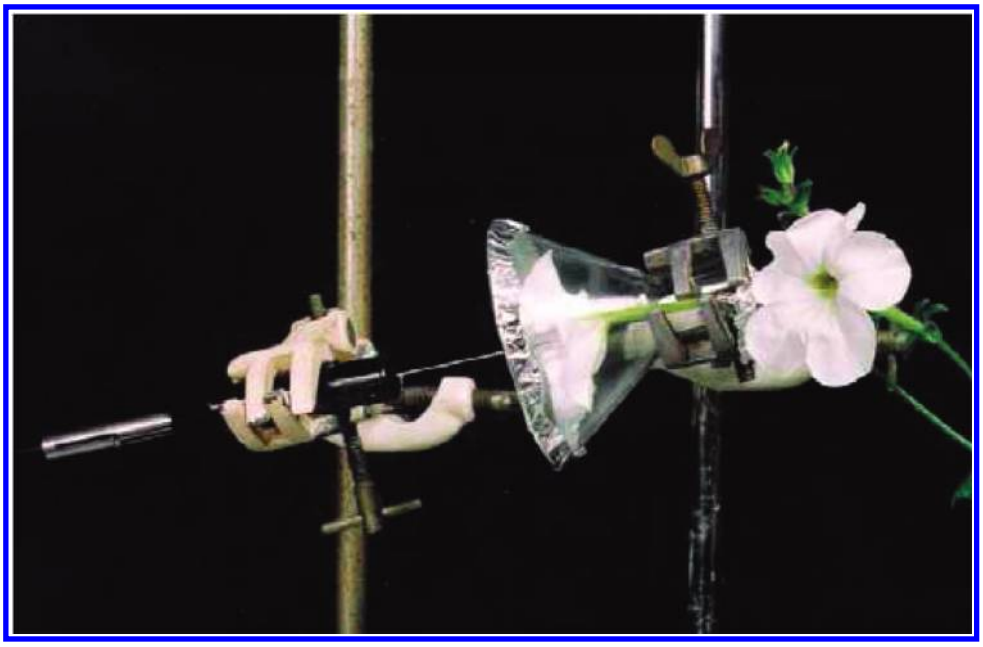

**Figure 15.** *In vivo* sampling of flower scent. Reprinted with permission from ref 266. Copyright 2003 Elsevier.

Table 2 presents recent applications of *in vivo* SPME for insect studies.

### 4.4. Plants

Plants emit VOCs that play important roles in their interactions with the environment and have a major impact on atmospheric chemistry.[253] SPME has been extensively used to sample VOCs emitted by plants and has facilitated studies of plant physiology and chemotaxonomics, interactions of plant with environment, and the search for new fragrances. In some cases, *in vitro* SPME was used for extracting volatiles from fruits,[254−259] flowers,[260−263] and trees,[264] but this type of sampling can be easily performed using *in vivo* mode to obtain more representative profiles, and the prevalence of *in vivo* approaches can be expected to increase in future.

For the sampling of flower scents, SPME is currently the predominant sample preparation method because of its superior perfomance.[265] A commonly used device for *in vivo* sampling of flower scent with SPME is shown in Figure 15.[266] The glass funnel can be replaced with a plastic bag as an enclosure. Through the study of scent production by petunia Mitchell flowers, Verdonk et al. demonstrated that SPME coupled to GC-MS is an outstanding tool to measure volatile production by flowers *in vivo*. The advantage of SPME analysis lies in its speed and simplicity, compared with trapping with solid adsorbents such as Tenax. Moreover, adsorbents such as Tenax may not provide the desired sensitivity in as short sampling period as used for SPME.[266] Similar methodology was used for the characterization of VOCs emitted from Chinese daffodil flowers. Twenty-seven compounds in the emission were initially identified, among which four compounds were proposed as biomarker molecules.[267]

Many factors may influence the production of BVOC by plants, such as abiotic factors (light intensity, water, season, etc.) or age of the leaves or plant, cultivars, and plant species. HS-SPME coupled to GC-MS was used to identify and monitor the emission

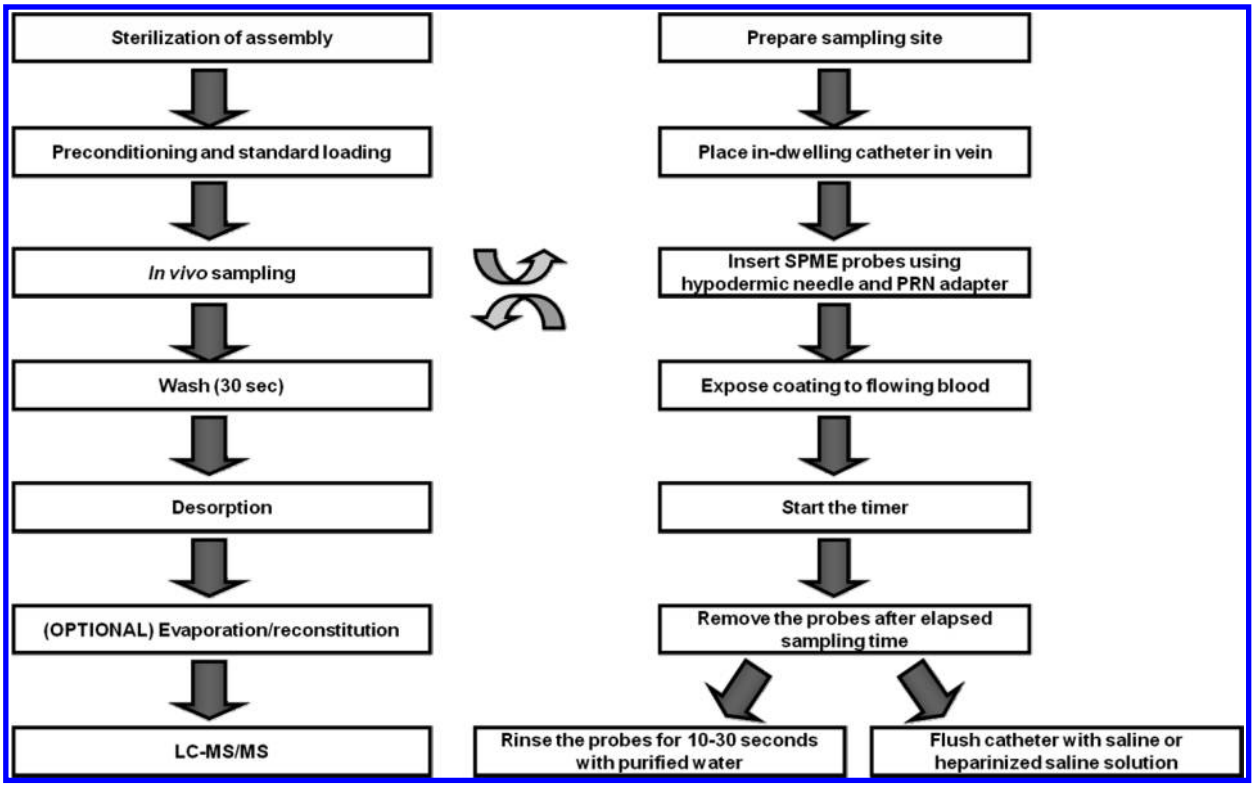


**Figure 16.** (A) Workflow of typical *in vivo* SPME procedure for animal studies and (B) detailed description of *in vivo* SPME sampling in large animals such as beagles.

patterns of biogenic volatile organic compounds from leaves of *Eucalyptus dunnii*, *Eucalyptus saligna*, and *Eucalyptus citriodora* in situ.[268] Short extractions (1 min) were performed every 30 min for periods of 8−10 h during 24 days. Forty-two compounds were detected, and 20 were identified in the headspace of *E. saligna* leaves, and 19 of 27 compounds were identified in the headspace of *E. dunnii* leaves. The emission pattern of (*E*)-$\beta$-ocimene and rose oxide suggests that they may play a bioactive role in *Eucalyptus*. As a simple, relatively inexpensive, noninvasive, fast extraction tool, SPME allows the monitoring of the BVOCs released by the plants over short intervals of time for long periods, to investigate the plant-plant, plant-herbivore-predator, or plant-environment interaction *in situ*. This type of long-term longitudinal studies is not accessible using conventional methodologies that require sacrifice of specimen and solvent extraction.

*In vivo* SPME methodology has also been used for the identification of volatile production of fruit,[269] branch,[270] or even whole plants.[271−274] These studies demonstrated that SPME sampling is relatively simple and inexpensive compared with traditional gas sampling and analysis methods. The integration of sampling and extraction eliminates the loss of analytes to the walls of sampling lines and instrumental parts, while direct desorption of the fiber in GC injector eliminates the need for solvent. Short exposure time makes fast and easy quantification. The portability of the device allows for the sampling of live plants using an enclosure in the field.[270,272]

Besides HS-SPME, direct SPME was used to measure allelochemical uptake by tomato plants *in vivo*.[275] The preconditioned SPME fiber was inserted into the stem of the test plant and the fiber was exposed to the stem fluid for 1 h. Exogenously applied 1,8-cineole was rapidly taken up by tomato. Tomato was also found to take up camphor, menthol, and coumarin, but not carveol.

Interest in speciation analysis of selenium has gown rapidly in the past years, especially in the use of chromatographic separation coupled with inductively coupled plasma mass spectrometry (ICP-MS).[276] *In vivo* SPME was used for sampling of selenium accumulating plant *B. juncea* to detect dimethylselenide and dimethyldiselenide in the plant's headspace using GC-ICP-MS detection.[277,278] The coupled technique of HS-SPME-GC-ICP-MS has proven suitable for the speciation of volatile selenium species in plants. The ultratrace detection limits achieved (1−10 ppt depending on the Se compound) permit the speciation of these compounds at very low levels in biological samples and require minimal sample treatment.[278]

Table 3 presents some recent applications of *in vivo* SPME for plant studies.

### 4.5. Animals

The reported applications of *in vivo* SPME for animal studies include measuring of various compounds in blood, muscle, breath, brain, and even rumen gas.

**4.5.1. Sampling of Animal Volatile Emissions.** SPME-GC-MS was used for sampling and analysis of VOCs in animal breath. Breath of morbid steers with respiratory tract infections and healthy steers were sampled with a system consisting of a face mask sampling device and SPME fibers.[284] A total of 21 VOCs were detected. The presence of acetaldehyde and decanal was associated with clinically morbid steers while methyl acetate, heptane, octanal, 2,3-butadione, hexanoic acid, and phenol were associated with healthy steers. The results suggest that noninvasive heath screening using breath analyses could become a useful diagnostic tool for animals and potentially humans as well.

*In vivo* HS-SPME was used for characterization of VOCs and odors in cattle rumen by using GC-MS-olfactometry.[285]

A novel device enabled SPME sampling and collecting of rumen gas samples from steers, through a cannula. Sampling times as long as 10 min were practical. Fifty VOCs from ten chemical groups were identified in the rumen headspace, which indicated that rumen gases can be an important potential source of aerial emissions of reactive VOCs and odor. The method is very useful for qualitative characterization of rumen gases, digestion, and its relationship to odor and VOC formation.

Frogs produce a remarkable array of noxious, distasteful, and highly toxic compounds, which, on secretion from granular skin glands, can serve to protect them from predators. A SPME-GC-MS technique was used to determine the defensive odors of Australian tree frog, *Litoria ewingi*.[286] SPME fibers were positioned above the frogs, and the animals were stressed by touching, prodding, and pinching with blunt forceps. Both the raw secretion and components isolated from it proved repellent to biting flies and were highly efficient as deterrents to predation by the water python *Liasis fiscus*. The study also proved the principal components of the odor are plant secondary compounds and that *L. ewingi* is reliant upon obtaining these compounds from its environment.

**4.5.2. SPME in Pharmacokinetic Studies.** SPME devices based on hydrophilic PPY[63] and PEG[142] or PEG/C18-bonded SPME[52,68] fiber coatings were used for direct extraction of diazepam and its metabolites from the flowing blood of beagle dogs. LC-MS/MS was used for analysis.[52,63,68,142] The fast microextraction technique caused minimal disturbance to the investigated system and additional information, such as the free concentration, could be obtained.[142] Figure 16 shows generalized in vivo SPME sampling workflow. More detailed description of experimental procedures and considerations for *in vivo* SPME sampling of flowing blood in beagles is described elsewhere.[287] These initial pharmacokinetic studies were performed on large animals such as beagles, because the size of the blood vessel was sufficiently large to permit direct introduction of SPME probe through a catheter. However, the inherent advantages of the blood-draw-free nature of SPME are particularly important for smaller animals such as rodents where there is limited blood volume available for withdrawal and analysis. For these smaller animals, the blood vessel size is too small to permit direct introduction of SPME probe without obstructing the blood vessel. To address this issue, a new polyurethane rodent sampling interface was designed and used to develop a sampling procedure for *in vivo* pharmacokinetic studies in rats.[70] The main concept of this sampling approach is illustrated in Figure 17. The interface is attached to a surgically inserted exteriorized carotid artery catheter. The interface also incorporates a PRN adapter for insertion of SPME probe. Blood is circulated through the interface during sampling using manual push-and-pull action with a syringe. Very recently, this sampling interface was further miniaturized to permit *in vivo* SPME sampling of mice for the first time.[182] The attempts to date to recirculate the blood through the interface resulted in clotting,[70] so further improvement of the sampling methodology is needed in order to eliminate the need for manual push/pull action with syringe. This can be accomplished through further development of interface to minimize clotting or by further miniaturization of SPME probes in order to permit direct insertion into blood vessel. As the analytical instruments become more sensitive, the latter option becomes more feasible and attractive but necessitates research into reproducible production of SPME coatings on very thin solid supports, an issue that has not yet been successfully resolved.

The main advantages of *in vivo* SPME over current methods are rapid sample preparation with excellent sample cleanup and significant reduction in animal use. Fewer animals can be used to obtain a full pharmacokinetic profile, and sampling can be simultaneously carried out at multiple sites in one animal without a reduction in blood volume. The technique can significantly decrease the turn-around time for acquiring pharmacokinetic profiles in small rodents, making it useful for the drug discovery process. Furthermore, the technique reduces exposure and handling of blood and eliminates dosing errors from pharmacokinetic profiles improving the reliability of analytical results.

**4.5.3. SPME and Tissue Analysis.** An *in vivo* SPME technique was successfully used for the detection of toluene levels in the brain of conscious, free-moving mice.[288,289] For measuring the pharmacokinetics of inhaled toluene in the brain of mice, a commercial SPME fiber was inserted into the hippocampus (CA1) through a cannula fixed onto the animal. BALB/c mice were exposed to toluene for 30 min. The pharmacokinetics of toluene in the brain of mice exposed to 50 ppm toluene showed that the toluene level decreased rapidly after the exposure and returned to control levels after 60 min. The technique does not require removal of the brain from the animal and can be performed without anesthesia thus giving it potential to become a useful tool for studies in the field of neurotoxicology.[289]

Monitoring the exposure of fish to various environmental pollutants such as pharmaceuticals and endocrine-disrupting chemicals is an emerging area of interest. However, using conventional approaches, this type of study usually involves sacrifice of multiple fish in order to extract the relevant tissues of interest and verify what type of xenobiotics are accumulating in a given species of fish. However, with *in vivo* SPME, this type of sampling can be carried out in a much simplified and nonlethal fashion as demonstrated recently both under laboratory and field conditions.[46,66,290] Self-made PDMS fibers were used for direct sampling of analytes in fish tissue for 20 min. After extraction, the fibers were desorbed in 50 $\mu$L of methanol for LC-MS/MS analysis. This method can be applied to extract pharmaceuticals simultaneously in fish muscle and adipose tissue using spatially resolved fibers described in section 2.3.1 and can be used to determine bioaccumulation factors.[46,290] The study expanded the SPME technique to *in vivo* sampling of analytes in semisolid tissues and is particularly promising from an environmental perspective due to the nondestructive nature of sampling.

**4.5.4. SPME and Metabolomics.** SPME methodology for global metabolomics studies of whole blood and plasma was recently developed.[71] In the initial *in vitro* study, the performance of the developed SPME technique was compared against conventional methodologies: (i) plasma protein precipitation with acetonitrile (PP), (ii) plasma protein precipitation with methanol/ethanol (PM), and (iii) ultrafiltration (UF) using a pooled human plasma sample.[71] In terms of metabolite coverage, SPME was found to perform better than ultrafiltration because it provides more balanced coverage of both hydrophilic and hydrophobic species as shown in Figure 11. This example shows that the majority of metabolites observed for ultrafiltration had retention times <10 min, indicating significant loss of hydrophobic species. Therefore, for studies where free concentration determination is important, SPME provides important advantages over ultrafiltration. In comparison to solvent precipitation methods (PM and PP), SPME provided similar coverage using short extraction times and best coverage among any method using long overnight extraction time in combination with negative ESI reverse phase global metabolite profiling method (Figure 11) with >3000 features detected after extraction using

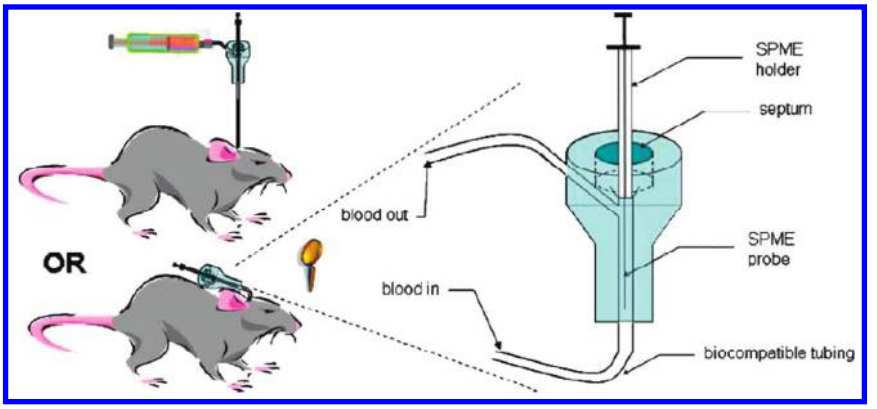


**Figure 17.** *In vivo* SPME sampling of rats: placement of SPME probe and interface connection to the carotid artery. Reprinted with permission from ref 70. Copyright 2008 Elsevier.

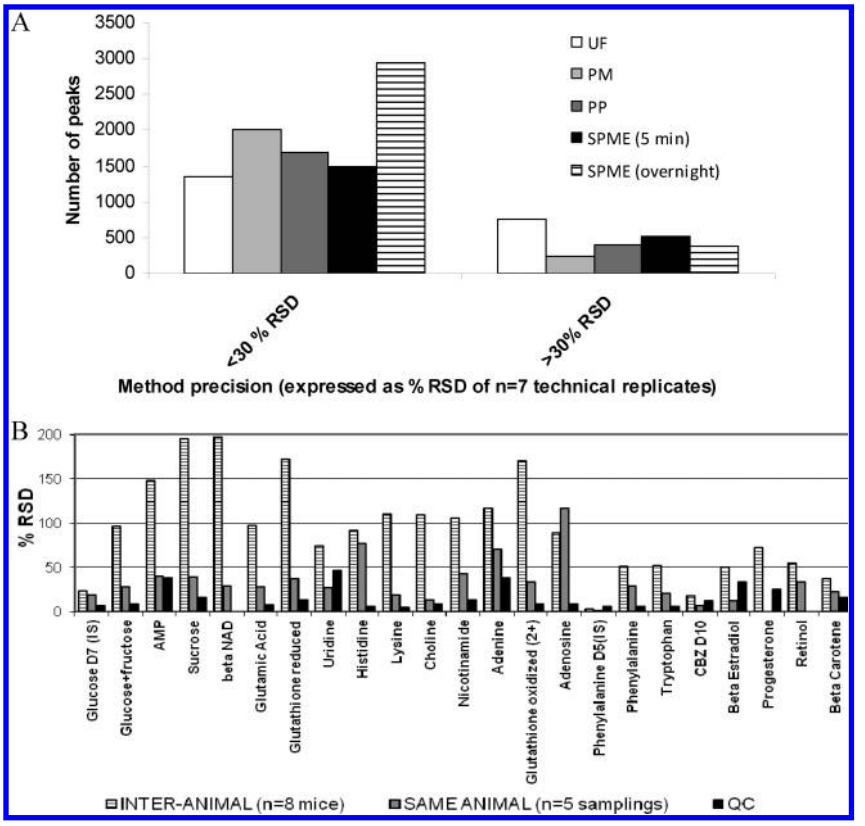


**Figure 18.** (A) Comparison of method precision of *in vitro* SPME using 5 min and overnight extraction times versus ultrafiltration (UF), solvent precipitation using methanol/ethanol (PM), and solvent precipitation using acetonitrile (PP). The results are shown for $n = 7$ independent preparations of pooled human plasma sample and analyzed using a global metabolite profiling method in negative ESI mode and using a pentafluorophenyl reverse phase column. (B) Comparison of analytical (repeated injections of QC sample) versus technical ($n = 5$ consecutive samplings of the same mouse) versus biological ($n = 8$ mice) variability obtained for a subset of identified metabolites in a global *in vivo* metabolomics study on mice.

a single SPME coating. In positive ESI reverse phase method, solvent precipitation with methanol/ethanol provided the most comprehensive coverage among the four methodologies tested. However, SPME coverage can be further enhanced by use of several complementary coatings. It is also important to mention that due to the nonexhaustive nature of SPME, signal intensities for SPME were significantly lower than those for conventional techniques. However, modern state-of-the-art analytical instrumentation is capable of detecting these small amounts of analytes extracted, thus permitting applications that were not achievable only a decade ago. In fact, the nonexhaustive nature of SPME, which results in small amounts extracted, is of utmost importance for *in vivo* sampling using SPME because it minimizes the disturbance to the system under study and permits the use of small probe dimensions making the technique less invasive than other more traditional approaches. Furthermore, lower overall signal intensities encountered in SPME methods result in two additional significant benefits: (i) reduced ionization suppression, which improves quantitation and data quality, and (ii) improved metabolite coverage because low intensity peaks are

not obstructed by the presence of highly intense peaks. On the other hand, low signal intensities can have an adverse impact on overall method precision, so this was investigated in more detail. Figure 18A shows example results for investigation of method precision using $n = 7$ independent preparations of same pooled plasma samples using each of the five specified methods. Method precision of SPME was found to be better than or comparable to conventional methodologies with a large proportion (80−92%) of metabolites showing RSD values below 30%, which is considered acceptable for global metabolomics studies.

Subsequently, the developed methodology was applied for *in vivo* mouse studies, and the above findings were further verified including metabolite coverage and method precision using short 2-min sampling times.[71] Figure 18B shows method precision for a set of identified endogenous metabolites including five independent consecutive samplings of the same animal using individual SPME probes (intra-animal variation), as well as the results for the sampling of eight different animals on the same day (interanimal variation). From this figure, it is clear that reproducibility of the SPME technique is good and that the normal biological variation can easily be detected even in this small subset of metabolites. Most importantly, *in vivo* results show additional advantages of *in vivo* SPME for metabolomics studies. Namely, *in vivo* SPME was able to capture metabolites that were missed using conventional methodologies based on blood withdrawal. In fact, >100 unique compounds were observed after *in vivo* SPME and not observed by any other method (blood withdrawal followed by *ex vivo* SPME, ultrafiltration, or solvent precipitation). Conversely, changes in concentration of some of metabolites and appearance of metabolites not detected by *in vivo* SPME show that methods based on blood withdrawal cannot ensure that the metabolome at the time of analysis is truly representative of the metabolome at the time of sampling due to incomplete inhibition of enzymatic activity by currently accepted and widely used methodologies. For example, SPME was able to capture $\beta$-NAD, which could not be detected by any of the other methods, and efforts are currently underway in our laboratory to further characterize the other species uniquely detected using *in vivo* SPME. These results show that SPME can successfully capture unstable or short-lived metabolites, thus making it a new and important tool in life science research.

In above proof-of-concept study, the effect of administration of carbamazepine was studied ($n = 4$ mice).[71] Principal component analysis of the resulting data set showed that the largest portion of variance in the data set was attributed to the variations between different animals, while the effect of the administration of the xenobiotic could be examined with the inclusion of third principal component (Figure 19). The high degree of animal variability (also termed biochemical individuality[291]) puts into question studies where different animals are used in control versus treatment groups, a situation that is fairly common for small rodent studies due to limited availability of biological fluids. In such experimental designs, the differences found between control and treatment groups may not be related to the question under study. In fact, in the same study, blood samples were collected after cardiac puncture and we could not accurately differentiate dosed versus control animals, thus not permitting reliable investigation of the influence of carbamazepine dosing when small animal cohorts are used ($n = 4$) and pre- and postdose samples from the same animal are unavailable. In contrast as shown in Figure 19, SPME permits sampling of the same animals before and after dosing thus simplifying data

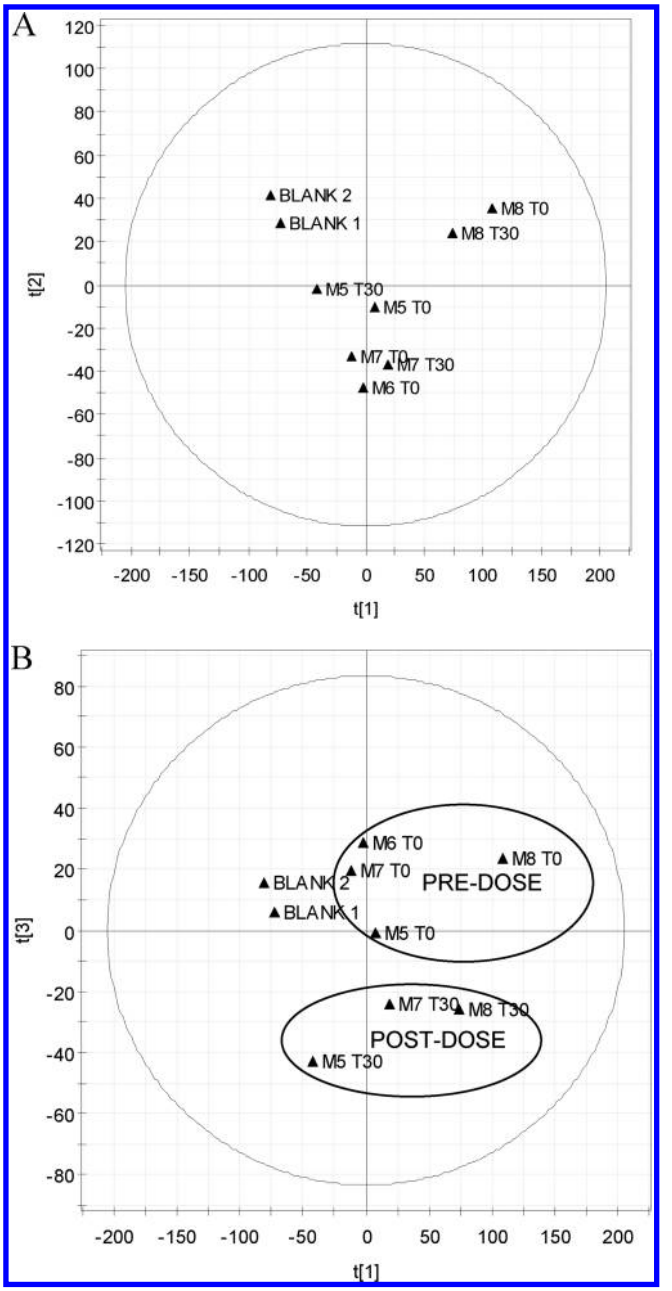


**Figure 19.** Global metabolomics study of carbamazepine administration to mice ($n = 4$) using *in vivo* SPME.[71] (A) 2D scores plot obtained for *in vivo* SPME sampling of mice (M5, M6, M7, M8) prior to (T0) and 30-min (T30) postdose showing PC1 versus PC2. (B) 2D scores plot of the same data set showing PC1 versus PC3. Blank injections are shown, while QC injections are omitted for clarity in all plots.

interpretation and removing the confounding influence of the use of different animals in treatment versus control groups. *In vivo* SPME also opens up new possibilities for study of biochemical individuality, because same animal can be sampled repeatedly and over long period of time to monitor various biological processes of interest. This means that temporal variations of various metabolites can be monitored (for example, diurnal, disease, or age-related) and differences between individuals can also be monitored. Figure 18B shows that the variability of metabolite concentrations is significantly lower for repeated samplings of the same animal versus biological variability of

**Table 4. Applications of *in Vivo* SPME in Animal Studies**

| analytes | samples | device | extraction mode/time/calibration | refs |
|---|---|---|---|---|
| pharmaceuticals | blood (dog vein) | custom-made PEG-C18 | direct/2 min/kinetic | 52,292 |
| pharmaceuticals | blood (carotid artery of rat) | custom-made PPY | direct/40 s/external, kinetic, and double extraction | 70 |
| pharmaceuticals | fish muscle | custom-made PDMS | direct/20 min/kinetic | 66 |
| toluene | mice brain | 85 μm PDMS/DVB/75 μm CAR/DVB | direct/2 min/converting by *in vitro* experiment | 288,289 |
| VOCs and odors | cattle rumen | 85 μm CAR-PDMS | HS/5 min/identification | 285 |
| pharmaceuticals | blood (dog vein) | custom-made PPY-C18, PEG-C18 | direct/30 s/external, kinetic | 142 |
| diazepam | blood (dog vein) | custom-made PEG-C18 | direct/5 and 2 min/equilibrium, kinetics | 68 |
| VOCs | bovine breath | 50/30 μm DVB/PDMS | HS/15 min/identification | 284 |
| VOCs | exhaled breath of rats | 100 μm PDMS, 65 μm PDMS/DVB, 75 μm PDMS/CAR | HS/2 h/identification | 293 |
| pharmaceuticals | blood (carotid artery of mouse) | biocompatible 45 μm C18 | direct/2 min/kinetic | 182 |

different animals, and this type of information can be easily captured by SPME. Reduced animal use is also important for studies utilizing precious, rare, and expensive animals such as genetic knockout mice, because repeated and multiple compartment sampling of the same animal can provide a wealth of high-quality information while lowering the overall experimental costs.

Table 4 presents some applications of *in vivo* SPME for animal studies.

### 4.6. Humans

For human studies, most applications of *in vivo* SPME to date mainly focused on VOCs from breath and skin, although *in vivo* SPME for blood analysis in a simulation system was also reported.[294] In this study, in-vein SPME was performed in an artificial vein system, which was built up from heart and lung machine components. Blood was provided from healthy volunteers. Determination of linezolid (0−15 μg/mL) was performed by SPME from the flowing system. The study showed that the amount of drug extracted by SPME did not depend on blood flow velocities when equilibrium extraction was used; therefore in-vein SPME could be employed for drug extraction using conventional venous access, which can be obtained easily in any patient. In principle, microdialysis could be used in a similar way as in-vein SPME. However, microdialysis requires venous access through appropriate catheters and specialized equipment making it more invasive than in-vein SPME, and extraction of antibiotic or antimycotic agents from blood does not work with standard MD equipment.[294] In-vein SPME has the potential to minimize blood requirements for diagnostic purposes and to speed up clinical drug analysis, if interfiber variation can be reduced.[294]

As a potential noninvasive diagnostic method, breath analysis has attracted much scientific clinical interest in the past years. Despite its advantages for routine biological monitoring, it has not become widely accepted as a tool in medical diagnostics or occupational hygiene. Suitable sampling methods and measurement techniques are the bottleneck.[295] SPME has been successfully employed for analysis of biomarkers in human breath. Volatile metabolites of *Mycobaterium tuberculosis* were monitored by a SPME-GC-MS method. By converting the free acid into methyl nicotinate, the detected methyl nicotinate is statistically significantly different in the breath of smear-positive patients compared with healthy subjects.[296] SPME was also found to be a fast and reliable enrichment method for the analysis of isoprene,[297] sulfur-containing compounds ($H_2S$, MeSH, EtSH, COS, and $CS_2$),[298] fentanyl,[299] propofol,[300] 2-pentyfuran,[301] and aldehydes[302] in human breath. Special devices were designed for the determination of VOCs in breath samples and end-exhaled breath samples with SPME.[303,304] A modified SPME device was applied to the quantitative determination of ethanol, acetone, and isoprene in human breath (Figure 20).[305] The calibration curves for the compounds are reproducible and linear over the concentration ranges found in human breath samples. The method is capable of detecting concentrations of acetone and isoprene reported for healthy subjects. The device is portable, economical, and easy to use in patient sampling.

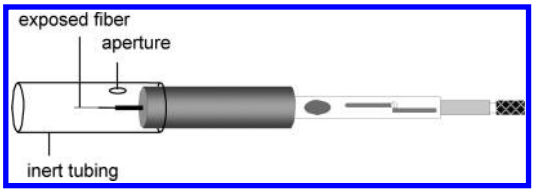


**Figure 20.** SPME device modified for breath analysis. Reprinted with permission from ref 305. Copyright 1997 American Chemical Society.

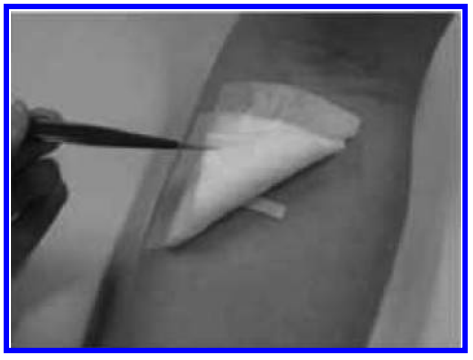

**Figure 21.** Sampling of human skin using thin PDMS film. Reprinted with permission from ref 82. Copyright 2008 Royal Society of Chemistry.

Lung cancer is a frequent cause of cancer-related deaths in the world. Conventional diagnostic methods for lung cancer are unsuitable for widespread screening due to expense and occasionally missed tumors.[306] Different methods have been developed to analyze VOCs in breath and to compare them in healthy subjects and lung cancer patients.[307] SPME-GC-MS was


dx.doi.org/10.1021/cr100203t |*Chem. Rev.* 2011, 111, 2784–2814

Table 5. Applications of *in Vivo* SPME in Human Studies

| analytes | samples | device | extraction mode/time/calibration | ref |
|---|---|---|---|---|
| VOCs | skin | PDMS membrane | direct/5−30 min/qualitative | 82 |
| isoprene | breath | 75 μm CAR/PDMS | HS/10 min/extenal | 297 |
| VOCs | breath | 65 μm PDMS/DVB | HS/10 min/identification and comparison | 303 |
| VOCs | end-exhaled breath | 100 μm PDMS | HS/1 min/external | 304 |
| ethanol, acetone, isoprene | breath | 65 μm PDMS/DVB | HS/1 min/external | 305 |
| VOCs | arm skin | 65 μm PDMS/DVB | HS/30 min/identification | 310 |
| 3-methybutanal | axillary odor | 50/30 μm DVB/CAR/PDMS | HS/50 min/qualitative | 312 |
| VOCs | skin | 50/30 μm DVB/CAR/PDMS | HS/30 min/identification | 313 |
| benzene | breath | 50/30 μm DVB/CAR/PDMS | HS/30 s/external | 317 |
| aroma release during eating cheese | breath | 100 μm PDMS | HS/60 s/identification and comparison | 318 |

employed for the identification of VOCs that represent lung cancer biomarkers.[306] SPME was also used for the determination of VOCs in the exhaled breath of a patient with lung cancer compared with those of healthy volunteers.[308,309] Some components could be found in the breath of lung cancer patients but were rarely detected in breath samples from healthy persons, although further follow-up large-scale confirmation studies are required to verify the utility of the proposed biomarkers. Studies comparing SPME-GC-MS with proton transfer reaction mass spectrometry (PTR-MS) showed that SPME-GC-MS had poor sensitivity for some compounds while PTR-MS could not identify compounds with certainty.[310,311]

Human skin emits a variety of volatile metabolites. SPME-GC-MS was used for the analysis of VOCs from human skin. By headspace sampling with a SPME fiber in a glass vial kept under the armpit, 3-methylbutanal was indentified in human axillary odor and the abundance of this compound varies significantly among individuals.[312] Skin VOCs from upper back and forearm and their changes due to aging were reported by a SPME-GC-MS method.[313] An active sampling device was designed for SPME sampling of VOCs from human arm skin, and 35 compounds were identified.[314]

A thermally desorbed PDMS membrane approach with analysis by gas chromatography-mass spectrometry was developed to sample VOCs arising in and on skin (Figure 21).[82] The membrane is 20 mm ×15 mm ×0.45 mm. The higher surface area and volume of the sampler increased the sensitivity of the method. An alternative method using commercial SBSE (Twister) devices and a special roller has also been described for large-scale metabolomics studies of human sweat.[315,316] These examples illustrate an important advantage of microextraction methods, which is the flexibility of device geometry. The most appropriate configuration can be designed and selected depending on the exact application requirements.

Table 5 presents some applications of *in vivo* SPME for human studies.

## 5. CONCLUSIONS

As a simple, miniaturized, fast, and environmentally friendly sampling and sample preparation technique, SPME approaches have been widely used for invasive and noninvasive *in vivo* studies. The target analytes investigated to date include environmental pollutants, pharmaceuticals, pheromones, metabolites, and proteins and show the versatility and capability of this technique. Future feasibility and applicability of *in vivo* SPME techniques depends on the development and commercialization of devices and extraction phases suitable for different types of applications. Proper design of SPME devices and accessorial systems can make the techniques more convenient and more applicable. The development of extraction phases can improve the sensitivity, selectivity, and biocompatibility of *in vivo* SPME techniques, and this aspect of technology is highly correlated to the development of material science. The development of SPME calibration methods in recent years has enabled accurate quantification even in situations when equilibrium cannot be reached or experimental conditions cannot be controlled or replicated for calibration purposes. Very recent research presented herein supports the use of solid-phase microextraction as an effective sample preparation method for global metabolomic studies of biofluids. In the future, this metabolomics workflow can also be extended to *in vivo* SPME sampling of tissues. Tissue metabolomics is particularly interesting for the study of damaged tissues such as tumors in search of novel biomarkers because the concentration of such biomarkers is expected to be higher in such tissue than in surrounding areas.[319] For this type of study, *in vivo* SPME presents a less-invasive sampling method over traditional methods, which require invasive biopsy followed by solvent extraction. SPME also provides improved spatial resolution sampling, which is important when dealing with such heterogeneous specimens, and spatially resolved fibers can play an important role in this type of application. Other interesting areas for exploration include single cell studies pending further miniaturization of SPME devices or the use of special coatings to trap known reactive metabolites and intermediates (for example, using glutathione or methoxylamine trapping agents[320,321]). The availability of *in vivo* SPME to simultaneously sample various compartments of freely moving animals (for example, blood, bile, and tissue such as liver, muscle, and adipose tissue) opens up new possibilities to investigate the fate of xenobiotics and metabolites in living systems. Such integrative multicompartmental studies are particularly important when studying fate, toxicity, and distribution of xenobiotics. The availability of *in vivo* SPME also facilitates long-term longitudinal studies particularly important for medical research (understanding the mechanism and progression of disease), because no sacrifice of animals is required. It can be expected that the impact of *in vivo* SPME will continuously increase in the future, and the technique will play an especially important role to reduce animal use and replace conventional methods requiring sacrifice of the animals under study. SPME techniques are particularly powerful in combination with portable separation/quatitation devices allowing completion of the whole analytical process on-site, where the sample is located. The recent trends in miniaturization and micromachining will facilitate the practical realization of this concept. Development of robust *in vivo* SPME devices

combined with on-site instrumentation will eventually lead to human application because no blood or tissue withdrawal is necessary, particularly for victims of accidents and critically injured persons, where time and repeated sampling is of the essence.

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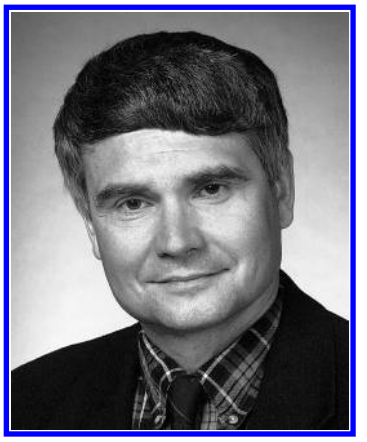
Dajana Vuckovic obtained her Hon. B.Sc. degree in Analytical Chemistry from the University of Toronto, Canada, in 2002. She spent three years as an R&D Chemist in pharmaceutical industry, and then completed Ph.D. studies in Analytical Chemistry at the University of Waterloo, Canada, in 2010 under the supervision of Dr. Pawliszyn. Her current research interests include the development of *in vivo* SPME for metabolomics as well as the development of high-throughput mass-spectrometry-based workflows for biomarker discovery, chemical proteomics, and bioanalysis. She is the recipient of several awards including Ontario Graduate Scholarship, NSERC Postdoctoral Fellowship, Johnson & Johnson Young Scientist Award and 2010 Douglas E. Ryan Graduate Student Award by Canadian Society for Chemistry.



Janusz Pawliszyn is a professor of analytical chemistry at the Department of Chemistry, University of Waterloo, Canada. He is an author of over 400 scientific publications and several books on SPME. He is a member of the Royal Society of Canada, an Editor of *Analytica Chimica Acta* and *Trends in Analytical Chemistry*, and a member of the Editorial Board of *Journal of Separation Science*. He received the 1995 McBryde Medal, the 1996 Tswett Medal, the 1996 Hyphenated Techniques in Chromatography Award, the 1996 Caledon Award, the Jubilee Medal 1998 from the Chromatographic Society, U.K., the 2000 Maxxam Award from Canadian Society for Chemistry, the 2000 Varian Lecture Award from Carleton University, the Alumni Achievement Award for 2000 from Southern Illinois University, the Humboldt Research Award for 2001, and 2002 COLACRO Medal; in 2006 he was elected to the most cited chemists by ISI, and in 2008 he received the A.A. Benedetti-Pichler Award from Eastern Analytical Symposium, the Andrzej Waksmundzki Medal from Polish Academy of Sciences, and the Manning Principal Award, in 2010 the Torbern Bergman Medal from the Swedish Chemical Society, the Ontario Premier's Innovation Award, the Marcel Golay Award, and the ACS Award in Separation Science and Technology, and in 2011 the PittCon Dal Nogare Award. He presently holds the Canada Research Chair and Natural Sciences and Engineering Research Council of Canada Industrial Research Chair in New Analytical Methods and Technologies. Currently his research focuses on simplification of on-site sampling and elimination of organic solvents from the sample preparation step to facilitate convenient on-site and *in vivo* monitoring and analysis, as well as whole column imaging detection in microseparation methods.

## ACKNOWLEDGMENT

This work was supported by the Natural Sciences and Engineering Research Council of Canada, Supelco, the Canada

Research Chairs Program, and National Natural Science Foundation of China (20877103, 21077135) and the Fundamental Research Funds for the Central Universities.

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