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In Vivo Solid–Phase Microextraction: An Efficient Sample Preparation Method for Plant and Tissue Bioanalysis

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Abstract
Generally, traditional bioanalytical methods including in vitro or ex vivo are associated with the limitations and drawbacks in the living systems analysis. However, the in vivo sampling technique is an excellent procedure to improve accuracy and performing the on–line and in–situ biological analyses. In this regard, solid–phase microextraction (SPME) as a simple, sensitive, solventless and noninvasive sample preparation technique has been considered by researchers in in vivo sampling, in recent years. This review briefly describes the use of in vivo SPME as a sample preparation method to study the living systems involving plants and animals (especially metabolomics and clinical researches). Also, biocompatible coatings and design innovations that use to enhance the sensitivity and functioning of the method have been investigated. Finally, the challenges facing the development in vivo SPME method are investigated and forthcoming trends for the better performance of bioanalytical method are offered.

Keywords
Solid–Phase Microextraction; In vivo; Coating; Bioanalysis; Metabolite.

1. INTRODUCTION

Generally, metabolomics demonstrated the inclusive analysis of metabolome (minor metabolites) in a living organism. Metabolomics scientifically studies the biochemical systems which make interior metabolites in a certain tissue. The identification of metabolites in living specimens is important to evaluate clinical diagnostics, illness situations, and behavior outcomes [1]. For instance, it is possible to find out the volatile organic compounds through the skin to follow the disease and its process of change [2].

A wide-ranging of biological samples, like body tissue, biological fluids, and cellular collection can be used for metabolomics studies. Proteins, salts, and other compounds exist in the matrix of biological samples that make them extremely complex matrices. Considering the metabolites have a variety of chemical types, it is vital to utilize a reliable and specific accurate technique to sampling procedure before high throughput analytical tools like liquid and gas chromatography-tandem mass spectrometry. According to these restrictions, the researchers have been offered sample pretreatment methods to investigate the metabolomics in biological samples [3–5]. Overall, in the metabolomics field, the organic solvents used to precipitate proteins to reach a floating sample for continuance analysis, besides to admit the extraction of targeted analytes in a multiphase system [6]. It is a crucial matter to approach the reproducible sample to analyze the method that combines the sampling and extraction performance simultaneously [7, 8]. This ideal procedure gets metabolome's real composition with quenching and pretreatment of the sample in a single step.

Solid-phase microextraction (SPME) is a simple and solvent-free sample pretreatment method introduced by Pawliszyn in 1989 [9]. The fundamentals and noteworthy developments of SPME have been discussed in several published reviews [7, 10–12]. The SPME method integrates the extraction, preconcentration, and introduction to analytical instruments in a single step [13]. The most important advantages of SPME for extraction of analytes are including simple handling, use of a large number of extraction phases with different structures and polarities, good repeatability, high enrichment factors, having two modes of extraction (direct and headspace modes), and the utilizing the autosamplers and also automation systems. SPME is applied as a highly sensitive method for extracting a wide variety of compounds in real samples [14–16].

In the pharmaceutical and pharmacological fields, there is a challenge to utilize a suitable bioanalytical method for qualitative and

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quantitative detection of drugs, metabolites and clinical researches. A specific method in bioanalytical inquiries especially solid tissue, is in vivo sampling, which is generally preferred to in vitro techniques. In recent years, SPME has been successfully developed for the sampling and bioanalytical analysis as in vivo and in vitro forms. Nevertheless, the in vivo method has a notable ability and more important in biological analysis. Moreover, the sample preparation by in vivo SPME obtains distinctive metabolic patterns as in–situ conditions in comparison with the in vitro metabolomics methods [17].

One important case in SPME for the bioanalysis purpose is the use of suitable and non-toxic fiber coatings, titled biocompatible fiber. Against the conventional extraction phases, biocompatible coatings have good characteristics which make them a fantastic alternative for non-destructive in vivo sampling. These advantages are including the appropriateness of SPME in direct mode, led to an introduction of fiber to complicated matrices without training procedure; high selectivity or even specifically analysis for extraction of compounds; In this regard, in vivo SPME is an excellent sampling method because of its simplicity and flexibility, especially capability of its coupling with different sampling interfaces. In comparison with other in vivo sampling techniques like microdialysis that needs some equipment (power supply, tubes, pumps, etc.) the SPME performance is free to specific apparatuses. Thus, the SPME method can implement suitably for on-site sampling without a time-consuming process and excess accomplice [18]. However, sensitivity and quantification limits are the significant procedural challenges in SPME in vivo sampling. The sensitivity of the method related to the type of selected extraction phase as a fiber coating. To overcome this challenge, new materials involving polymers are developed to improve the extraction efficiency and obtain a higher selectivity for in vivo SPME sampling.

The main objects in this review are the investigation of new and efficient performances of SPME in vivo sampling. Also, some features related to the design of in vivo SPME devices in this field are reviewed. The development of the extraction phase types and applicability for the in vivo metabolomics, lipidomics, and clinical researchers are discussed. Subsequently, the challenges and also perspective of in vivo SPME method are summarized.

2. MATRIX COMPATIBLE COATINGS FOR IN VIVO SPME

Up to now, many commercial and homemade coatings have been used in the SPME method as an individual or combined sorbent materials. The usual fiber coatings as extraction phase are polydimethylsiloxane (PDMS), divinylbenzene (DVB), carbowax (CW), polyacrylonitrile (PAN), carboxen (CAR), octadecyl bonded silica (C18), hydrophilic-lipophilic balance (HLB) and some types of templated resin (TPR) [6, 19, 20]. According to the compound and matrix types, there are some commercial fiber coatings with several chemical structures, polarities, and thicknesses [19, 21]. The SPME method is performed in two modes of direct immersion (DI) and the headspace (HS). Typically, the DI–SPME method is used either for simple matrix, or complicated samples after the pretreatment process such as centrifuge, dilution, and filtration steps. The DI–SPME analysis for samples with high matrices complexity is commonly accompanied by decreasing extraction efficiency due to the deposition of macromolecules such as proteins on the fiber surface. This phenomenon will diminish the simplicity and efficiency of the method and also limited the capability to analyze the complex samples [22].

To develop the SPME fibers, researchers have been focused on the fabrication of the biocompatible coatings that are preserved from the deposition of particles or macromolecules on the fiber surface. Biocompatibility term refers to the ability of the extraction phase to direct exposure in living organisms with good selectivity and no side effects [23]. One of the current concerns that arise in bioanalytical samples is fouling phenomena, persuaded by matrix effects. The devices related to SPME coating are categorized in the biocompatible term, too. The applied devices in the in vivo SPME technique should be nontoxicity effects and performed without any significant injurious. The most commonly matrix compatible coatings favorably used in SPME devices are PDMS [24], polyaniline (PANI) [26], polyacrylonitrile (PAN) [25], polymeric ionic-liquids (PILs) [27], and polytetrafluoroethylene amorphous fluoroplastics (PTFE AF) [28]. Besides, highly repeatable C18 commercial fiber is much less expensive than the other commercial fibers and usually used as a mixed coating in SPME [29].

Qui et al. [26] proposed a fiber coating including graphene oxide, C18 and poly (diallyl dimethylammonium chloride) on a quartz surface via PANI. The SPME fiber was applied to preconcentrate the acidic pharmaceutical traces in the dorsal-epaxial of alive fish muscle before the HPLC–MS/MS analysis [9]. The robustness fiber exhibited good reproducibility and a higher extraction efficiency than polyacrylate (PA) and PDMS commercially fibers. The results confirmed the applicability of the fiber for the

determination of the pharmaceutical drug residues in in vivo SPME sampling.

Recent developments of in vivo HS–SPME showed that the good skill of the technique for the investigation of volatile and semivolatile metabolites. The commercially available PDMS/DVB and CAR/PDMS fibers have been normally used in headspace mode for semi-volatile and polar compounds like volatile organic compounds (VOCs) or volatile organic metabolites (VOMs) [30]. Nevertheless, Risticevic et al. [31] applied an extra thin PDMS layer as DVB/CAR/PDMS coating for the in vivo SPME method in direct mode. They used the new fiber to analyze 198 metabolites in an apple sample successfully. Despite the appropriate performance of the DVB/CAR/PDMS fiber in DI–SPME mode (to extract VOMs and VOCs), the CAR/PDMS and DVB/PDMS fibers are usually used in HS–SPME mode. HS–SPME applications for VOMs analysis has some advantages including high sensitivity, simultaneous extraction and enrichment, noninvasive SPME device, high throughput of analysis, adaptability with in vivo sampling, and finally on-site sampling.

As mentioned earlier, C18 is one of the prevailing chemical materials used for the fiber coating. C18 solid particles as a binder combined have been applied in SPME in vivo sampling to obtain higher extraction efficiency [32]. Polyacrylonitrile (PAN) is another efficient polymer used as a biocompatible coating, which was applied to the extraction of drugs from human plasma, for the first time [25]. In the next works, PAN was combined with various sorbent particles to improve the analysis of metabolomics and lipidomics from tissue samples [10]. In this regard, Mirnaghi and co-workers [33] used PAN with C18 particles in SPME as a 96-well plate prob. The capability of the PAN–C18 SPME blades were also tested for the in vivo sampling of fish to study the modifications of metabolite in different ecological circumstances or endogenous tension. The results revealed the sensitivity and efficiency of the combined extraction phase [34].

In recent years, innovative corporate biocompatible coatings named mixed-mode (MM), such as C18 plus benzene sulfonic acid have been introduced to neurotransmitters, metabolome, lipidome and such that compounds [35–39]. Now, these fibers are produced commercially and suitable as an interface in the LC instrument's introduction. The results confirm that the efficiency of MM SPME fibers to the determination of metabolites is two times better than that of the conventional C18 fiber. The use of MM coatings provides a higher sorption capability of hydrophilic analytes. Win-Shwe and co-workers [40] utilized a wire with a diameter of 200 μm coated via the MM extraction phase (with a thickness of about 45 μm) as in vivo SPME sampler. The prepared fiber was used to investigate the neurotoxicity effects of toluene on mice. For this reason, after the coverage of toluene, the concentration variations of taurine and glutamate compounds in the hippocampus section of mice were evaluated. The other part of the wire which was uncoated, equipped with a portion of rubber septum, cut fittingly to right introduced into the commercially CMA design cannula. Additionally, the C18 and benzenesulfonic acid as mixed-mode fiber (with a coating thickness of 45 μm) were used for untargeted metabolomics analysis in deep brain stimulation (DBS) in rats [41]. The DBS behavior on brain chemistry was studied at minor molecules level by non-lethal nature. The occurred chemical changes resulted in amino acids and lipids metabolism. The MM extraction phase has also been applied to the analysis of bronchoalveolar washing fluid [37], the extracellular fluid of the brain in two forms as moving and through the surgery [38, 35], and in liver and lung of a pig [36]. The in vivo sampling by MM coatings was performed satisfactorily without any matrix effects in tissues and biofluids.

In recent years, in some efforts, the selectivity of in vivo SPME fibers was improved for the extraction of specific compounds. In this regard, some carbohydrate compounds at very low concentration levels were detected in Aloe due to the interaction between the boronate-affinity and cis-diol containing compounds [42]. Also, highly specific coatings have been developed for the analysis of different compounds such as pesticides, heavy metals, drugs, and food safety in live biological samples [43, 45, 46].

The advanced biocompatible SPME probes have prepared as direct immersion mode in both the in vitro and in vivo performances in the complex biological tissues. There are some problems with the performance of traditional fibers and devices for sampling procedures from living systems. In this regard, researchers have been investigated the effect of various factors on probes performance such as swelling inflation in organic solvents and the weakness of fibers function, stripping of the coating and clogging the analytical instruments, and irreversible adsorption of macromolecules to the extraction phase. For solving these difficulties, some modifications in sampling devices would be performed. For example, a sharp ending fiber was used instead of the classical coatings to more conveniently

introducing the in vivo SPME probe into some solid tissues like the muscles. One important challenge in the use of biocompatible fibers for in vivo tissue analysis is coating stability or robustness [46]. In this regard, Poole and co-workers [45] examined the deposition strategies regarding the coating process for on-site fish sampling on recessed support.

Among the various in vivo tissue analysis, non-destructive brain sampling is the most important and highly sensitive in SPME in vivo. In this regard, the analysis of neurotoxic chemicals [47], neurotransmitters [48], and drugs [49], in brain tissue were performed. The first study of in vivo sampling to obtain the profile of a vast variety of metabolites in primate brains was performed on the macaque sample [48]. In this research, the detection of neurotransmitters in rhesus macaque brains was performed by the HLB–styrene divinylbenzene sulfonic acid (HLB–SCX) coating on recessed support with a diameter of lower than 200 µm. Sometimes in vivo sampling of the brain, there is a prerequisite to investigate some neurotransmitters simultaneously. For example, in the case of in vivo SPME sampling of macaque monkeys' brains during goal-directed behavior, the researchers performed some modifications in the fiber performance [50]. To obtain the precise tests and sampling/detection performance in several brain areas, they were miniaturized the SPME fiber and compare the detection levels of targeted analytes. For this reason, after testing of some proposed designs, the final design probe with a total diameter of about 200 µm was selected, because of higher performance and reduced invasiveness. The device was consisting of a blunt tip and a recessed hydrophilic-lipophilic balanced coating. The blunt tip of SPME fiber was positioned in the distinct brain sections by the management of a stereotactic software operation equipped with guiding cannula.

One important matter in the in vivo SPME of the brain is a temporal resolution that restricts the method sensitivity. This challenge relates to the capacity of sorbent profited by the length and thickness of the extraction phase. By applying the longer and thicker of the extraction phase, the higher sensitivity is obtained. Although, in general applications, two important parameters including the sensitivity and resolution of the method would be balanced [51].

3. IN VIVO SPME FOR PLANT METABOLITES

Up to now, plant material has been used for human safety, and employed as prevention or treating illnesses. Therefore, the healthiness of herbs and eatable plants are valuable, and sampling and analyzing the living plants have been interested in researches [52]. All fruits and plant regions such as leaves, flowers, stems, and roots emit VOCs as naturally or by a reaction to the environmental condition changes like temperature, light, water overflowing or drought [53, 54]. In recent years, the SPME method satisfactory employed for sampling and analyzing the botanicals and their metabolites in plant varieties, especially for the VOCs detection [1,2,15]. For example, orchid flowers as botanical were tested and analyzed by SPME–GC/MS in headspace mode. The results demonstrated that the method can conveniently distinguish the VOCs fingerprint in different orchid origins [55].

Among scientific research methodologies, the SPME technique has significantly improved to extract volatile metabolite over the past few years. In vivo SPME for analyzing volatile metabolites in plants has approved several innovative sampling strategies, especially for in-situ analysis [10, 44]. The first in vivo submissions of SPME was the extraction of volatile metabolites from existing plants [56, 57]. For instance, volatile and semi-volatile metabolites of Aristolochia ringens Vahl species have been analyzed by in vivo and ex vivo SPME floral scent sampling [58]. The authors discovered that the chemical species with an unpleasant odor (undecanal, nonanal, decanal, and 1-methylbutanoic acid) were the main materials extracted via in vivo SPME, whereas the aldehydes with low molecular weight were absent in the crushed flowers-derived extractions in ex vivo sampling. The implementation of a new SPME probe composed of functionalized carbon nanotubes/phenylboronic acid with high selectivity has been reported for the determination of carbohydrates in plants samples tissues. Many natural specimens such as Malabar Spinach and Aloe-Vera have been investigated in this study. The proposed in vivo SPME technique successfully detects glucose in spinach, as well as some other carbohydrates such as mannose, rhamnose, galactose, and glucose compounds in the Aloe-Vera sample. Given that the conventional SPME fiber typically displays a low tendency to polar compounds, the study by Chen et al. suggests a remarkable approach for in vivo screening of carbohydrates in plants [58].

It should be noted that appropriate sampling systems are pivotal to discover characteristics of volatile metabolites in living plants and such other samples. The use of interconnected chambers to quarantine the analysis system, the process of employing fiber alongside empty sampling chambers, as well as assessing the transfer impact on the composition of volatile metabolites, are all necessary for accurate volatile metabolite extraction. In this respect, in a study of

Mitchell floral scent production, Verdonk et al. [57] demonstrated that in vivo SPME along with gas chromatography-mass spectrometry is a suitable system for measuring volatile compounds from flowers. The instrument exploited to analyze the floral scent by in vivo SPME is displayed in Fig. 1. In this system, a glass funnel has been placed around the growing flower, and an aluminum foil has been used to confine the funnel to hamper any air circulation. SPME device has been fixed in place next to glass funnel using the SPME hollow needle through aluminum foil, placing the fiber close to the flower.

Musteata and co-workers [60] evaluated the capability of in vivo SPME for in-situ analysis. They employed hand-made biocompatible SPME fibers alongside liquid chromatography-mass spectrometry to achieve in vivo metabolic fingerprinting on twenty Amazonian plant kinds. The employed coatings in this study were PAN, C16 RP–Amide and HS-F5. Also Sang et. al [61] were analyzed the VOCs released from Daffodil flowers using a device similar to that displayed in Fig. 1. In another experiment, unscented dispersion (due to the lack of suitable substrates or precursors of scented compounds) of some plucked flowers were investigated by in vivo headspace SPME–GC–MS [62].

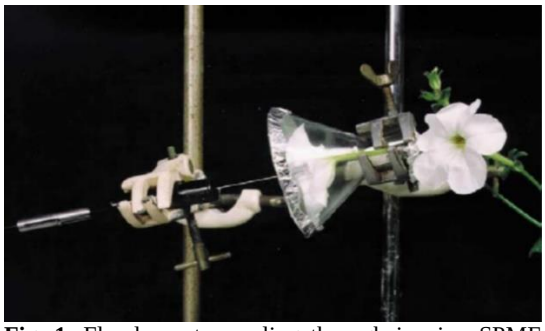

Fig. 1. Floral scent sampling through in vivo SPME [57].

Despite the study of plant metabolites with the headspace SPME technique has proper performance, SPME in direct immersion mod as a complementary method has also been applied for in vivo sampling [59, 63]. In this regard, Risticevic et al. [31] have compared the in vivo DI–SPME and HS–SPME modes in the case of apple sample. The results showed the DI–SPME mode eliminated the difficulty of HS–SPME for the extraction of unstable metabolites with hydrophobic properties, in addition to significantly enhance the analysis of metabolites. In a noteworthy study on plant cells' analysis as a conceptual proof study, Piri et al. [64] studied the increase spatial resolution by directing a single onion cell. In this study, the exploited a tip of a polypyrrole SPME fiber (with the coating length of 150 μm and coating thickness of 5 μm) to qualitatively analyze of luteolin and quercetin [64]. Moreover, to extract carbamate pesticides, Zhou et al. [65] incorporated PDMS/DVB fiber directly into the leaf of a green plant and then characterized the extracted species using LC-MS. Comparative results revealed that in vivo SPME is more sensitive, accurate and precise in comparison with the microdialysis method which requires the utilization of additional equipment (Fig. 2).

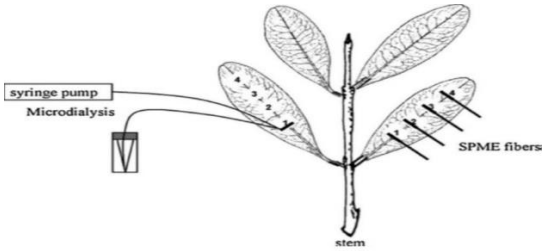

Fig. 2. In vivo SPME and MD comparison in green leaf sampling [65].

4. IN VIVO and IN SITU SPME CLINICAL RESEARCH

Through high selectivity in vivo sampling of complex living species, biocompatible SPME coatings have created an unprecedented circumstance for SPME in blood metabolome analysis [66]. Recent studies on living tissues have demonstrated that greatly reactive small molecules tend to undergo spontaneous oxidation. Also, lipidomes (lipid collection), particularly phosphocholine and fatty acids, are the most abundant components in tissues that have been collected and stored for analysis purposes [67]. Nowadays, in vivo lipidomic studies are focusing on living tissue biopsy. Since it mainly focusses on biomarkers analysis, one of the most important purposes of researches is to achieve the correct form of metabolomic/lipidomic analysis which avoided the false positive or negative results. In cases where discovered biomarkers include short-living species (immediate energy sources), in vivo sampling is preferred over the routine in vitro methods.

In vivo SPME has been utilized as a non-lethal method to detect drugs in tissues. Slight quantities of analytes are extracted by in vivo sampling of a living system employing the SPME probe, thus allowing the measurement of the target analyte concentration through minimizing chemical equilibrium alterations [68]. In a recent study, SPME's application has been investigated as a suitable method for the simple analysis of complex lipids. Zebrafish lipidomics has been magnificently conducted through in vivo SPME

with a biocompatible probe [69]. Based on this report, in vivo sampling has facilitated the efficient attainment of bioactive lipids such as fatty acyls which are undetectable through ex vivo SPME or solvent-based extraction techniques. In this study, Roszkowska and co-workers have confirmed the benefits of in vivo SPME through comparing in vivo sampling of SPME for fish samples, and solid-liquid extraction (SLE) of the same fish tissue afterward preservation for one year. The comparison declared that particular lipids, especially fatty acyls, only existed in the extracts obtained from in vivo SPME sampling, while sterols and glycerophospholipids with negligible concentrations were detected only using SLE. The results revealed the applicability of the technique to determine the wide-ranging of lipids and have pronounced potential in bioanalytical compounds, particularly biomarkers [5].

To analyze the polar and non-volatile components in tissues or muscles, the DI–SPME mode is employed through implanting fiber into the tissue. the direct immersion mode is the most sophisticated analysis of this kind. This microextraction strategy requires robust and flexible fibers with small sizes. Above all, the use of biocompatible coatings is essential to prevent any toxic or harmful effects on the living system. Additionally, to better performance for absorbing target analytes, the coatings should not absorb any protein in living tissue. In vivo SPME sampler is a needle-attached probe that is placed inside a port, and the fiber coating exposed to the bloodstream through piercing the port septum during sampling. after the optimized time for the extraction, the fiber is withdrawn and subsequently, the sampler is detached from the port. In these cases, the extraction time is generally short. As previously reported in the case of in vivo sampling for dogs, a two-minute extraction time was enough to extract the benzodiazepines in the blood sample by 10 μm thickness polypyrrole fiber [70].

In several studies, the SPME for rapid and accurate determination of bioaccumulation of new pollutants (pesticides, medicines, and personal care products) in fish muscle has been investigated as in vitro, and field studies without the need for animal dissection [16, 71–74]. Moreover, to monitor the misuse of antibiotics, the in vivo SPME method has been employed to the bioanalysis of pharmacological agents in a particular live fish tissue as a model. SPME needles have been exploited to determine pharmaceutical remains especially in the dorsal-axial muscles of fish besides adipose tissue, and precise accuracy of results have been achieved through repeated in vivo tissue sampling [75].

SPME in direct immersion mode has been efficiently employed for the metabolomics and lipidomics investigations as well as protein detection in mouse brain tissue, also lung, liver, and other fish tissues. To increase simplicity and avoid tissue-damaging, instead of 23 or 24 gauge needles which are mostly used for in vitro SPME, the probe was inserted into a subcutaneous injection needle. Boiko et al. [35, 36] employed the microprobe for the analysis of a large part of endogenous analytes through liver transplantation or surgery on liver and lung tissues. The SPME probe, which was placed right into the peripheral vein of the living animal, was able to screen changes in drugs and their metabolites and also measure their concentration quantitatively.

In another study, some endogenous neurotransmitters including serotonin, dopamine, glutamic and g-aminobutyric acids were analyzed in the striatum of a rat brain by in vivo SPME method. The condition changes on the neurotransmitters in brain tissue were monitored after the injection of fluoxetine as single-dose intraperitoneal [48]. The results appeared that the injection of fluoxetine had no influence on the neurotransmitters except for serotonin. However, serotonin concentration was increased up to 400% in comparison with the preliminary level because of that the fluoxetine blocked the performance of the transporter serotonin by hindering the serotonin reuptake in the tissue brain [48].

Lately, C18 particles as extraction phase was used in SPME to estimate the changes of drug concentration in striatal and cortex areas of the rat brain tissue before the LC-MS/MS analysis [48]. In this study, the concentration levels of carbamazepine and cimetidine in two steady and dynamic states were monitored in the striatum and cortex of the rat brain.

Additionally, the employment of thin fiber (TF) in SPME combines the preconcentration and sampling in one step. In vivo SPME method employing the TF sampling in 5-minutes followed by gas and liquid chromatography separation–detection systems have enabled the analysis of a vast variety of compounds with various nature characterizations [11]. To enhance the sensitivity of the in vivo SPME relative to traditional extraction phases, Togunde et al. [76] utilized the SPME method with a thin-film blade for fish sampling, which resulted in increased surface area and also increased the sampling speed.

SPME applications in indicating metabolomics features of brain tumors revealed variations in different tumor types based on chemometrics analysis [79]. Commonly, while a sliced tumor or biopsy tissue is examined, the sample preparation procedure is time-consuming. This necessitates the incorporation of larger support through

increasing the surface area such as a thin film or blade which enhances extraction efficiency and sensitivity in addition to devoid the increasing of the extraction time (Fig. 3).

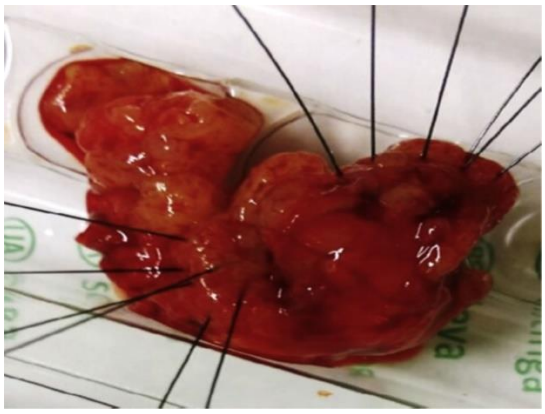

Fig. 3. The in vivo sampling of brain tumor directly after slicing [79].

A recent study at the University of Waterloo confirmed that in vivo SPME is an efficient technique for the extraction of nonpolar metabolites in the brain mice. The scientists are also dedicated enables monitoring of changes in hydrophobic metabolites after the treatment [41]. This study confirmed SPME's capability in the simultaneous monitoring of changes in several categories of metabolites and revealed new information regarding metabolic pathways affected by DBS. On the other hand, the screen changes of polar compounds at trace concentration levels associated with some difficulties in the SPME method. Three major constraints in this issue were included the minor size of the probe, the low tendency of polar compounds to the fiber surface, and the application of the MS detection system in full scan mode.

The application of SPME in the extraction of unsteady metabolites, including beta-nicotinamide adenine dinucleotide, adenosine monophosphate, and glutathione through in vivo SPME bioanalysis in the blood sample, has recently been investigated. For instance, Garolinska et al. [80] utilized C18-PAN fiber with LC-MS to study human milk lipidome. Lipids analysis was performed after 5-minute extraction followed by chromatography analysis. In this study, main lipid types (such as glycerolipids, glycerophospholipids, sphingolipids, fatty acyls, prenol lipids, and sterols) were extracted from human milk. This technique also proved the ability of the method to differentiate human milk, formulated milk and cow's milk by characterizations of lipid categories.

Moreover, through in vivo analysis, SPME has been exploited to determine doxorubicin concentrations during in vivo pulmonary injection for the treatment of malignant lung metastases [81]. This study illustrated SPME capability in improving temporal and spatial resolution by focusing on the monitoring changes in drug concentration throughout the process. Besides, the distribution changes of the drug through districts of the body including lung was monitored. Following an investigation on the practicality of the in vivo SPME technique, this advanced method has also been utilized in tissue matrix for screening the biomarkers and medications throughout liver and lung transplant surgical procedure (Fig. 4) [35].

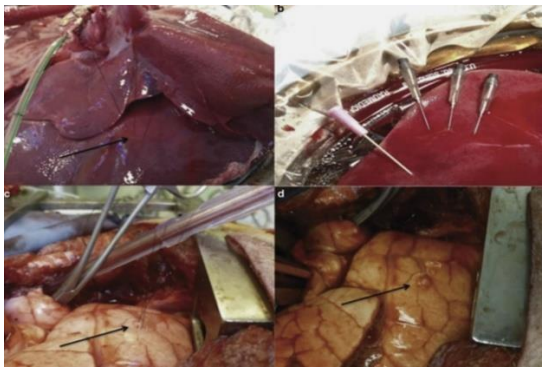

Fig. 4. Liver tissue sampling by in situ SMPE with non-assembled fibers (a), needle-assembled fibers (b), in vivo sampling of lung by non-assembled fibers (c), and a tissue sample after sampling procedure [35]

The predominant in vivo SPME approaches in the human body have been mainly the analysis of VOMs and VOCs from samples such as human saliva, skin, and breath. HS–SPME sampling in the study of VOCs in humans has significantly reduced background signals and improved the repeatability of the analysis. In this regard, the analysis of VOCs using the in vivo SPME technique confirmed the possible lethality from captan consumption [82]. This technique has also been employed in garlic and alcohol consumption biomarkers detection [83]. In another study, rapid breath analysis has been achieved by SPME in the human body at disease conditions with atherosclerosis of bladder tissue [84].

In vivo SPME has recently been expanded to evaluate the endogenous substances with high volatility such as application in breast cancer cell lines to investigate infection conduits [85], and fungi and bacteria metabolic fingerprinting [86, 87]. In this regard, in vivo SPME is monitored the metabolites for biochemical analysis and determined biomarkers throughout a surgery [35]. In the latest research, the selectivity of the fiber

was enhanced via functionalization with a DNA aptamer and applied to extract and isolate specific proteins from human plasma. In this study, SPME has been successfully utilized to investigate thrombin in plasma [88].

Up to now, the in vivo SPME has been successfully implemented for a wide range of samples. Several in vivo SPME techniques for endogenous substance analysis and tissue bioanalysis are listed in Table 1.

Overall, it can be asserted that due to its non-destructive properties and convenient sample preparation, the in vivo SPME provides an excellent implement for quick detection of cardiovascular diseases, cancer, and cerebral complications, and such studies' interpretation will assist the detection of diseases at the near future.

Table 1. Selected researches applying in vivo SPME method in sample analysis.

Sample type	Analytes	Coating	SPME	Instrumental analysis	Ref
Blood	Benzodiazepines	PPY[a] and PEG[b]	DI[c]	LC–MS/MS	70
Blood	Carbamazepine and its metabolite,carbamazepine-10,11-epoxide	C18	DI	LC–MS/MS	89
Human breast cancer (BC) cell lines	VOMs[d]	DVB/CAR/PDMS	HS[e]	GC–MS	85
Human plasma	Thrombin	Apt-PANCMA[f]	DI	LC–MS	88
Breath	VOCs[g]	CAR/PDMS	HS	GC–MS	84
Skin	Untargeted analysis	DVB-CAR-PDMS	HS	GC–MS	90
Skin	DMSO[h], AMS[i], allyl-mercaptan	PDMS	HS	GC–MS	83
Skin	Untargeted analysis	PDMS-DVB	HS	GC–MS	91
Human saliva	Endogenous steroid	PDMS/HLB[j]/C18	DI	GC–MS	92
Human viscera content	Captan and its metabolites	PDMS	DI	GC–MS	82
Fish muscle	Bisphenol A, Ibuprofen, Diclofenac, Naproxen, Gemfibrozil, Carbamazepine, Fluoxetine, Norfluoxetine	C18	DI	LC–MS/MS	68
Fish	Metabolite profiling	PAN-C18	Blade SPME	LC–MS/MS	67
Pufferfish	etrodotoxin	Al_2O_3@GO-PLGA@PNA[k]	DI	LC–MS/MS	93
Rainbow trout	Geosmin, 2-methyl isoborneol	PDMS	DI	GC–MS	94
Lung, liver	Endogenous metabolites profiling	C18 with benzenesulfonic acid	DI	C–MS	35
Brain	Carbamazepine	C18	DI	LC–MS/MS	77
Macaque brain	Neurotransmitters	HLB-SCX[l]	DI	LC–MS/MS	48
Rat brain	GABA[m], GLU[n], Dopamine, and serotonin; untargeted metabolomics	C18 with benzenesulfonic acid	DI	LC–MS/MS	38
Ophrys sphegodes	Hydrocarbon, aldehyde, furan	DVB/CAR/PDMS	HS	GC–MS	55
Phragmites australis	Methyl *tert*-butyl ether	CAR/PDMS	HS	GC–MS	95
Apple	Metabolites	DVB/CAR/PDMS	DI	GC–GC–TOF[o] MS	31
Aloe	Mannose, rhamnose, glucose	Boronic acid decorated defective metal-organic framework (B-D-MI-100)	DI	HPLC–UV[p]	42

[a] Polypyrrole; [b] Polyethylene glycol; [c] Direct-immersion; [d] Volatile organic metabolites; [e] Head-space; [f] Aptamer-functionalized poly (acrylonitiile-co-maleic acid); [g] Volatile organic compounds; [h] Dimethylsulfoxide; [i] Allyl methyl sulfide; [j] Hydrophilic lipophilic balanced particles; [k] Al_2O_3@graphene oxide-poly(lactic-co-glycolic)@polynoradrenaline [l] Styrene divinyl benzene sulfonic acid; [m] Gamma aminobutyric acid; [n] Glutamic acid; [o] Time of flight; [p] Ultraviolet

4. CONCLUSION

SPME is a worthy sampling strategy which has been effectively employed for in vivo and in vitro analysis of endogenous substances (metabolites, lipidomics, proteins, hormones, messenger compounds, neurotransmitters, etc.). This bioanalytical sample preparation method was used satisfactorily in many living systems like humans, insects, animals, plants, microorganisms, cells, etc. The technique encompasses advantages including simplicity, low detection limit, high throughput, biocompatible coating features, non-destructive sampling, and high enrichment capability. Coupling SPME with gas or liquid chromatography can mediate prompt biospecimen analysis and in–situ samplings which is impossible by the other methods. The simplicity of the procedure is its non-invasive nature, making it as a diagnostic tool especially for monitoring the clinical disorders.

One of the uppermost advances in SPME is the usage of several remarkable biocompatible coating substances with different physical and chemical properties. This opportunity has turned SPME into a powerful tool in bio-sampling without basal tissue interference, which analyzes the endogenous substances provides by adaptable coatings (in DI or HS modes). The selectivity of the technique is directly associated with the correct selection of the extraction phase. The choice of the fiber type is significant for untargeted metabolomics studies involving a wider range of analytes.

Despite the phenomenal privileges of the in vivo SPME technique, there are some challenges toward developing and improving its performance, which require additional investigation and applied research in the future. Regarding this matter, new strategies for manufacturing coatings with high selectivity are a priority. In addition, to this date, developed SPME coatings have been mostly reported for the separation and enrichment of small molecules. However, efficient coatings for microextraction of macromolecules such as glycoproteins, genomes' cells, microRNAs and exosomes are limited which require further studies.

Furthermore, effective approaches for coupling SPME with portable MS or GC–MS are pivotal toward developing this approach, which is valuable for field analysis and online investigations. Finally, miniaturizing the sampling device to explore the targeted and untargeted compounds in microenvironments and even in a single cell with high resolution is another paramount challenge in this bioanalysis technique. Although the SPME has been proposed for microorganisms and individual cells, the compounds which can preconcentrated and extracted in this field are rather restrained. Also, increasing the sensitivity as well as enhancing the performance of the miniaturized SPME fiber should be considered. Undoubtedly, studies regarding this matter and considering the challenges will enhance the SPME method capability as a powerful tool for in vivo sampling and analysis of living organisms in the future.

REFERENCES

[1] J.D. Wulfkuhle, L.A. Liatta, E.F. Petricoin, Proteomic applications for the early detection of cancer, *Nat. Rev. Cancer*. 3 (2003) 267–275.

[2] T. Abaffy, R. Duncan, D.D. Riemer, O. Tietje, G. Elgart, C. Milikowski, Differential volatile signatures from skin, naevi and melanoma: a novel approach to detect a pathological process, *BMC Bioinformatics*. 16 (2015) 158–170.

[3] D. Vuckovic, Current trends and challenges in sample preparation for global metabolomics using liquid chromatography–mass spectrometry, *Anal. Bioanal. Chem*. 403 (2012) 1523–1548.

[4] Z.G. Gong, J. Hu, X. Wu, Y.J. Xu, The Recent Developments in Sample Preparation for Mass Spectrometry-Based Metabolomics, *Crit. Rev. Anal. Chem*. 47 (2017) 325–331.

[5] D. Vuckovic, Improving metabolome coverage and data quality: Advancing metabolomics and lipidomics for biomarker discovery, *Chem. Commun*. 54 (2018) 6728–6749.

[6] N. Reyes-Garcés, E. Gionfriddo, Recent developments and applications of solid phase microextraction as a sample preparation approach for mass spectrometry-based metabolomics and lipidomics, *Trends Anal. Chem*. 113 (2019) 172–181.

[7] H.L. Lord, X. Zhang, F.M. Musteata, D. Vuckovic, J. Pawliszyn, In vivo solid-phase microextraction for monitoring intravenous concentrations of drugs and metabolites. *Nat. Protoc.* 6 (2011) 896–924.

[8] Q.H. Zhang, L.D. Zhou, H. Chen, C.Z. Wang, Z.N. Xia, C.S. Yuan, Solid-phase microextraction technology for in vitro and in vivo metabolite analysis, *Trends Anal. Chem.* 80 (2016) 57–65.

[9] R.P. Belardi, J. Pawliszyn, Application of chemically modified fused silica fibers in the extraction of organics from water matrix samples and their rapid transfer to capillary columns, *Water Pollut. Res. J. Can*. 24 (1989) 179–191.

[10] N. Reyes-Garcés, E. Gionfriddo, G.A. Gómez-Ríos, M.N. Alam, E. Boyacl, B. Bojko, V. Singh, J. Grandy, J. Pawliszyn, Advances in Solid Phase Microextraction and

Perspective on Future Directions, *Anal. Chem*. 90 (2018) 302–360.
[11] E.A. Souza-Silva, E. Gionfriddo, J. Pawliszyn, A critical review of the state of the art of solid-phase microextraction of complex matrices II. Food analysis, *Trends Anal. Chem.* 71 (2015) 236–248.
[12] B. Bojko, N. Reyes-Garcés, V. Bessonneau, K. Goryński, F. Mousavi, E. Souza Silva, J. Pawliszyn, Solid-phase microextraction in metabolomics, Trends Anal. Chem. 61 (2014) 168–180.
[13] H. Kataoka, H.L. Lord, J. Pawliszyn, Applications of solid-phase microextraction in food analysis, *J. Chromatogr. A.* 880 (2000) 35–62.
[14] D. Vuckovic, I. Lannoy, B. Gien, Y. Yang, F.M. Musteata, R. Shirey, In vivo solid-phase microextraction for single rodent pharmacokinetics studies of carbamazepine and carbamazepine- 10,11-epoxide in mice, *J. Chromatogr. A*. 1218 (201) 13367–3375.
[15] J. Pereira, C.L. Silva, R. Perestrelo, J. Gonçalves, V. Alves, J.S. Câmara, Re-exploring the high-throughput potential of microextraction techniques, SPME and MEPS, as powerful strategies for medical diagnostic purposes. Innovative approaches, recent applications and future trends, *Anal. Bioanal. Chem*. 406 (2014) 2101–2122.
[16] X. Zhang, K.D. Oakes, S. Wang, M.R. Servos, S. Cui, J. Pawliszyn, In vivo sampling of environmental organic contaminants in fish by solid-phase microextraction, *Trends Anal. Chem.* 32 (2012) 31–39.
[17] E. Cudjoe, B. Bojko, P Togunde, J. Pawliszyn, In vivo solid-phase microextraction for tissue bioanalysis, *Bioanalysis*, 21 (2012) 2605–26194.
[18] K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, Quantitative liquid chromatography/mass spectrometry for the analysis of microdialysates. *Talanta* 74 (2008) 458–469.
[19] G. Vas, K. Vékey, Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis, *J. Mass Spectrom.* 39 (2004) 233–254.
[20] J. Pawliszyn, SPME commercial devices and fiber coatings, In: Handbook of Solid Phase Microextraction, (Ed, J. Pawliszyn) Chemical Industry Press, China (2009) pp. 315-324.
[21] J.V. Hinshaw, C. Serveron, "*Solid-Phase Microextraction", LC·GC Europe, GC Connections*. Orlando (2003) pp. 2–5.
[22] N.H. Godage, E. Gionfriddo, A critical outlook on recent developments and applications of matrix compatible coatings for Solid Phase Microextraction, *Trends Anal. Chem*. 111 (2019) 220–228.
[23] M. Vert, Y. Doi, K.H. Hellwich, Terminology for biorelated polymers and applications (IUPAC recommendations 2012). *Pure Appl. Chem*. 84 (2012) 1–10.
[24] É.A. Souza Silva, J. Pawliszyn, Optimization of fiber coating structure enables direct immersion solid phase microextraction and high-throughput determination of complex samples, *Anal. Chem*. 84 (2012) 6933–6938.
[25] M.L. Musteata, F.M. Musteata, J. Pawliszyn, Biocompatible solid-phase microextraction coatings based on polyacrylonitrile and solid-phase extraction phases., *Anal. Chem*. 79 (2007) 6903–6911.
[26] J. Qiu, G. Chen, S. Liu, T. Zhang, J. Wu, F. Wang, J. Xu, Y. Liu, F. Zhu, G. Ouyang, Bioinspired Polyelectrolyte-Assembled Graphene-Oxide-Coated C18 Composite Solid-Phase Microextraction Fibers for in Vivo Monitoring of Acidic Pharmaceuticals in Fish, *Anal. Chem*. 88 (2016) 5841–5848.
[27] C. Cagliero, H. Nan, C. Bicchi, J.L. Anderson, Matrix-compatible sorbent coatings based on structurally-tuned polymeric ionic liquids for the determination of acrylamide in brewed coffee and coffee powder using solid-phase microextraction, *J. Chromatogr. A*. 1459 (2016) 17–23.
[28] E. Gionfriddo, E. Boyaci, J. Pawliszyn, New Generation of Solid-Phase Microextraction Coatings for Complementary Separation Approaches: A Step toward Comprehensive Metabolomics and Multiresidue Analyses in Complex Matrices, *Anal. Chem*. 89(2017) 4046–4054.
[29] G. Ouyang, D. Vuckovic, J. Pawliszyn, Nondestructive Sampling of Living Systems Using in Vivo Solid-Phase Microextraction, *Chem. Rev*. 111 (2011) 2784–2814.
[30] E.S. Silva, J. Pawliszyn, Optimization of Fiber Coating Structure Enables Direct Immersion Solid Phase Microextraction and High-Throughput Determination of Complex Samples, *Anal. Chem*. 84 (2012) 6933–6938.
[31] S. Risticevic, E.A. Souza-Silva, J.R. DeEll, J. Cochran, J., Pawliszyn, Capturing plant metabolome with direct-immersion in vivo solid phase microextraction of plant tissues, *Anal. Chem*. 88 (2016) 1266–1274.
[32] D. Vuckovic, R. Shirey, Y. Chen, L. Sidisky, C. Aurand, K. Stenerson, J. Pawliszyn, In vitro evaluation of new biocompatible coatings for solid-phase microextraction: implications for drug analysis and in vivo sampling applications, *Anal. Chim. Acta* 638 (2009) 175–181.

[33] F.S. Mirnaghi, Y. Chen, L.M. Sidisky, J. Pawliszyn, Optimization of the coating procedure for high throughput 96-blade solid phase microextraction system coupled with LC–MS/MS for analysis of complex samples. *Anal. Chem*. 83 (2011) 6018–6025.

[34] V. Bessonneau, J. Ings, M. McMaster, R. Smith, L. Bragg, M. Servos, J. Pawliszyn, In vivo tissue sampling using solid-phase microextraction for non-lethal exposome-wide association study of CYP1A1 induction in Catostomus commersonii, *Environ. Res*. 151 (2016) 216–223.

[35] B. Bojko, K. Gorynski, G.A. Gomez-Rios, J.M. Knaak, T. Machuca, E. Cudjoe, V.N. Spetzler, M. Hsin, M. Cypel, M. Selzner, M.Y. Liu, S. Keshjavee, J. Pawliszyn, Low invasive in vivo tissue sampling for monitoring biomarkers and drugs during surgery, *Lab. Investig.* 94 (2014) 586–594.

[36] B. Bojko, K. Gorynski, G.A. Gomez-Rios, J.M. Knaak, T. Machuca, V.N.;Spetzler, E. Cudjoe, M. Hsin, M. Cypel, M Selzner, M.Y. Liu, S. Keshavjee, J. Pawliszyn, Solid phase microextraction fills the gap in tissue sampling protocols, *Anal. Chim. Acta* 803 (2013) 75–81.

[37] V. Bessonneau, B. Bojko, A. Azad, S. Keshavjee, S. Azad, J. Pawliszyn, Determination of bronchoalveolar lavage bile acids by solid phase microextraction liquid chromatography-tandem mass spectrometry in combination with metabolite profiling: comparison with enzymatic assay, *J. Chromatogr*. A 1367 (2014) 33–38.

[38] E. Cudjoe, B. Bojko, I. Lannoy, V. Saldivia, J. Pawliszyn, Solid-phase microextraction: a complementary in vivo sampling method to microdialysis, *Angew. Chem. Int. Ed*. 52 (2013) 12124–12126.

[39] A. Roszkowska, M. Yu, V. Bessonneau, L. Bragg, M. Servos, J. Pawliszyn, Metabolome profiling of fish muscle tissue exposed to benzo[alpha]pyrene using in vivo solid-phase microextraction, *Environ. Sci. Technol. Lett*. 5 (2018) 431–435.

[40] T. Win Shwe, D. Mitsushima, D. Nakajima, S. Ahmed, S. Yamamoto, S. Tsukahara, M. Kakeyama, S. Goto, H. Fujimaki, Toluene induces rapid and reversible rise of hippocampal glutamate and taurine neurotransmitter levels in mice, *Toxicol. Lett*. 168 (2007) 75–82.

[41] N. Reyes-Garces, "*Solid Phase Microextraction as a Sample Preparation Tool for Targeted and Untargeted Analysis of Biological Matrices*" Ph.D. Thesis, University of Waterloo, (2017).

[42] G. Chen, X. Fang, Q. Chen, J. Zhang, Z. Zhong, J. Xu, F. Zhu, G. Ouyang, Boronic acid decorated defective metal-organic framework nanoreactors for highefficiency carbohydrates separation and labeling, *Adv. Funct. Mater*. 27 (2017) 1702126.

[43] S. Wang, K.D. Oakes, L.M. Bragga, J. Pawliszyn, G.D. Mark, R. Servos, Validation and use of in vivo solid phase micro-extraction (SPME) for the detection of emerging contaminants in fish, *Chemosphere* 85 (2011) 1472–1480.

[44] G. Ouyang, D. Vuckovic, J. Pawliszyn, Nondestructive sampling of living systems using in vivo solid-phase microextraction, *Chem. Rev*. 111 (2011) 2784–2814.

[45] J.J. Poole, J.J. Grandy, M. Yu, E. Boyaci, G.A. Gomez-Rios, N. Reyes-Garces, B. Bojko, H.V. Heide, J. Pawliszyn, Deposition of a Sorbent into a Recession on a Solid Support To Provide a New, Mechanically Robust Solid-Phase Microextraction Device, *Anal. Chem*. 89 (2017) 8021–8026.

[46] O.P. Togunde, H. Lord, K.D. Oakes, M.R. Servos, J. Pawliszyn, Development and evaluation of a new in vivo solid-phase microextraction sampler, *J. Sep. Sci*. 36 (2013) 219–223.

[47] E.A. Souza Silva, S. Risticevic, J. Pawliszyn, Recent trends in SPME concerning sorbent materials, configurations, and in vivo applications, *Trends Anal. Chem*. 43 (2013) 24–36.

[48] S. Lendor, S.A. Hassani, E. Boyaci, V. Singh, T. Womelsdorf, J. Pawliszyn, A solid phase microextraction-based miniaturized probe and protocol for extraction of neurotransmitters from brains in vivo, *Anal. Chem*. 91 (2019) 4896–4905.

[49] J. Xu, R. Wu, S. Huang, M. Yang, Y. Liu, Y. Liu, R. Jiang, F. Zhu, G. Ouyang, Polyelectrolyte microcapsules dispersed in silicone rubber for in vivo sampling in fish brains, *Anal. Chem*. 87 (2015) 10593–10599.

[50] S.A. Hassani, S. Lendor, E. Boyaci, J. Pawliszyn, T. Womelsdorf, Multi-Neuromodulator Measurements Across Fronto-Striatal Network Areas of the Behaving Macaque using Solid-Phase Microextraction. https://doi.org/10.1101/534651.

[51] W. Filipiak, B. Bojko, SPME in clinical, pharmaceutical, and biotechnological research - How far are we from daily practice?, *Trends Anal. Chem*. 115 (2019) 203–213.

[52] F. Zhu, J. Xu, Y. Ke, S. Huang, F. Zeng, T. Luan, G. Ouyang, Applications of in vivo

and in vitro solid-phase microextraction techniques in plant analysis: A review, *Anal. Chim. Acta* 794 (2013) 1402–1410.

[53] H.J. Yan, H. Zhang, Q.G. Wang, H.Y. Jian, X.Q. Qiu, J.H. Wang, K.X. Tang, Isolation and identification of a putative scent-related gene RhMYB1 from rose, *Mol. Biol. Rep*. 38 (2011) 4475–4482.

[54] T. Kaewtathip, S. Charoenrein, Changes in volatile aroma compounds of pineapple (Ananas comosus) during freezing and thawing, *Int. J. Food Sci. Technol*. 47 (2012) 985–990.

[55] A. Manzo, S. Panseri, I. Vagge, A. Giorgi, Volatile fingerprint of Italian populations of orchids using solid phase microextraction and gas chromatography coupled with mass spectrometry, *Molecules* 19 (2014) 7913–7936.

[56] C. Zini, F. Augusto, T.E. Christensen, B.P. Smith, E.B. Caramão, J. Pawliszyn, Monitoring biogenic volatile compounds emitted by Eucalyptus citriodora using SPME., *Anal. Chem*. 73 (2001) 4729–4935.

[57] J.C. Verdonk, C. Ric de Vos, H.A. Verhoeven, M.A. Haring, A.J. van Tunen, R.C. Schuurink, Regulation of floral scent production in petunia revealed by targeted metabolomics, *Phytochemistry* 62 (2003) 997–1008.

[58] E.E. Stashenko, S.A. Ordonez, N.A. Marin, J.R. Martinez, Determination of the Volatile and Semi-volatile Secondary Metabolites, and Aristolochic Acids in Aristolochia ringens Vahl, *J. Chromatogr. Sci*. 47 (2009) 817–821.

[59] G. Chen, J. Qiu, J. Xu, X. Fang, Y. Liu, S. Liu, S. Wei, R. Jiang, T. Luan, F. Zeng, F. Zhu, G. Ouyang, A novel probe based on phenylboronic acid functionalized carbon nanotubes for ultrasensitive carbohydrate determination in biofluids and semi- solid biotissues, *Chem. Sci.* 7 (2016) 1487–1495.

[60] F.M. Musteata, M. Sandoval, J.C. Ruiz-Macedo, K. Harrison, D. McKenna, W. Millington, Evaluation of in vivo solid phase microextraction for minimally invasive analysis of nonvolatile phytochemicals in Amazonian plants, *Anal. Chim. Acta*. 933 (2016) 124–133.

[61] G. Song, J. Xiao, C. Deng, X. Zhang, Y. Hu Use of solid-phase microextraction as a sampling technique for the characterization of volatile compounds emitted from Chinese daffodil flowers, *J. Anal. Chem*. 62 (2007) 674–679.

[62] Z.Y. Yang, S. Endo, A. Tanida, K.J. Kai, N. Watanabe, J. Agric, Synergy Effect of Sodium Acetate and Glycosidically Bound Volatiles on the Release of Volatile Compounds from the Unscented Cut Flower, *Food Chem*. 57 (2009) 6396–6401.

[63] X. Fang, G. Chen, J. Qiu, J. Xu, J. Wang, F. Zhu, G. Ouyang, Determination of four salicylic acids in aloe by in vivo solid phase microextraction coupling with liquid chromatography-photodiode array detection, *Talanta* 184 (2018) 520–526.

[64] H. Piri-Moghadam, F. Ahmadi, G.A. Gómez-Ríos, E. Boyaci, N. Reyes-Garcés, A. Aghakhani, B. Bojko, J. Pawliszyn, Fast Quantitation of Target Analytes in Small Volumes of Complex Samples by Matrix-Compatible Solid-Phase Microextraction Devices, Angew. Chemie - Int. Ed. 55 (2016) 7510–7514.

[65] S.N. Zhou, G.F. Ouyang, J. Pawliszyn, Comparison of microdialysis with solid-phase microextraction for in vitro and in vivo studies, *J. Chromatogr. A* 1196 (2008) 46–56.

[66] D. Vuckovic, S. Risticevic, J. Pawliszyn, In Vivo Solid-Phase Microextraction in Metabolomics: Opportunities for the Direct Investigation of Biological Systems, *Angew Chem. Int. Ed. Engl.* 50 (2011) 5618–5624.

[67] V. Bessonneau, J. Ings, M. McMaster, R. Smith, L. Bragg, M. Servos, J. Pawliszyn, In vivo microsampling to capture the elusive exposome, *Sci. Rep*. 7 (2017) 44038.

[68] S. Wang, K.D. Oakes, L.M. Bragg, J. Pawliszyn, G. Dixon, M.R. Servos, Validation and use of in vivo solid phase micro-extraction (SPME) for the detection of emerging contaminants in fish. *Chemosphere* 85 (2011) 1472–1482.

[69] A. Roszkowska, M. Yu, V. Bessonneau, L. Bragg, M. Servos, J. Pawliszyn, Tissue storage affects lipidome profiling in comparison to in vivo microsampling approach, *Sci. Rep*. 8 (2018) 6980.

[70] F.M. Musteata, M.L. Musteata, J. Pawliszyn, Fast in vivo microextraction: a new tool for clinical analysis. *Clin. Chem*. 52 (2006) 708–715.

[71] S.N. Zhou, K.D. Oakes, M.R. Servos, J. Pawliszyn, Application of Solid-Phase Microextraction for In Vivo Laboratory and Field Sampling of Pharmaceuticals in Fish, Environ. *Sci. Technol*. 42 (2008) 6073–6079.

[72] X. Zhang, K.D. Oakes, S. Cui, L. Bragg, M.R. Servos, J. Pawliszyn, Tissue-Specific In Vivo Bioconcentration of Pharmaceuticals in Rainbow Trout (Oncorhynchus mykiss) Using Space-Resolved Solid-Phase Microextraction, *Environ. Sci. Technol*. 44 (2010) 3417–3422.

[73] G. Ouyang, W. Zhao, L. Bragg, Z. Qin, M. Alaee, J. Pawliszyn, Time-Weighted Average Water Sampling in Lake Ontario with Solid-Phase Microextraction Passive Samplers, *Environ. Sci. Technol*. 41 (2007) 4026–4031.

[74] O.P. Togunde, K.D. Oakes, M.R. Servos, J. Pawliszyn, Determination of Pharmaceutical Residues in Fish Bile by Solid-Phase Microextraction Couple with Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS), *Environ. Sci. Technol*. 46 (2012) 5302–5309.

[75] J. Xu, S. Huang, R. Wu, R. Jiang, F. Zhu, J. Wang, Bioinspired polydopamine sheathed nanofibers for high-efficient in vivo solid-phase microextraction of pharmaceuticals in fish muscle, *Anal. Chem*. 87 (2015) 3453–3459.

[76] O.P. Togunde, K. Oakes, M. Servos, J. Pawliszyn, Study of kinetic desorption rate constant in fish muscle and agarose gel model using solid phase microextraction coupled with liquid chromatography with tandem mass spectrometry. *Anal. Chim. Acta* 742 (2011) 2–9.

[77] E. Cudjoe, C. Hamani, E. Hoque, "*Solid Phase microextraction: the potential for in vivo monitoring of endogenous small polar molecules in biological systems",* ASMS Conference. Denver (2011) pp. 112–116.

[78] B. Bojko, E. Cudjoe, G.A. Gomez-Rios, SPME – Quo vadis? *Anal. Chim. Acta* 750 (2012) 132–151.

[79] P.Z. Gorynska, K. Chmara, K. Gorynski, D. Paczkowski, M. Harat, B. Bojko, A new strategy for brain tumour metabolomic analysis, *Med. Res. J*. 3 (2018) 15–22.

[80] D. Garwolińska, W. Hewelt-Belka, J. Namieśnik, A. Kot-Wasik, Rapid Characterization of the Human Breast Milk Lipidome Using a Solid-Phase Microextraction and Liquid Chromatography-Mass Spectrometry-Based Approach, *J. Proteome Res*. 16 (2017) 3200–3208.

[81] N. Looby, B. Bojko, G.A Gomez-Ríos, M. Tascon, A. Roszkowska, K. Gorynski, J. Pawliszyn, "*Comprehensive sampling and sample preparation: analytical techniques for scientists*", *XXII International Mass Spectrometry Conference, Florence*, Italy (2018) pp. 296–312.

[82] A.K. Gottzein, F. Musshoff, B. Madea, Systematic toxicological analysis revealing a rare case of captan ingestion, *J. Forensic.Sci*. 58 (2013) 1099–1103.

[83] R. Jiang, E. Cudjoe, B. Bojko, T. Abaffy, J. Pawliszyn, A non-invasive method for in vivo skin volatile compounds sampling, *Anal. Chim. Acta*. 804 (2013) 111–119.

[84] R. Kramer, A. Sauer-Heilborn, T. Welte, C.A. Guzman, M.G. Höfle, W.R. Abraham, A rapid method for breath analysis in cystic fibrosis patients, *Eur. J. Clin. Microbiol. Infect. Dis*. 34 (2015) 745–751.

[85] C.L. Silva, R. Perestrelo, P. Silva, H. Tomas, J.S. Camara, Volatile metabolomic signature of human breast cancer cell lines, *Sci. Rep*. 7 (2017) 43969.

[86] F. Mousavi, B. Bojko, J. Pawliszyn, Development of high throughput 96-blade solid phase microextraction-liquid chromatrography-mass spectrometry protocol for metabolomics, *Anal. Chim. Acta* 892 (2015) 95–104.

[87] P.F. de Lima, M.F. Furlan, F.A. Ribeiro, S.F. Pascholati, F. Augusto, In vivo determination of the volatile metabolites of saprotroph fungi by comprehensive two-dimensional gas chromatography, *J. Sep. Sci*. 38 (2015) 1924–1932.

[88] F. Du, M.N. Alam, J. Pawliszyn, Aptamer-functionalized solid phase microextraction-liquid chromatography/tandem mass spectrometry for selective enrichment and determination of thrombin, *Anal. Chim. Acta*. 845 (2014) 45–52.

[89] D. Vuckovic, I. de Lannoy, B. Gien, In vivo solid-phase microextraction for single rodent pharmacokinetics studies of carbamazepine and carbamazepine-10,11-epoxide in mice. J. *Chromatogr. A* 1218 (2011) 3367–3375.

[90] M. Gallagher, C. Wysocki, J. Leyden, A. Spielman, X. Sun, G. Preti, Analyses of volatile organic compounds from human skin. *Br. J. Dermatol*. 159 (2008) 780–791.

[91] Z.M. Zhang, J.J. Cai, G.H. Ruan, G.K. Li, The study of fingerprint characteristics of the emanations from human arm skin using the original sampling system by SPME–GC/MS. *J. Chromatogr. B* 822 (2005) 244–252.

[92] V. Bessonneau, E. Boyaci, M. Maciazek-Jurczyk, J. Pawliszyn, In vivo solid phase microextraction sampling of human saliva for non-invasive and on-site monitoring, *Anal. Chim. Acta*. 856 (2015) 35–45.

[93] Y. Tang, S. Huang, J. Xu, G. Ouyang, Y. Liu, PLGA-based nanofibers with a biomimetic polynoradrenaline sheath for rapid in vivo sampling of tetrodotoxin and sulfonamides in pufferfish, *J. Mater. Chem. B* 6 (2018) 3655–3664.

[94] Z. Bai, A. Pilote, P.K. Sarker, G. Vandenberg, J. Pawliszyn, In vivo solid-phase microextraction with in vitro calibration: determination of off-flavor

components in live fish, *Anal. Chem*. 85 (2013) 2328–2332.

[95] N. Reiche, F. Mothes, P. Fiedler, H.A. Borsdorf, solid-phase microextraction method for the *in vivo* sampling of MTBE in common reed (*Phragmites australis*), *Environ. Monit. Assess*. 185 (2013) 7133–7144.

ریزاستخراج فاز جامد درون‌تنی: یک روش کارآمد آماده‌سازی نمونه برای آنالیز زیستی گیاهان و بافت‌های زنده

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چکیده

روش‌های قدیمی آماده‌سازی نمونه و تجزیه زیستی در زمینه آنالیز سیستم‌های زنده در شرایط آزمایشگاهی با محدودیت‌ها و نارسایی‌هایی همراه هستند. نمونه‌برداری درون‌تنی (in vivo) با هدف بهبود صحت و ارتقای عملکرد آنالیزهای زیستی به‌صورت درجا (in-situ) و درمحل (on-site)، تکنیک مناسبی برای برطرف‌سازی مشکلات روش‌های سنتی است. در این زمینه در سال‌های اخیر از میان روش‌های آماده سازی نمونه، ریزاستخراج فاز جامد (SPME) در شرایط درون‌تنی با بهره‌گیری از امتیازاتی همچون سادگی اجرا و غیرتخریبی بودن، مورد توجه ویژه محققین قرار گرفته است. این بررسی به‌طور خلاصه به کاربرد ریزاستخراج فاز جامد درون‌تنی به عنوان یک روش آماده‌سازی نمونه در سیستم‌های زنده شامل گیاهان و جانوران (به ویژه مطالعه متابولیت‌های سیستم‌های زیستی و تحقیقات بالینی) می‌پردازد. همچنین، پوشش‌های زیست‌سازگار و نوآوری‌های ریزاستخراجی که به‌منظور ارتقا حساسیت و عملکرد روش، به‌کار گرفته شده مورد بررسی قرار می‌گیرد. در نهایت، چالش‌های پیش رو در مسیر توسعه این تکنیک بررسی شده و پیشنهاداتی در زمینه پیشبرد فعالیت‌های کاربردی آن ارائه می‌گردد.

واژه‌های کلیدی

ریزاستخراج فاز جامد؛ درون‌تنی؛ تجزیه زیستی؛ پوشش؛ متابولیت.

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