In Vivo SPME for Bioanalysis in Environmental Monitoring and Toxicology

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Abstract

Solid-phase microextraction (SPME) is a well-established sample preparation technique in the field of environmental and toxicological studies. The application of SPME has extended from the headspace extraction of volatile compounds to the capturing of short-lived and unstable components of the ecosystem extracted from the living organism via direct immersion of SPME probes into the tissue (in vivo SPME). The development of biocompatible coatings and availability of different calibration approaches enables in vivo sampling of exogenous and endogenous compounds from the living plants and animals without the need for tissue collection. In addition, new geometry designs such as thin-film coatings, needle trap devices, recession needles, coated tips or blades has increased the sensitivity and robustness of in vivo sampling. Here, we present the fundamentals of in vivo SPME technique, including the types of extraction mode, geometry design of the coatings, calibration methods and data analysis methods used in untargeted in vivo SPME. We also discuss recent applications of in vivo SPME in environmental studies and in the analysis of pollutants in plant and animal tissues in addition to in vivo human saliva, breath and skin analysis. In summary, in vivo SPME technique shows great potential for both targeted and untargeted screening of small molecules in the living organisms exposed to the surrounding environment.

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G. B. Jiang and X. D. Li (eds.), *A New Paradigm for Environmental Chemistry and Toxicology*, https://doi.org/10.1007/978-981-13-9447-8_3

Keywords

SPME • In vivo sampling • Environment • Toxicology • Contaminants • Calibration • Bioanalysis

1 Introduction

Solid-phase microextraction (SPME) was first introduced in 1989 as a novel sample preparation technique for environmental analysis. Since then, SPME has received particular attention as breakthrough "green" technology—not only in the field of environmental and toxicological studies but also in food analysis and biomedical research (Arthur and Pawliszyn 1990; Bojko and Pawliszyn 2014; Souza-Silva et al. 2015). One of the principal features that significantly distinguishes SPME from other techniques is that SPME integrates sampling, sample clean-up, and analyte pre-concentration into a single step, a feat which cannot be achieved with the use of traditional sample preparation protocols. This unique integrative feature of SPME not only facilitates fast, simple, and efficient extraction of analytes of interest from a variety of matrices, its superior clean-up enables easy coupling of SPME to different instrumental methods such as GC-MS and LC-MS, among others. Furthermore, new advances in SPME have enabled the direct coupling of SPME to MS for measurements of extracted compounds, an attractive option that shortens total analysis times while reducing errors related to sample handling (Reyes-Garcés et al. 2018).

While SPME has gained considerable attraction in environmental studies as a leading method for analysis of volatile organic compounds (VOCs), the applicability of the technique has broadened over the past few years to allow for the analysis of a wide variety of environmental pollutants, including pharmaceutical and personal care products (PPCPs), pesticides, and metal–organic compounds, as well as the analysis of endogenous compounds of plants and

animals exposed to contaminants (Llompart et al. 2019; Wang et al. 2009; Zhang et al. 2018). SPME has also been optimized for several on-site applications, such as analysis of toxins present in inanimate and animate components of the environment and evaluation studies concerning the impact of emitted pollutants on the functioning of ecosystems.

Owing to its advantageous in vivo capabilities, SPME has also been largely applied in environmental in vivo studies, where non-lethal extraction of small molecules can be performed on living organisms (Ouyang et al. 2011b; Vuckovic et al. 2011). This feature of SPME facilitates monitoring of the fate of pollutants in living organisms and allows for investigations concerning the distribution and accumulation of pollutants within an individual organism as a response to the exposome. In vivo sampling via SPME additionally enables the capture of short-lived and unstable metabolites by stabilizing highly reactive small molecules of endogenous and exogenous origin, thus preventing their degradation during sample handling and storage. Recently, SPME technique has been also applied for direct measurements of free drug concentration in solid tissue by a series of experiments performed in the laboratory and also in silico by using a mathematical model developed in COMSOL Multiphysics (Huq et al. 2019). The applied strategy facilitated calculations of local depletion of the analyte by a coating and also the mass transfer kinetics of SPME coating. The extraction by SPME coating did not affect the free concentration of the drug in solid tissue as the depletion of drug concentration surrounding the fiber was negligible, therefore the measurements of analyte distribution with the use of SPME technique are feasible. Therefore, in vivo SPME does not disturb homeostasis within investigated systems, as only a small portion of metabolites is extracted from the system under study (negligible depletion), thus enabling repeated SPME sampling of the same tissue or organ in individual organisms (Reyes-Garcés et al. 2018). More importantly, the capture of low-molecular-weight compounds via in vivo SPME may provide additional information concerning metabolic changes in exposome-wide association studies (EWAS) and also in a toxicological analysis. Important compounds derived from natural or anthropogenic sources of contamination, such as biomarkers of exposure, or toxicants and their metabolites, could be extracted with the use of this technology.

This chapter begins with an overview of the fundamentals of in vivo SPME and related techniques. We next discuss the most important topics regarding in vivo SPME: development of in vivo SPME devices, calibration methods, and data analysis for in vivo SPME sampling. A review of the main applications of this technique in environmental monitoring and toxicology studies of plant, animal, and human systems is then presented. This chapter closes with future perspectives of SPME and its potential in environmental studies.

2 Fundamentals of in Vivo SPME

2.1 In Vivo SPME and Related Techniques

SPME can be performed in headspace (HS) or direct immersion (DI) mode for extraction of analytes from a variety of matrices. Extraction is carried out for a predetermined period of time with the use of an SPME device (e.g., blades, fibers) coated with an extraction phase (Godage and Gionfriddo 2019; Pawliszyn 2012; Vuckovic 2013). Application of HS-SPME and DI-SPME in environmental and toxicological studies has been reported for analysis of different elements (living and inanimate) of the ecosystem, including water, soil, plants, and animals. Following extraction, the SPME device can be directly introduced to GC for thermal desorption or desorbed using an optimized solvent that is subsequently injected into LC–MS, or directly coupled to MS.

The availability of different SPME devices and their biocompatibility make SPME technology a convenient tool for in vivo analysis, with minimum invasiveness to living organisms (Vuckovic et al. 2010). The availability of different calibration approaches for quantitative analysis enables optimal extraction and analysis of a broad range of analytes with different polarities from environmental and biological samples. Given that a derivatization step is necessary for the analysis of nonvolatile pollutants, an on-fiber derivatization technique, developed to combine the derivatization reaction and extraction steps during SPME, is also available for such applications (Martos and Pawliszyn 1998). In-tube SPME, on the other hand, enables direct analysis of nonvolatile compounds in aqueous matrices, and has been applied in the targeted analysis of environmental pollutants (Moliner-Martinez et al. 2015).

SPME can also be optimized for in vivo sampling via coating optimization. A matrix-compatible, PDMS-overcoated SPME fiber can be directly used in living systems for in vivo sampling, whereas coatings with HLB particles can extend the range of extracted compounds, making such coatings ideal for comprehensive or untargeted analyses of potential pollutants (Gionfriddo et al. 2017; Godage and Gionfriddo 2019). The constant development of SPME coatings aims to both improve coverage of compounds and minimize the invasiveness of the procedure on sampled animals during in vivo sampling.

The free concentration of a given pollutant in a living system can reflect in vivo exposure levels, while its total

concentration might reflect the long-term risk of the pollutant with respect to different living systems. While SPME fibers can directly measure free concentrations via non-exhaustive extraction, attainment of both the free concentration and total concentration of a given pollutant would allow for a more comprehensive and dynamic investigation of their environmental behavior and risk (Boyacı et al. 2018). In such cases, needle trap devices (NTD), which allow for exhaustive extraction, can be used to this end as passive sampling devices in air pollution monitoring (Lord et al. 2010). The NTD approach uses small needles containing a packed sorbent bed to briefly trap both fluid-borne analytes and particles. Similar to the SPME fiber, NTD can be directly coupled to a GC injection system to release the adsorbed analytes. An analytical approach exploiting both NTD and SPME fiber can thus enable parallel measurements of free concentrations and total concentrations of pollutants in air, enabling a more comprehensive evaluation of risk for living systems with respect to pollutants found in the environment (Niri et al. 2009).

Thin-film SPME (TFME) was developed in recent years for applications that demand higher extraction efficiency and sensitivity (Jiang and Pawliszyn 2012). TFME employs a larger surface area to extraction phase volume ratio so as to increase surface contact with the sample. Different TFME membranes are available for coupling with GC and LC instrumentation. Depending on the aim of research and the specific group(s) of compounds under study, analysts may choose to employ either thermally stable TFME membranes for gas chromatography or solvent-stable TFME membranes for liquid chromatography. The cold fiber technique presents yet another approach to increasing method sensitivity (Menezes et al. 2013). Briefly, the technique works by introducing cold air into a needle-based SPME apparatus. This causes a temperature difference between the extraction phase and sample, which enables faster and higher extraction of compounds into the cooling SPME fiber. The cold fiber technique has been applied in soil and sediment analyses as a way to enhance method sensitivity for volatile compounds (Martendal and Carasek 2011; Ghiasvand et al. 2006). These two methods, TFME and cold fiber SPME, can also be combined to further enhance method sensitivity for specific applications, such as to measure fragrance compounds in air (Jiang and Pawliszyn 2014).

Other geometries of SPME are also available for specific in vivo environmental analysis purposes. For instance, recession needles protect the extraction phase from mechanical damage due to the presence of a recession notch in the needles where the coating is housed (Poole et al. 2017). This research has been used in the untargeted analysis for living fish. Coated tips and mini tips, on the other hand, can be employed for extraction from small sample amounts (<10 uL) such as blood from mice (Piri-Moghadam et al. 2016; Vasiljevic et al. 2019). In addition to the advantages described above, such technologies also offer direct coupling with mass spectrometry for high-throughput analysis, and can be applied for nontarget analysis, features that are highly beneficial in exposome studies (Gómez-Ríos et al. 2018; Augusto Gomez-Rios et al. 2017). Prior to in vivo sampling, simulation-based experiments can be carried out to optimize the geometry of the device in view of the intended purpose of the research and the target sample (Alam et al. 2015).

2.2 Calibration Approaches

SPME is a non-exhaustive extraction method in which only a free fraction of the analyte is extracted from the sample matrix. Such a feature is particularly important in environment-wide association studies (EWAS) and toxicological studies, given that it is the unbound fraction of the toxicant that determines its activity in living systems. Quantitative analysis of targeted compounds is carried out by first determining the relationship between extracted amounts by the SPME coating and the analyte concentration in the biomatrix. To this end, various calibration methods have been developed to quantify concentrations of target analytes in biological samples (Ouyang and Pawliszyn 2008). In the equilibrium calibration method, the analyte in the extraction phase equilibrates with that in the sample matrix, and the extracted amount in the extraction phase can be expressed as

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

where n_e is the amount extracted, C_0 is the initial concentration of the target analyte in the sample, V_s is the sample volume, V_f is the volume of extraction phase, and K_{es} is the distribution coefficient of the analyte between the extraction phase and the sample matrix. However, in most in vivo SPME applications, the volume of the sample matrix is very large ($V_s \gg K_{fs}V_f$), and Eq. (1) can be rewritten to the following equation:

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

In addition, under equilibrium conditions, calibration is independent of hydrodynamic variables, such as blood flow in a living system. However, due to the extended amount of time required for some compounds to equilibrate with the fiber, other calibration methods are often preferred for field and on-site SPME analysis applications, namely on-fiber kinetic calibration and sampling rate calibration (Bai et al. 2013). The kinetic calibration model has been used in several animal studies, and is based on the preloading of the fiber with a deuterated analog, which after introduction to a

biological matrix is desorbed from the fiber while the analytes from the sample matrix are extracted. The free concentration of the analyte is then calculated based on the isotropy of desorption of the deuterated analog from the extraction phase and the simultaneous extraction of the analyte from the sample. Use of this calibration method improves the accuracy and precision of analysis while also accounting for the influence of certain environmental factors, such as temperature, on extracted amounts. However, one of the main limitations of this calibration approach is that it is not suitable for some in vivo studies, e.g., human studies, since the introduction of exogenous substances to the living system is forbidden. In such cases, use of pre-equilibrium extraction approach may be considered for in vivo studies. In this calibration method, the linear regime of the SPME extraction process is required and the rate of mass transfer (sampling rate) must remain constant throughout the duration of sampling. Several factors, including sample matrix and the type of SPME coating employed, influence the ratio between the concentration of target analyte in the sample matrix and the extracted amount of analyte. Use of this diffusion-based calibration method allows for much shorter extraction times in comparison to the equilibrium extraction approach. This method has been employed in in vivo SPME fish studies; briefly, sampling rates were determined in laboratory conditions, then used to determine concentrations of analytes on-site (Ouyang et al. 2011a). The main advantage of this calibration approach is the elimination of standard addition or preloading of the SPME fiber. However, several factors in the analyzed matrix, such as blood flow and fluid content, may affect the amount of extracted analyte.

2.3 Data Analysis for in Vivo SPME

A major hindrance in SPME-based untargeted analysis is the annotation of obtained data (Domingo-Almenara et al. 2018). Since SPME can be performed on-site and in vivo, the scope of extracted and analyzed chemicals is beyond our current knowledge. This is called the "unknown unknown" paradox for research performed at the metabolites level: we want to identify unknown analytes as "markers", but since unknown compounds are annotated in a database, we have no prior knowledge of these unknown compounds to identify them. Thus, we become restricted to identifying "known" compounds. For exposome studies, the major restriction in data interpretation is that although we already have databases with thousands of small molecules, we still don't know whether there are other short-life compounds or trace metabolites totally ignored by the current knowledge scope, especially exogenous compounds (Vuckovic et al. 2011).

To address this issue, reaction/structure directed analysis was developed for SPME-based analysis (Yu et al. 2019). Instead of using inductive rules and statistical properties from known compounds, statistical properties of peaks from real samples are considered for data mining. The qualitative information obtained from high-resolution mass spectrometry is the accurate mass-to-charge ratio (*m/z*) of compounds. However, multiple compounds could share the same mass-to-charge ratios, while a single compound could also generate multiple mass-to-charge ratios such as adducts, neutral loss, or isotopologues. In this case, the single mass-to-charge ratio is not a very useful parameter for the annotation step. However, distances between mass-to-charge ratios could indicate certain types of reactions or structures; such an approach is called paired-mass distance (PMD) analysis. For instance, a PMD of 15.99 Da is always associated with an oxidation process. A randomly generated dataset would not show a PMD of 15.99 Da, while in real samples, a PMD of 15.99 Da will likely always appear with high frequency. In addition, PMDs can also reveal structure information for particular compounds. For instance, a PMD of 14.02 Da is associated with a-CH2-bond, while the PMD 42.01 Da is associated with a peptide bond. In this case, the use of local statistical properties (frequency of PMDs) to screen reaction level bio-information is preferred over trying to annotate each unknown compound. By quantitively checking structure or reaction level changes from in vivo SPME sampling, we may capture the changes of short live metabolites' profile.

3 Application of in Vivo SPME

Several organic compounds, such as PAHs, pesticides, PPCPs, and inorganic compounds (such as heavy metals) are constantly introduced to the ecosystem via a number of routes. Contaminants circulating in water and/or residing in sediments and soil eventually enter living organisms, where they affect the functioning of these biological systems (Miller et al. 2018). Even at very low concentrations, the constant persistence of such compounds in living organisms can exert adverse effects by acting on specific cellular processes at the genome, proteome, and metabolome levels. Due to their potential toxic, carcinogenic, and mutagenic activities, such chemicals have been labeled as emerging contaminants, indicating that their levels and distribution in the environment should be tightly controlled. Therefore, methods that will facilitate fast and efficient extraction and analysis of these environmental toxicants are highly desired.

Different analytical methodologies have been developed to investigate and determine trace levels of environmental toxins in animals and plants' tissues. Typical sample preparation protocols include tissue homogenization followed by extraction of analytes with the use of organic solvents, whereupon the sample extract is cleaned-up prior

to instrumental analysis. In order to reduce the number of sampling/sample preparation steps, and so as to avoid sample collection that necessitates the sacrifice of living organisms, in vivo SPME has been introduced as a promising tool for non-lethal sampling of organic contaminants on-site. As already mentioned, in vivo SPME offers several advantages over conventional sample preparation protocols for extraction of analytes from complex matrices in field studies. Moreover, SPME can be directly coupled to GC-MS or LC-MS to provide close to real-time information about the types of toxicants present in living organisms as well as their levels in the analyzed matrices (Fig. 1). Given the advantages offered by in vivo SPME, application of this technique in the analysis of environmental contaminants in living plants and animals has been frequently reported (Xu et al. 2016; Zhang et al. 2016).

3.1 In Vivo SPME in Plant Analysis

Given that plants are important components of the food chain for many species, there is growing concern regarding the exposure of plants to contaminants present in the surrounding environment, including water, soil, and air. Once introduced to the plant body, toxins are distributed and reside in different parts of plants. Once plants are consumed, such toxins can also be forwarded to other hosts within the food chain, and affect the health status of those organisms. Over the past years, multiple classes of emerging contaminants, including PAHs and PPCPs, have been detected in edible parts of plants from contaminated regions. However, extraction and quantitation of toxins in plants remain a challenging task due to the very complex nature of this matrix. Therefore, analytical methodologies that can track tissue distribution of contaminants in plants, such as SPME, are gaining increasing attention in food safety and environmental studies (Musteata et al. 2016). For instance, in vivo SPME was employed to monitor the fate of environmental contaminants such as chlorinated VOCs in living plants (Zhu et al. 2013). The optimized procedure facilitated extraction of methyl tert-butyl ether (MTBE), a component of a fuel, and prevented loss of this volatile compound, a loss previously observed during the traditional sample pre-treatment procedure (Reiche et al. 2013). The optimized technique facilitated monitoring of the level of MTBA present in reeds as a function of the season and water concentration levels. The accumulation and distribution of organochloride pesticides (OCPs) and organophosphorus pesticides (OPPs) via in vivo SPME were also investigated (Qiu et al. 2016). Custom-made PDMS fibers were introduced to different organs of living Malabar spinach plants, and concentrations of analyzed pesticides were calculated using the sampling rate calibration approach. Several factors, including the distribution concentration factor (DCF), have been used to assess the translocation and accumulation of toxins in different organs in order to improve our understanding of

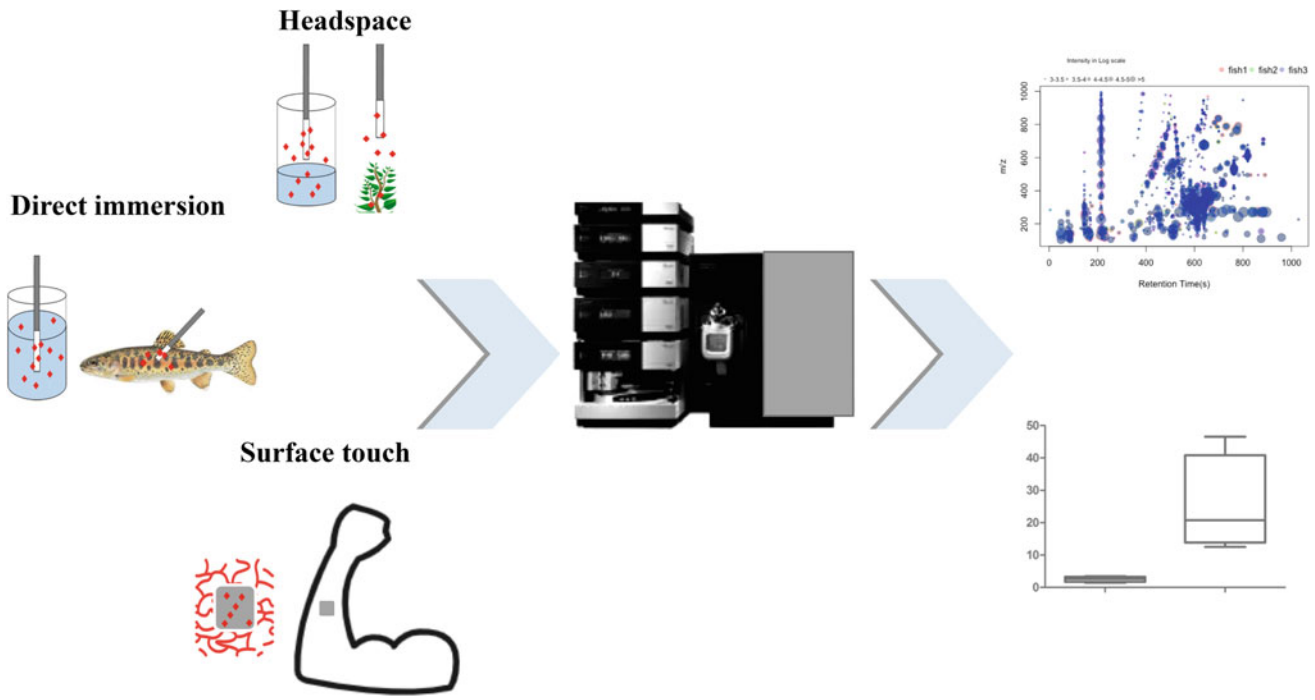

Fig. 1 Application of SPME sampling along with the instrumental analysis and targeted/untargeted screening of metabolites in plant, animal and human studies

contaminant behavior in living plants. In addition, in vivo SPME was also applied for the analysis of metabolome profile and for detection of alterations in metabolite composition in fruits during maturation on the tree (Risticevic et al., under review). Several classes of esters were upregulated in very mature 'Honeycrisp' apples, where the level of estragole was increasing with fruit ripening. Moreover, novel bioactive molecules, namely Amaryllidaceae alkaloids were extracted and successfully detected in the analyzed apples by two-dimensional gas chromatography–time-of-flight mass spectrometry (GCxGC-ToFMS). The applied in vivo SPME protocol facilitated a metabolite quenching and extraction of unique fruit components at different maturity stages. These were not previously reported in apples, possibly due to the use of sample preparation approaches that could disturb real metabolite composition in fruits via induction of enzymatic degradation and oxidation processes.

3.2 In Vivo SPME in Animal Studies

SPME has been considered an easy and efficient tool for in vivo analysis of contaminants present in the tissues and organs of animals exposed to environmental toxins. The ability to monitor the distribution of trace contaminants in living organisms is a significant advantage of SPME over conventional techniques, where complex sample preparation prior to instrumental analysis may result in a loss of compounds, especially those characterized by a volatile and/or highly reactive nature. One of the first reports describing the application of in vivo SPME in toxicological analysis in animal species detailed a pharmacokinetic study of a VOC, toluene, in the hippocampus of mice exposed to this toxin via inhalation (Nakajima et al. 2006). It had been previously observed on animal models that toluene can cause severe or chronic toxicity related to central nervous system disturbances. However, due to the fast vaporization of toluene during the traditional sampling steps, data regarding the exact level and pharmacokinetics of this compound in the brain had been inconsistent. Measurements of levels of toluene via SPME probes directly inserted into the hippocampus of freely moving rats provided reliable information regarding the half-life of this toxin. In vivo SPME followed by GC-MS instrumental analysis revealed that the concentration of toluene in mice brains reached a maximum within 30 min, and decreased rapidly in the next 90 min upon exposure to the VOC. In another study, in vivo SPME sampling was applied for analysis of the effects of intraperitoneally administered toluene on the composition of amino acids in the brain of living mice (Win-Shwe et al. 2007). The attained results helped to shed light into the relationship between high doses of toluene and increasing levels of glutamate and taurine in the hippocampus suggesting that exposure to this toxin leads to the activation of neuroprotective mechanisms in the brain.

Few laboratory and field-based studies have also demonstrated the suitability of SPME technology for the extraction of emerging contaminants present in aquatic organisms. For instance, in vivo SPME has been applied for measurement of a variety of PPCPs in fish muscles. Quantitative analysis of a wide range of pharmaceuticals, including carbamazepine, naproxen, diclofenac, gemfibrozil, bisphenol A, fluoxetine, ibuprofen, and atrazine, was carried out with use of an SPME technique initially optimized in laboratory conditions (Wang et al. 2011). In controlled lab exposures, concentrations of the targeted compounds in fish were found to be related to exposure concentrations in effluents in a dose-dependent manner. Next, the optimized technique was applied to a field study of wild fish exposed to municipal wastewater effluents (MWWE). In vivo SPME allowed for monitoring of dynamic bioaccumulation processes of selected contaminants in the tissues of living organisms. Wild fish sampling via in vivo SPME enabled extraction of a selected group of emerging contaminants of concern from the tissues of several fish species, yielding results comparable to those attained via traditional monitoring methods. Togunde et al. developed an SPME method for in vivo sampling of the dorsal–epaxial muscle of rainbow trout (*Oncorhynchus mykiss*) in an 8-day laboratory exposure to selected pharmaceuticals (carbamazepine, fluoxetine, sertraline, paroxetine, atorvastatin, diclofenac, and venlafaxine). Concentrations of extracted analytes were measured in vivo with custom-made SPME fibers (Togunde et al. 2013). Due to the low invasiveness of the SPME probes used in this study, the optimized method was also applied for an on-site study of wild fish exposed to MWWE. The uptake and bioconcentration of waterborne contaminants, such as pharmaceutical residues in the muscles of Muskellunge (*Esox masquinongy*) were measured as a part of this wild fish study. In another study, Ouyang et al. developed a rapid in vivo SPME approach based on the sampling rate calibration method for both laboratory and field studies (Ouyang et al. 2011a). The SPME probe was inserted in fish dorsal–epaxial muscle for 20 min for extraction of certain pharmaceuticals (atrazine, carbamazepine, and fluoxetine). The method developed in the laboratory also facilitated the detection of trace levels of analytes within muscles in wild fish, yielding analytical results comparable to those of traditional sample preparation techniques involving lethal sampling followed by tissue liquid extraction with the use of organic solvents.

Recently, in vivo SPME was applied in an EWAS for monitoring of toxicants as well as for an assessment of the biochemical response of sixty white suckers (*Catastomus commersonii*) to exposure to potential contaminants

(Roszkowska et al. 2019). SPME probes were placed into dorsal–epaxial muscle for untargeted analysis of contaminants present in tissues of fish collected in the oil sands development region and outside the deposit (pulp and paper mill discharge region). Several organic compounds potentially related to industrial and workplace toxins, such as aliphatic and aromatic hydrocarbons, pesticides and PPCPs, including unstable and highly reactive compounds, were extracted via SPME probes from almost all sampling regions. In addition, this study revealed the presence of petroleum-related compounds in the fish muscle tissue, providing important information regarding the exposome of the organism to the surrounding environment as well as alterations in the biochemical profile of fish muscles caused by such exposures. The applied in vivo SPME technology for wild fish studies presents an interesting alternative to other techniques for biomonitoring studies aiming to track alterations in organisms exposed to environmental contaminants.

3.3 In Vivo SPME in Human Studies

In vivo sampling of different human matrices, including biofluids and solid tissues, can reveal real-time changes in certain metabolic pathways and also the presence of exogenous compounds as a response to environmental exposures (Vereb et al. 2011). SPME and related techniques have been applied in such studies as minimally invasive approaches capable of capturing short-lived species and endogenous compounds. For instance, HS-SPME has been used as a noninvasive diagnostic tool in breath analysis, where a commercially available SPME device was modified for direct, real-time extraction and quantitative determination of ethanol, acetone, and isoprene in human breath (Grote and Pawliszyn 1997). Recently, needle trap device (NTD) technology coupled with thermal-desorption photoionization time-of-flight mass spectrometry (TD-PI-TOFMS) was used during in vivo breath sampling to evaluate human exposure to smoke (Kleeblatt et al. 2015). In this work, several xenobiotic substances (benzene, toluene, styrene, and ethylbenzene) were successfully extracted and identified in the breath of smoking individuals. Another interesting human matrix for application of the in vivo SPME technique is saliva. As a complex biological matrix that can be easily collected in a noninvasive manner, saliva is essentially composed of filtered blood, and is thus potentially able to reflect human real-time exposure conditions to pollutants. In a nontargeted EWAS, a TFME device was immersed into saliva for in vivo monitoring of saliva with the aim of investigating associations between environmental exposure and chronic diseases (Bessonneau et al. 2013). To further increase the sensitivity of the saliva sampling method, TFME was also applied in in vivo saliva extraction by placing the TFME device directly in the mouth of participants for a 5 min sampling period (Bessonneau et al. 2015; Shigeyama et al. 2019). The method was validated for simultaneous quantification of 49 prohibited substances, and can be used in doping tests. This optimized technique facilitated detection of trace levels of endogenous steroid hormones. Skin odor, on the other hand, is capable of revealing environmental exposure conditions as well as alterations in endogenous metabolites; to this end, HS-SPME has been used for in vivo skin analysis of human fragrance profiles (Duffy et al. 2017). In contrast to the SPME fiber, a TFME membrane can be directly applied onto the skin surface, enabling better sensitivity for semi- and low- volatility compounds in in vivo analysis (Jiang et al. 2013). Due to the minimal invasiveness of this technique, its non-depletive extraction mode and biocompatibility, in vivo SPME shows great potential for real-time monitoring of exposure of human tissues and organs to environmental contaminants and other toxins.

4 Conclusions and Future Perspectives

Increasing concern for ecosystem protection continues to extend the application of SPME in in vivo environmental and toxicological studies to the analysis of the accumulation and metabolism of contaminants, as well as to a wide range of exposome studies. SPME technology facilitates tracing of the behavior of certain toxins in living systems while also offering the possibility for monitoring of biochemical responses of organisms to exposure to environmental contaminants. In vivo SPME sampling provides more accurate and reliable information about the distribution of target compounds as it enables minimally invasive, real-time extraction of small molecules from living systems without the need for sample pre-treatment or animal sacrifices. This is especially important for low-abundance populations of animals and plants, for which traditional sampling can be problematic. Development of novel SPME devices and extraction phases as well as direct coupling of SPME probes to MS will extended the application of SPME techniques in EWAS and toxicological analyses; this in turn can provide further insight into the composition of exogenous substances and endogenous profiles of individual organisms. Use of this technique in cause–effect relationship studies of metabolome response of living organisms to exposure to contaminants may allow for the identification of novel biomarkers of the exposome, and provide crucial information necessary for a better understanding of the persistence and effects of pollutants in the environment.

Acknowledgements This monograph was developed within the framework of the Environment Canada through the Environmental Damages Fund (grant EC-129114).

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