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Reviews

Current Developments and Future Trends in Solid-phase Microextraction Techniques for Pharmaceutical and Biomedical Analyses

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Sample preparation is important for the isolation and concentration of desired trace components from complex matrices. Sample preparation is the most labor-intensive and error-prone process in analytical methodology, and greatly influences the reliable and accurate determination of analytes. The integration of sample preparation with various analytical instruments is most conveniently achieved by using microextraction techniques and/or microdevices. Solid-phase microextraction (SPME) is both simple and effective, enabling miniaturization, automation and high-throughput performance. Moreover, SPME has reduced analysis times, as well as the costs of solvents and disposal. This review describes current developments and future trends in novel SPME techniques, including fiber SPME, in-tube SPME and related new microextraction techniques. Especially innovative SPME approaches, including multi-well high-throughput sampling, ligand-receptor binding study for pharmacokinetics, direct *in vivo* sampling, chip-based microfluidic system, and new sampling techniques using intelligent carbon nanotube and temperature-response polymer in pharmaceutical and biomedical analysis are focused items.

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1 Introduction

Quantitative analysis of therapeutic drugs and their metabolites in biological matrices, including plasma, serum, whole blood, urine, saliva, and tissues, has been utilized extensively in pharmacokinetics, pharmacodynamics and metabolomics studies, and is becoming more important in assessing the therapeutic and toxic effects of drugs and in the discovery and development of more selective and effective drugs.[1] Drugs of abuse, illicit drugs and incidental/accidental intoxication by drugs and poisons are often analyzed in clinical and forensic toxicology.[1,2] In addition, biological and clinical studies involve the analysis of endogenous substances, such as neurotransmitters, hormones and various bioactive compounds. These substances are useful as diagnostic or prognostic biomarkers in disorders or healthcare, and are often analyzed during population screening,

disease diagnosis, and biomonitoring of therapy, and for predicting therapeutic responses.[3] Therefore, simple and rapid bioanalysis has become a significant challenge in pharmaceutical development, clinical control, doping inspection, and forensic chemistry.[1-3]

Over the last decade, there have been many technological breakthroughs in analytical methodology and instrumentation. Among these modern analytical techniques, liquid chromatography coupled with mass spectrometry is considered to be the benchmark for quantitative/qualitative analysis, due to specificity, sensitivity and speed. These analytical techniques, however, also have limitations, including matrix effect, compromised selectivity and reduced sensitivity for the analyte of interest in various complex matrices. For example, biological materials, such as blood and urine, often contain proteins, inorganic salts, and organic compounds, with many analytes in these samples present in trace amounts. Therefore, sample preparation is usually necessary to extract, isolate, fractionate, and/or concentrate the analytes from complex matrices, and these methods may greatly influence the reliable and accurate analysis of these materials.[4-14] Thus, sample preparation is the most error-prone part of the process, and has been regarded as a bottleneck in the development of sensitive, selective and precise analytical methods. The goal of sample preparation is to eliminate interfering compounds from the matrix using a minimum number of steps, resulting in a reproducible methodology. An ideal sample-preparation technique should be simple, fast, selective, efficient, solvent-free, inexpensive, easy to automate, suitable for miniaturization or downsizing of the analytical system, and safe for both the operator and the environment. It should also give reproducible and high recoveries without degradation of the analyte, and be compatible with a wide range of separation methods and applications.

These purposes may be best accomplished by microextraction techniques or microdevices that integrate sample preparation with various analytical instruments. Solid-phase microextraction (SPME) is particularly remarkable due to its simplicity and effectiveness. SPME, first developed by Arthur and Pawliszyn[15] in 1990, is based on the adsorption or absorption of analytes directly from an aqueous sample onto a fused-silica fiber coated on the outside with an appropriate stationary phase. The extraction process is usually non-exhaustive, and only a small fraction of the initial amount of analyte is separated and introduced into an analytical instrument. SPME is fast, solvent-free and easily coupled to gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Moreover, SPME has many advantages over traditional sample-preparation methods, such as liquid–liquid extraction and conventional solid-phase extraction (SPE). SPME techniques have been used in many fields, including food, environmental, biological, pharmaceutical, and forensic analyses, with thousands of articles published to date. Furthermore, technological innovations in materials science and robotics, and an in-depth understanding of biological matrices have led to the development of many new strategies in SPME techniques. The details of SPME techniques have been described in a number of reviews[16-52] and SPME books.[53-55] In addition, we reviewed recent advances in SPME techniques in biomedical analysis, and summarized their applications to pharmacotherapeutic, forensic and diagnostic analyses.[37] In this review, we present current developments and future trends in novel SPME techniques, including fiber SPME, in-tube SPME and other new SPME related techniques. Especially, innovative applications of microextraction techniques to multi-well high-throughput sampling, ligand-receptor binding study, direct *in vivo* sampling, chip-based microfluidic system, and new sampling techniques using intelligent carbon nanotube and temperature-response polymer in pharmaceutical and biomedical analysis are focused items.

2 Current Developments of SPME Techniques in Bioanalysis

SPME techniques can be classified roughly into static batch equilibrium microextraction and dynamic flow through equilibrium microextraction methods (Fig. 1). Fibers (fiber SPME) and capillary tubes (in-tube SPME) coated with an appropriate stationary phase are usually used for SPME, though alternative microextraction techniques have been developed using stirring bars (SBSE), thin films (TFME), microsyringes (syringe SPME) and pipette tips (in-tip SPME). New developments include the use of newly designed coatings and devices with improved extraction efficiency, and the use of new systems that interface with various analytical instruments and automated on-line systems. Various SPME methods have been developed to determine the compounds present in biological samples, including urine, serum, plasma, whole blood, saliva, breath and hair.[16-55] This section reviews these novel SPME techniques according to their configurations and implementations, the development of new coatings and devices, and their more recent applications in bioanalysis.

2·1 Fiber SPME techniques

A fiber SPME device (Fig. 1A) consists of a fiber assembly with a built-in extraction fiber inside a needle and an assembly holder. Retractable SPME fibers containing fused silica (1 or 2 cm long) are now commercially available. When the fiber is inserted into the sample, the target analytes partition from the sample matrix into the polymeric stationary phase coated onto the outer surface of the fiber until equilibrium is reached. In contrast to conventional SPE with packed-bed columns, this arrangement combines all steps of sample preparation into a single step. Two fiber SPME techniques can be used in the extraction of analytes: direct immersion (DI) of SPME fiber into a liquid sample matrix; and headspace (HS) extraction, in which the sample matrix is heated in a vial to volatilize the analytes and the fiber is placed just above the sample matrix. Fiber SPME is routinely used in combination with GC or GC-MS by thermal desorption in the GC injection port, and can be successfully applied to a wide variety of volatile and semi-volatile organic compounds in gaseous, liquid and solid samples. It can also be coupled directly with HPLC and LC-MS to analyze weakly volatile or thermally labile compounds not amenable to GC; also an SPME/HPLC interface equipped with a small-volume solvent chamber has been utilized for solvent desorption prior to HPLC analysis. These techniques can be performed manually or automatically, with automation of fiber SPME methods accomplished using the commercially available Combi-PAL (CTC Analytics; Zwingen, Switzerland), MPS 2 (Gestel Inc.; Mulheim and der Ruhr, Germany), TriPlus (Thermo Fisher Scientific; Milan, Italy), and Concept 96 (PAS Technology; Magdala, Germany) autosamplers. These systems can be easily programmed to perform various sample preparation steps, such as dilution, agitation and extraction, thus providing a number of advantages, including reduced times for routine analysis and development of analytic methods, faster sample throughput and greater reproducibility. The Concept 96 robotic system is especially suitable for automated high-throughput analysis with a multi-fiber SPME configuration (Sect. 3·1), and

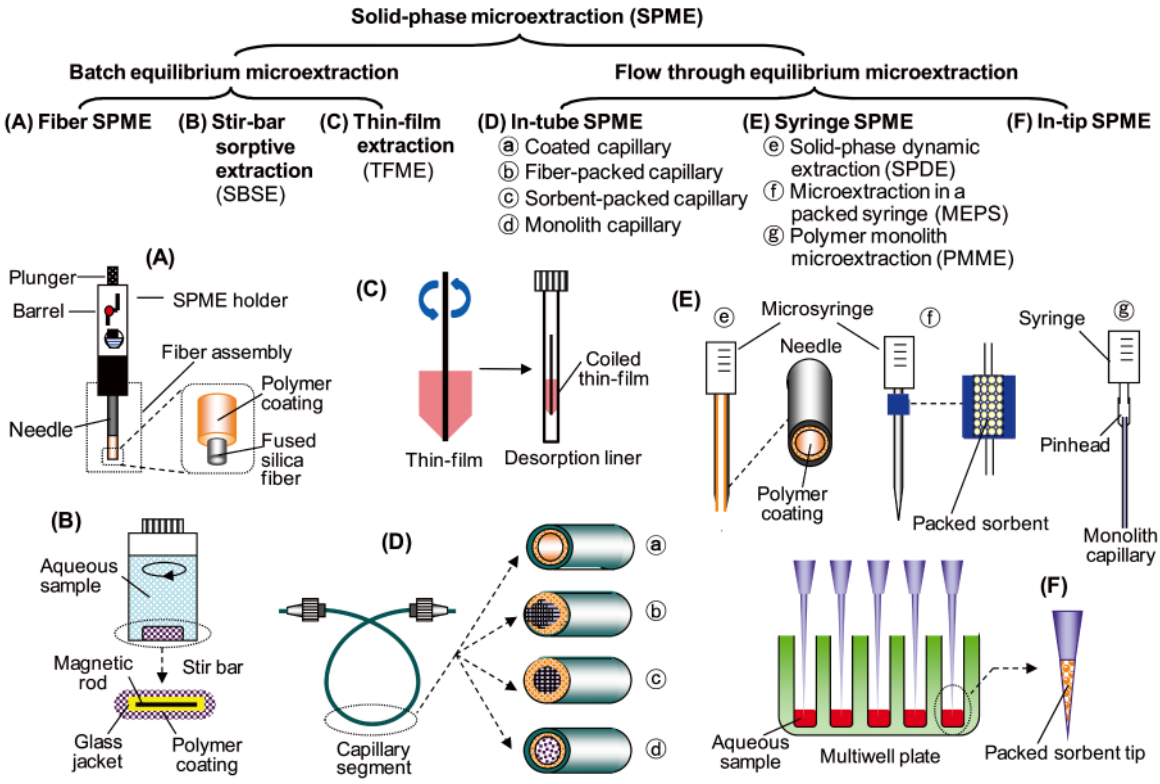

Fig. 1 Classification and configurations of SPME methods.

is utilized in drug-protein binding studies (Sect. 3·2). The details of automated SPME sampling systems are also reviewed.[20,24,27,35]

Success of SPME is determined by the physicochemical properties and the thickness of the coating. A number of fiber coatings, which offer a range of analyte solubilities and porosities, are commercially available. These include apolar polydimethylsiloxane (PDMS) for the extraction of non-polar analytes, more polar polyacrylate (PA) for the extraction of polar analytes (especially phenols), PDMS–divinylbenzene (PDMS-DVB) for the extraction of polar analytes (especially amines), Carboxen–PDMS (CAR-PDMS) for the extraction of volatile/low molar mass analytes, Carbowax–DVB (CW-DVB) for the extraction of polar analytes (especially alcohols), CW–template resin (CW-TPR) for the extraction of polar analytes, and DVB-CAR-PDMS for the extraction of a broad range of analytes. The StableFlex-type fiber has a flexible fused-silica core, and is less breakable. Furthermore, new superelastic metal fiber assemblies are developed to enhance the durability and shape memory, and to more robustly perform several hundreds of analyses. Fiber coatings are also available in increasing thicknesses of 7 – 150 µm, which increases the partitioning ratio of the target analytes, but also increases the equilibration times. HS- or DI-SPME techniques using PDMS fibers have recently been used to extract illicit drugs, recreational drugs (amphetamines and cocaine),[56] opioids (codeine, morphine and acetyl morphine)[57] and cannabinoids[58] from hair, urine and plasma samples and to subsequently analyze them by GC-MS. The analysis of drugs in hair samples has become particularly popular in recent years, with possible applications in forensic and clinical toxicology for the retrospective detection of chronic drugs of abuse. Other applications of fiber SPME methods in pharmaceutical, biomedical and forensic analysis have been summarized in previous reviews.[16-37]

2·2 Other batch equilibrium microextraction techniques

Some variations of static batch equilibrium SPME are based on the configuration of the extraction device, such as a coated stirring bar and thin film. Stir-bar sorptive extraction (SBSE)[41-43] (Fig. 1B) is a new sample-preparation technique that overcomes the limited capacity of SPME fibers. Magnetic PDMS-coated stirring bars are now commercially available as Twister® stir-bar (Gerstel; Mülheim, Germany), similar to SPME but with a thicker layer (0.3 – 1.0 mm), resulting in a phase 50 – 250 times greater than that in SPME. However, SBSE essentially requires manual handling. For DI-SBSE, the stirring bar is placed in a suitable volume of a liquid sample in a vial or other container, and the sample is stirred until the partition equilibrium time is reached. The stirring bar is then easily removed with tweezers, rinsed with purified water to remove adsorbed sugars, proteins, and other sample components, and dried with clean paper tissue to remove residual water droplets. For HS-SBSE, the stirring bar is placed on the headspace of a liquid or solid sample; special devices to hold the stirring bar in place are available. Thermal desorption with GC and solvent desorption in combination with LC can be used. An SBSE/GC-MS method has been developed to detect basic drugs in blood, urine and tissue samples for routine drug screening in forensic toxicology.[59] Recently, a highly sensitive analytical method using SBSE with *in situ* derivatization and thermal desorption GC-MS was developed for the simultaneous measurement of trace amounts of phenolic xenoestrogens in human urine samples.[60] Furthermore, SBSE in combination with HPLC-UV could be used for therapeutic drug monitoring of carbamazepine, phenytoin and phenobarbital in plasma samples.[61]

Although the sensitivity of the SPME method can be improved by increasing the volume of the extraction phase, increased thickness of only the extraction phase would require a much

longer equilibration time because the extraction rate is controlled by the thickness of the coating. Thin-film microextraction (TFME)[35] (Fig. 1C) was recently developed to increase the mass uptake rates and the sensitivities of SPME. A thin sheet of PDMS membrane with a large surface area was found to have a much higher extraction phase volume than other SPME configurations, such as fibers and rods. To conveniently introduce the membrane to the analytical instrument, the membrane can be attached to a holding rod; after extraction, the membrane can be rolled around the rod and introduced into the injection system for desorption of extracted components. This approach is especially applicable to hydrophobic semivolatile components with high distribution constants. Recently, a TFME SPME/LC-MS/MS method was developed to measure free and conjugated testosterone and epitestosterone in urine samples for clinical diagnosis and therapy.[62] Furthermore, a new configuration of the C_{18} thin-film extraction phase has been applied to the LC-MS/MS analysis of benzodiazepines in spiked urine samples. High-throughput analysis was achieved by using a robotic autosampler, which enabled parallel analyte extraction in a 96-well plate format (Sect. 3·1).[63]

2·3 In-tube SPME techniques

In-tube SPME[44,45] using a capillary column (Fig. 1D) was developed for the miniaturization, automation, high-throughput performance, on-line coupling with analytical instruments and to reduce solvent consumption. Unlike fiber SPME, in-tube SPME typically uses a short inner-wall coated fused-silica capillary (Fig. 1D-a). In contrast, fiber-packed (Fig. 1D-b), sorbent-packed (Fig. 1D-c) and rod-type monolith (Fig. 1D-d) capillaries have been developed to improve extraction efficiency and specificity. The fiber-packed format consists of a capillary tube packed with fibrous rigid-rod heterocyclic polymers,[46,47] and sorbent-packed and rod-type monolith formats consist of micro-LC capillary columns packed with the extracting phase. In these formats, analytes are absorbed or adsorbed onto the outer surface of the packed fibers and sorbents. In-tube SPME operating systems can be categorized as flow-through extraction systems, in which solutions are passed continuously in one direction through an extracting capillary column; or as draw/eject extraction systems, in which the sample solution is repeatedly aspirated into and dispensed from an extracting capillary column. The extracted analytes can be desorbed by introducing a stream of mobile phase or a static desorption solvent, and then analyzed by an off-line or on-line system coupled with a GC, LC or CE. In-tube SPME can be automated to directly extract target analytes in aqueous matrices by column-switching techniques.

Several commercial GC capillary columns are currently available for in-tube SPME.[45] Silica-modified columns have been found to be more suitable for the analysis of nonpolar compounds. Sufficiently bonded and cross-linked liquid-phase type capillaries can prevent the loss of phase when the solvent is passed through the capillary. An adsorptive-coated Supel-Q-PLOT capillary was found to be more efficient for the analysis of steroid compounds[64] due to its large surface area and enhancing mass-transfer kinetics, and has been used for the anti-doping analysis of urine samples by in-tube SPME/LC-MS. Furthermore, an automated on-line in-tube SPME coupled with LC-MS using a CP-pora PLOT capillary was developed for the determination of nicotine, cotinine and related alkaloids in urine and saliva.[65] This method is useful for monitoring tobacco smoking and for estimating the uptake of nicotine and tobacco-related toxicants in individuals being treated for nicotine addiction or dependency. Other recent applications of in-tube SPME methods in pharmaceutical, biomedical and forensic analyses have been summarized in previous reviews.[7,11,19-23,31,37]

2·4 Other flow through equilibrium microextraction techniques

Some variations of dynamic flow through equilibrium SPME are based on the configuration of the extraction device, such as a microsyringe (syringe SPME) (Fig. 1E) and pipette tip (in-tip SPME)[47,51,52] (Fig. 1F). Syringe SPME methods include solid-phase dynamic extraction (SPDE)[11,23,28] (Fig. 1E-e), microextraction in a packed syringe (MEPS)[48–50] (Fig. 1E-f) and polymer monolith microextraction (PMME)[10,66,67] (Fig. 1E-g). Dynamic SPDE sampling is performed automatically by passing the headspace through the tube using a syringe. An automated SPDE technique using a hollow needle with an internal coating of PDMS instead of a capillary column may be suitable for headspace extraction coupled with GC-MS, and can be used for the determination of illicit drugs, such as amphetamines, cannabinoids and methadone, in hair samples.[68,69] MEPS is a new technique for sample preparation coupled on-line with GC-MS and LC-MS. This form of miniaturized SPE uses a procedure similar to in-tube SPME and SPDE. A new on-line MEPS sample-preparation technique has also been applied to the analysis of local anesthetics in human plasma.[70,71] PMME sampling using a short monolithic capillary is performed by connection to a plastic syringe using a plastic pinhead connector.[72] In PMME, a syringe infusion pump is used to drive the sample and desorption solution through the monolithic capillary. The eluate is then collected in a vial and subsequently analyzed by LC or CE.[73–76]

An in-tip SPME packed sorbent at the top of a micropipette (Fig. 1F) is a miniaturized version of the conventional SPE technique. This microextraction technique has recently been used for the purification, concentration, and selective isolation of proteins and peptides in genomics, proteomics, and metabolomics studies. The entire extraction process is accomplished by repeated aspirating/dispensing cycles using a manual micropipettor. Compared with conventional SPE, tip extraction is easier and faster, relatively inexpensive, and uses disposable materials. Although extraction is usually performed off-line, packed pipette tips have the advantage of automatically processing several samples in parallel and are especially useful for high-throughput analysis of 96-tip arrays (Sect. 3·1). The extraction tips are now commercially available as ZipTip (Millipore; Bedford, MA), Omix (Varian; Palo Alto, CA), and NuTip and MonoTip C_{18} tip (GL Sciences; Tokyo, Japan). MonoTip C_{18} tips containing fixed C_{18}-bonded monolithic silica gel has been used to extract several drugs from urine and plasma samples.[77-79] Recently, an in-tip SPME using Omix C_{18} coupled with LC-MS/MS was developed for the determination of illicit drugs, such as stimulants, hallucinogens, ketamine and phencyclidine, in oral fluids.[80] Furthermore, a high-throughput in-tip SPME using 96-well pipette tips containing a chemically bonded monolithic methacrylate sorbent was developed for drug analyses in plasma (Sect. 3·1).[81,82]

2·5 New coatings and devices for SPME

Polypyrrole[34] and polythiophene coatings are intrinsic conducting polymers, which are positively charged and can be utilized for the efficient extraction of polar, aromatic and anionic compounds. Polypyrrole coating has higher extraction efficiencies than commercial SPME coatings. Polypyrrole and polythiophene-coated fibers have been utilized to extract antibiotics used to treat multi-resistant *Staphylococcus aureus*

from blood[83] and adrenolytic drugs from plasma.[84] Immunoaffinity coatings, containing covalently immobilized antibodies on the surface of a fused-silica fiber, are useful for the selective and sensitive extraction of analytes.[85,86] Molecularly imprinted polymers (MIPs)[87-90] are stable, selective and cross-linked synthetic polymers synthesized by the copolymerization of a monomer with a cross-linker in the presence of a template molecule, and used to coat SPME fibers, inner surfaces of capillaries and sorbent particles. Testosterone-imprinted SPME fiber was developed for the selective extraction of anabolic steroids in urine samples and their analysis by GC-MS.[91] The principle of restricted access materials (RAM),[92] in which commercially available 35-μm LiChrospher RP-18 alkyl-diol-silica and ion exchange diol silica RAM particles are glued to a cleaned stainless-steel wire, has been adopted for direct SPME extraction from blood. This RAM-based SPME approach was able to simultaneously separate proteins from a biological sample, while directly extracting the active components from a natural drug. MIP and RAM particles have also been used for packed PEEK capillary, syringe and tip SPME techniques. Recently, biocompatible in-tube SPME coupled with HPLC-fluorescence detection was developed for the therapeutic monitoring of interferon α in plasma samples.[93] Protein-coated silica RAM has also been used to prepare lab-made biocompatible capillaries that enable the direct injection of biological fluids and the simultaneous exclusion of macromolecules by a chemical diffusion barrier and drug preconcentration.[93] Furthermore, a pencil lead fiber with a custom-designed unique extraction phase was found to be useful for SPME sampling of trace amounts of methamphetamine from human saliva.[94]

In contrast, sol-gel porous silica coatings,[95,96] in which organic structures are deposited onto inorganic polymeric structures, have been introduced to overcome some of the problems of commercial fibers, such as solvent instability and swelling, low operating temperature and stripping of the coating. In the sol-gel coating technique, hydroxyl-terminated siloxane polymers or polymers mixed with polyethylene or polypropylene glycol are bonded to Si-OH groups on the fused-silica surface. The sol-gel technology can be utilized effectively to selectively coat SPME fibers and capillaries. Ionic and molecular recognition by these materials can be achieved by controlling the pore size and morphology of the silicate host structure; by introducing specific functional groups such as crown ethers, calixarenes and β-cyclodextrin into the dense framework; or by utilizing molecular imprinting or templating strategies. A fast and sensitive method based on DI-SPME and using a sol-gel derived fiber and post-derivatization on the fiber coupled with GC-MS was developed to analyze fatty acids in the sputum of 21 patients with pulmonary tuberculosis.[97] A new SPME method using an ion liquid-based fused-silica fiber[98] was developed to assay for amphetamine and methamphetamine in human urine.[99] Although the extraction efficiency of ion liquid fiber is lower than that of 100 μm PDMS, this method is simple, fast and sensitive due to its ability to select a wider number of both cations and anions, and has advantageous physical and chemical properties, including hydrophobicity, viscosity, thermal stability, selectivity and vapor pressure.

Various highly efficient monolithic materials[66,67,100] were recently prepared by *in situ* hydrolysis and polycondensation. The hydrophobic polymeric bone structure and the acidic pendant groups of poly(methacrylic acid-ethylene glycol dimethacrylate) make it superior for extracting basic analytes from aqueous matrices. In combination with LC or CE, various monolithic capillary columns have been used as in-tube SPME devices to determine the drug concentrations in biological samples. The biocompatibility of these monolithic structures allows the direct analysis of drugs of abuse in urine samples with no manipulations other than dilution and/or centrifugation, simplifying the entire process. A new MIP monolith has been developed for in-tube SPME of 8-hydroxy-2′-deoxyguanosine, a biomarker of *in vivo* oxidative DNA damage.[101] These monolithic capillaries showed excellent reusability and high stability at extreme pH, due to a low-pressure drop that allowed a high flow-rate to achieve high-throughput, and good binding capacity based on its higher porosity. Furthermore, an imprinted porous polymer monolith fiber coated with home-made capillary glass[102] and poly(ST-DVB) carbon monolith fiber carbonized by heating the porous polymer[103] showed higher extraction efficiency than other commercially available SPME fibers. More recently, a boronate affinity monolith,[104,105] and C_{18} and titania-coated silica monolithic tips,[106] as sorbents for PMME, were used for the selective extraction of hydrophilic proteins and phosphopeptides from biological samples. Applications of these methacrylate monolithic tips were extended to the extraction of pindolol, metoprolol and local anesthetics from human plasma.[82] The details of these new SPME coatings have also been reviewed.[21,33,107-111]

3 Innovative SPME Approaches in Pharmaceutical and Biomedical Analysis

This section reviews more recent innovative SPME approaches in pharmaceutical and biomedical applications, including high-throughput sampling using multi-well plate techniques, direct *in vivo* SPME sampling, SPME for a ligand-receptor binding study, a chip-based microfluidic system, and new intelligent coating devices, such as fiber coated with multi-walled carbon nanotubes and capillary coated with temperature-response polymer.

3·1 Multi-well high-throughput sampling

Many biological applications generate numerous samples for analysis, and the total analysis time may prove to be impractical when these samples are analyzed sequentially. Therefore, parallel extraction and desorption of multiple samples on a multi-well plate format would be more efficient. This may be accomplished by new automated multi-well high-throughput sampling systems using small multiple SPME fibers and multiple micropipette tips.[20,24,25,28,33-35,37,112]

The Concept 96 autosampler described in Sect. 2·1, can be utilized for the robotic automation of the extraction, agitation and liquid desorption steps of multi-fiber SPME for many samples in parallel, resulting in very accurate control of the timing of SPME steps and reproducible positioning of all the fibers in the wells. A 96-fiber device based on a commercially available pin-tool replicator is compatible with this well-plate system. An automated method for the preparation of multiple samples simultaneously has been developed utilizing an SPME multi-fiber device, two orbital shakers, and a three-arm robotic system, followed by LC-MS/MS analysis. An optimized multi-fiber SPME LC-MS/MS method was subsequently fully validated for the high-throughput analysis of benzodiazepines in human whole blood.[113] This method allowed the automated preparation of 96 samples in 100 min, which represents the highest throughput of any SPME technique to date, while achieving excellent accuracy, precision and sensitivity. An automated multi-fiber SPME can improve the assay precision and reproducibility by reducing human intervention, allowing

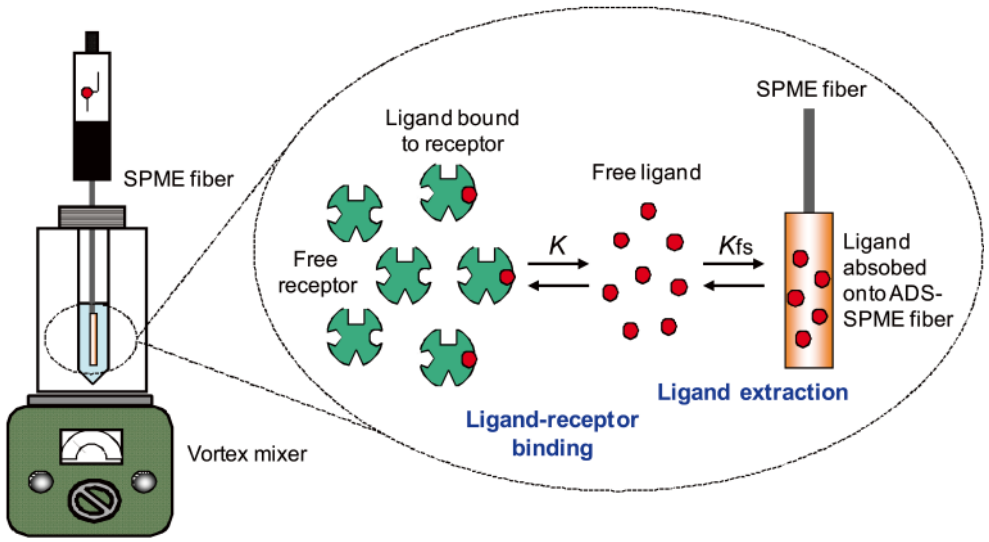

Fig. 2 Experimental setup for the determination of free concentrations and binding constants. K, Binding constant between ligand and receptor; K_{fs}, partition coefficient of free ligand between fiber and solution. Reproduced from Ref. 25 with permission.

for high-throughput screening suitable for drug discovery and *in vivo* monitoring.

Recently, a 96-thin film device with large surface areas as extraction phases for TFME (Sect. 2·2) was developed for parallel extractions from a 96-multi-well plate. The Concept 96 robotic system has adopted the thin-film configuration for automated high-throughput analysis. The absolute recoveries of benzodiazepines from phosphate buffer were significantly higher using thin-film extraction phases made from C_{18} particles than using the multi-fiber rod configuration.[54] Agitation and mass-transfer properties of benzodiazepines in each well were improved at higher agitation speed (900 rpm), with a minimum extraction time of 10 min. For both urine and PBS samples, excellent intra-well and inter-well reproducibilities and limits of detection and quantitation were observed for all analytes.

A modified device has also been developed to connect the Chromolith capillary column to the end of a 96-well plate.[114] Empty capillaries, 2.4 mm in length and with an inner diameter of 75 mm, are inserted into the bottom of each well of a 96-well V-shaped polypropylene plate. The plate incorporated capillaries with an inter diameter of 200 mm, which were filled with reversed-phase modified Chromolith. This technique, combined with a MALDI system, has been utilized for peptide analysis. The two-plate extraction improved the process stability and enabled high-throughput analysis. Furthermore, the in-tip SPME can simultaneously handle 96 samples within 2 min in commercially available systems that use a 96-well format furnished with polymer monolithic tips and a robot.[51,115] Using tips developed in-house, a packed 96-tip assembly was used to successfully extract drugs in human plasma[115] and vitamin D_3 in serum.[116] Local anesthetics, anticancer drugs, and β-blockers in human plasma could be also successfully detected by this tip extraction coupled with LC-MS/MS.[82]

3·2 Ligand-receptor binding study

Interactions between ligands and receptors (usually proteins) to form molecular complexes are vital in all basic life sciences.[25] Drug binding to specific plasma transport proteins is an integral step in many intermolecular interactions. Determining the amount of drug bound to protein is important in pharmacodynamics and pharmacokinetics studies in order to understand drug metabolism and partitioning and to optimize the treatment of individual patients, as well as being an essential step in drug discovery and in clinical phases of drug development. All of these binding constants and interactions can be investigated by measuring the concentrations of the bound and/or free form of the drug.

SPME can be utilized practically to assess drug-protein binding. Using fiber SPME, the free concentration of drug can be calculated as the ratio between the amount of analyte extracted and the binding constant of the fiber (fiber constant). Briefly, in the presence of an SPME fiber, an amount m (moles) of a drug is extracted from the solution, with the amount on the fiber being in equilibrium with the free concentration of the drug (Fig. 2). The free concentration of drug remaining in the solution (C_{free}) can be expressed as m/f_c, where f_c is the fiber constant, and represents the product of the partition coefficient of the drug (between fiber and solution without binding matrix) and the volume of the fiber (for liquid coatings) or the active surface of the fiber (for solid coatings). Using special materials for the extracting phase, the large receptor molecules are prevented from being coextracted. The amount of ligand extracted can be quantified by any method that can be coupled to SPME, including GC, LC, CE, MS, or radiometry. The method has been successfully utilized for the *in vivo* and *in vitro* pharmacokinetic measurements of free concentrations of ligand.[113,117–125] Whole-blood and free concentrations of ligand, easily measured by SPME, are of utmost importance in therapeutics, since they correlate with the pharmacological effects of drug and are more significant than plasma concentrations. Using PDMS and PA coatings, the binding of several drugs with different polarities and binding constants to human plasma and serum albumin has been determined, indicating that this method is thermodynamically sound, requiring a small volume of sample and a short analysis time.[118] Since the coating of the SPME fibers might also adsorb proteins, the preparation of coatings that prevent protein adsorption (biocompatible coatings) represents an important step towards increasing the applicability of SPME for the analysis of biological samples.

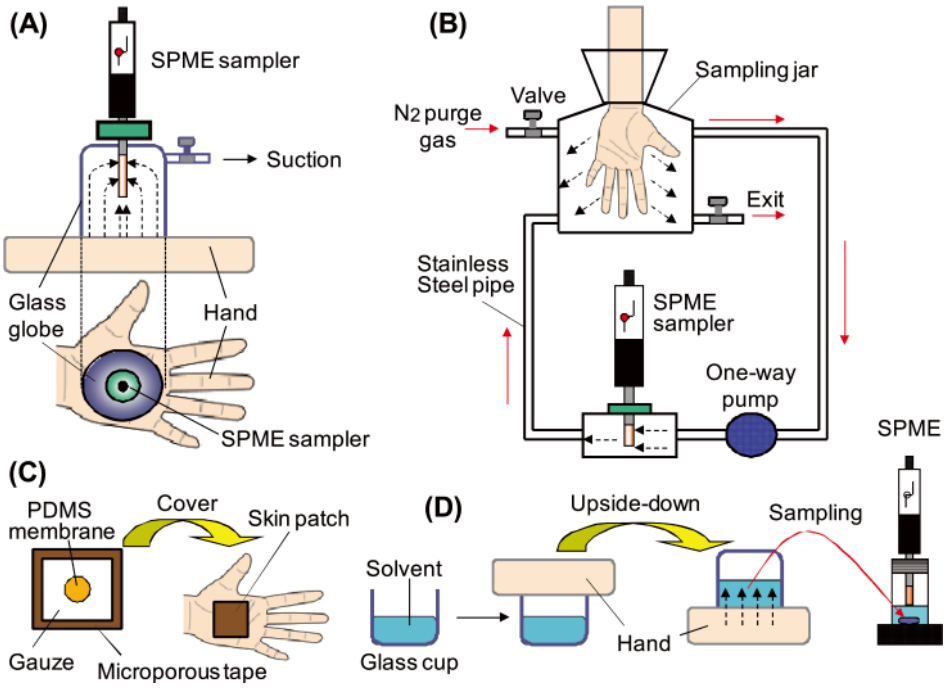

Fig. 3 Direct sampling systems of volatile compounds emitted from the skin. (A) Direct SPME in sealed glass globe, (B) direct SPME in flow sampling chamber, (C) direct SPME in skin patch using PDMS membrane and (D) liquid sampling in glass cup.

3·3 Direct *in vivo* biogas sampling

Hundreds of substances (*e.g.*, carboxylic acids, alcohols, aldehydes, aliphatics, esters, ketones, amines, mercaptans) are synthesized by metabolism, and emanate from human breath and skin. Any changes in metabolism equilibrium can alter human emanations, and their individual constituents can act as informative biomarkers to identify criminals and diagnose several diseases. *In vivo* SPME can be used to sample volatile and non-volatile emanations, such as breath exhalations and skin emissions using commercially available devices placed in direct contact with body parts or by sampling gases.

The analysis of human breath can provide benefits, such as better patient acceptance and reductions in blood samples, and is useful for monitoring the drug concentration, screening for toxicological exposure and detecting disease. Volatile organic compounds (VOCs) in human breath are good biomarkers for the diagnosis of cancer[126-132] and the detection of *Helicobacter pylori*.[133] Tedlar bags and specific breath sampling tubes, such as Bio-VOC® and Alveolar breath gas samplers, are usually used for SPME sampling of human breath, and the analytes in collected gas are extracted by direct exposure of SPME fiber in bag sand tubes. VOCs and organic metabolites in exhaled breath from healthy volunteers, smokers, nonsmokers and lung cancer patients with and without treatment, have been measured by SPME coupled with GC-MS in order to differentiate healthy volunteers from lung-cancer patients.[125,127] An analysis of volatile aldehydes in breath samples by HS-SPME on-fiber derivatization/GC-MS found that exhaled concentrations of C_5 – C_9 aldehydes were significantly higher in lung-cancer patients than in smokers and healthy controls, suggesting that exhaled aldehydes reflect aspects of oxidative stress and tumor-specific composition and metabolism.[129] The analysis of human breath by HS-SPME/GC-MS has also been used to monitor intravenously administrated anesthetics, such as propofol.[134]

Four approaches for direct *in vivo* SPME sampling of skin emissions, include a sealed glass globe (Fig. 3A) or funnel,[135] a flow sampling chamber (Fig. 3B),[136] a skin patch[137,138] using PDMS membrane (Fig. 3C) and liquid sampling in a glass cup (Fig. 3D). In the first and second approaches, SPME fibers are placed directly over the skin emissions; in the third approach, PDMS membranes can be placed in direct contact with skin; the fourth approach can be used to further increase the extraction of more polar and non-volatile compounds. Using direct SPME in flow sampling chambers (Fig. 3B), VOCs were extracted onto PDMS/DVB fibers exposed to emanations from human arm skin for 30 min at room temperature, and the levels of VOCs were used to study the fingerprint characteristics of human odors.[136] New devices using sorptive tape[137] and PDMS membrane[138] have also been utilized for direct SPME sampling of skin emissions. For example, a skin patch of PDMS membrane (Fig. 3C) was developed to trap VOCs from skin. The sampling-patch ($20 \times 15 \times 0.45$ mm) was placed onto skin, covered with cotton wool pads, secured to the skin with microporous tape, and sealed in thermal desorption tubes, which were stored in airtight containers at 4°C before GC-MS analysis. This method is currently being used to determine VOC profiles in patients with skin cancer, fibrotic skin disorders, wound healing and infection. We have also developed a new SPME sampling technique to measure volatile odor mercaptans exhaled from breath, palm skin and saliva. Dimethylsulfide and dimethyltrisulfide, which may be biomarkers for breast cancer, were detected in breath exhalations and saliva and in skin emissions, respectively. In these assays of volatile compounds, PDMS/DVB fibers were usually used for fiber SPME.

3·4 Direct *in vivo* drug monitoring

In vivo SPME can be used to monitor and quantify intravenous concentrations of drugs and metabolites in animals, without the need to draw blood samples in pharmacokinetic,

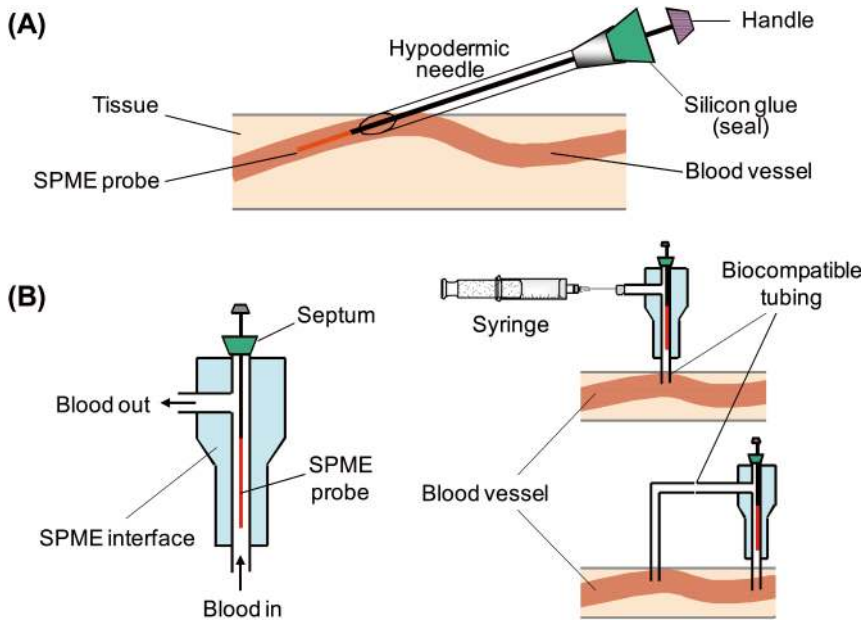

Fig. 4 Direct *in vivo* SPME systems for sampling from flowing blood vessels. (A) Direct insertion of a SPME device into a blood vessel and (B) placement of an SPME device and surgically connection of an interface into the carotid artery.

bioaccumulation, metabolomic and environmental toxicologic studies.[24,25,27,30,35,39,40,87,117-125] Two configurations of proposed *in vivo* SPME sampling systems are illustrated (Fig. 4). For larger animals, such as dogs, the SPME device should be small enough for direct insertion into a peripheral vein, whereas, for smaller animals, including rodents, a special interface is used for probe insertion due to smaller blood vessel size. In addition to blood sampling, *in vivo* SPME has also been successfully used for the sampling of tissues, including brain, muscle, adipose tissue and liver. *In vivo* SPME analysis greatly simplifies and shortens the analytical effort and time required, and limits the exposure of laboratory personnel to blood. It also offers the potential of monitoring dynamic processes, providing faster results and capturing short-lived and/or unstable analytes.[39]

The most complex *in vivo* sampling procedures include direct exposure of the SPME fiber to the bloodstream (Fig. 4A). The fiber coating is either carefully rubbed against the sample or inserted with a special *in vivo* device. Direct exposure devices must incorporate a mechanically strong, flexible and unbreakable fiber core, and their materials must be biocompatible so as to avoid toxic reactions caused by direct exposure to biological sample and to minimize the possibility of surface fouling of the coating during the short experimental sampling times. This may be achieved by covering the surface of the sorbent with a known biocompatible polymer, such as polypyrrole,[117] polyethylene glycol[120,121] or polyacrylonitrile,[87] which permits the diffusion of low-molecular weight analytes to the sorbent, while excluding high-molecular weight species, such as proteins. *In vivo* SPME probes have diameters ranging over 100 – 200 µm, the coating thicknesses ranging over 5 – 200 µm and coating lengths varying from 1 – 15 mm. Modified devices with 1 – 2 cm long coatings housed inside a hypodermic needle can be particularly useful, and the small dimensions of these devices minimize tissue damage. *In vivo* SPME sampling[119-123] of flowing blood is faster than the current methods based on drawing blood, with the former having sampling times of 2 min for external calibration and 30 s for standard on fiber approaches. *In vivo* sampling can minimize the errors associated with sample preparation, and can limit the exposure of personnel to hazardous biological samples. This *in vivo* SPME method was used to obtain full pharmacokinetic profiles of diazepam in rats and mice,[118] with results well correlated with those of a standard analytical method based on blood drawing. SPME sampling greatly facilitates animal handling, and eliminates the loss of blood almost completely. This leads to less stress on animals, making the pharmacokinetic data more biologically relevant, and resulting in the use of fewer animals to obtain reproducible data.

Due to the small size of rodent blood vessels, which prevents direct insertion of SPME probes (Fig. 4B),[118] *in vivo* sampling of small rodents requires an interface. Y-shaped interfaces, designed to allow the recirculation of blood to the animal, are prone to clotting problems, such that adequate blood flow through the interface cannot be maintained for prolonged periods of time. An alternative approach, in which only one tube is connected to a catheter, was found to work much better, and blood flow in this design is provided by manual push/pull with a syringe. For example, these fibers were used in the *in vivo* pharmacokinetic determination of diazepam and carbamazepine in rats and mice.[119-122,125] The catheters were plugged and exteriorized at the nape of the neck, with the lower tube of the interface connected to the carotid artery catheter, and the upper tube of the interface either recirculated to the carotid artery catheter or connected to a syringe. After administration of a drug by bolus injection into the jugular carotid artery catheter, the SPME device was placed through the septum into the interface and exposed to flowing blood, and the drug and its metabolites were analyzed by LC-MS/MS.

3·5 Chip-based microfluidic system

Due to limited amounts of samples, bioanalytical methods seek to reduce time, size, cost, and contamination. The development of a completely integrated microfluidic system for total analysis in genomics, proteomics and bioinformatics is

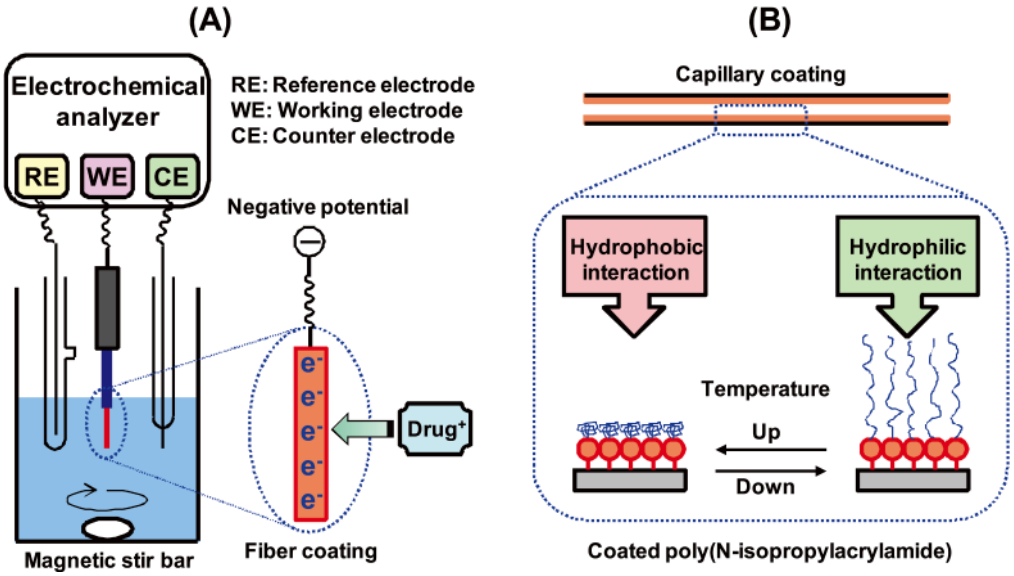

Fig. 5 Schematics of (A) EE-SPME device and (B) in-tube SPME device coated with temperature responsive polymer, and their fundamental mechanism. Reproduced from Refs. 150 and 151 with permission.

therefore of great interest. A microfluidic device, so-called "lab-on-a-chip", can integrate one or several laboratory functions on a single chip only a few square centimeters in size. This new device promises a reduced analysis time and reagent consumption, low cost, short reaction time, the ability to integrate multiple functions onto a single device, easy automation and the potential for high-throughput analysis.[139-142] The chip-based microfluidic concept has greatly promoted the use of nanotechnology in laboratories and hospitals.[140] Many microfluidic systems have been developed to handle multiple procedures for miniaturized analysis, including nucleic acid purification, polymerase chain reaction (PCR), and electrophoretic separation. These chip-based microfluidic systems can be widely employed for the purification and enrichment of biological molecules, including DNA, proteins, and peptides, to determine metabolic profiles and genetic transformation.[139-148] Recently, a microfluidic blood-sampling device using a double-layer PDMS chip was developed for automated collection of nL-size blood samples from mice, and applied to *in vivo* quantitative small-animal PET analysis.[145] The challenges in this technology are the design of the chip and the incorporation of proper materials for diverse purposes. Silica or silica-related materials and polymer monoliths are used as microfluidic devices. For example, a microchip containing a monolith has been integrated with an ion-trap mass spectrometer using a modified commercial interfacing system, and has been shown to be effective for the purification of imipramine from urine, with an up to 208-fold enrichment.[143] All of these characteristics offer a great opportunity to overcome the difficulties of repeated blood sampling, and to reduce the total blood loss in an animal, factors important for pharmacokinetic studies.

3·6 New intelligent coating nano-materials

A multi-walled carbon nanotubes/Nafion-coated fiber was developed for an electrochemically enhanced SPME (EE-SPME).[149,150] The EE-SPME device is constructed as a three-electrode system (Fig. 5A). By applying a mild negative potential (–0.6 V) to the EE-SPME device, the extraction of basic drugs from an aqueous medium was enhanced by electrophoresis and complementary charge interaction. The EE-SPME method can be coupled with a sophisticated chromatographic system, such as GC-MS, and may become useful for clinical, forensic and pharmaceutical drug analysis, since it is simple, straightforward and sensitive, and does not require any other sample pretreatment. This technique was successfully utilized to enhance the extraction of several basic drugs, such as methamphetamine, amphetamine, ephedrine, 3,4-methylenedioxyamphetamine, atropine, methadone, cocaine, codeine, acetylcodeine and papaverine, at concentrations of 157 to 2199 fold greater than prior to extraction. Further work will focus on EE-SPME with a positive potential, to enhance the extraction of negatively charged drugs from an aqueous medium (*e.g.*, drugs containing a carboxylic or phenolic group).

Recently, a silica nanoparticle-deposited capillary, bonded by 3-(triethoxysilyl) propyl methacrylate, and then modified with poly(*N*-isopropylacrylamide) (PNIPAAm) by polymerization, was developed as a new extraction device for in-tube SPME.[151] The PNIPAAm containing both hydrophilic imide groups and hydrophobic isopropyl groups exhibits a lower critical solution temperature (LCST, about 32°C) in aqueous solution, and possesses switchable hydrophilic-hydrophobic characteristic by simply altering the environmental temperature (Fig. 5B). When the temperature is lower than LCST, the hydrophilic groups in PNIPAAm can combine with water through hydrogen bonding and the polymer chains stretch, endowing the PNIPAAm hydrophilic property. While, when the temperature is higher than LCST, the hydrophobic interaction between polymer chains increases, leading the polymer chains to curl up. This temperature responsive character was applied to the capillary coating for the in-tube SPME/HPLC analysis of three synthetic estrogens from milk samples. The PNIPAAm nanoparticles have a high specific surface area, expecting a relatively high enrichment capability; the extraction efficiency of these estrogens were increased to above 40°C.

4 Conclusions and Future Trends

Sample preparation has always been at the forefront of pharmaceutical and biochemical research, for newer and more effective methods of extracting analytes from complex matrices. Current trends in sample-preparation techniques include simplification, automation, miniaturization, expedition, high-throughput performance, on-line coupling to analytical instruments, cost-effectiveness and safety. These trends have led to the development of new solvent-less microextraction techniques, such as SPME, which provide high-throughput and easy coupling with automated analytical instruments. To date, various configurations and implementations of SPME coupled with GC, LC or CE analysis have been developed for efficient sampling/sample preparation, including fiber, stirring bar, thin film, capillary, needle, pipette tip, and microchip devices. These SPME techniques have been successfully applied around the world to a wide range of bioanalytical investigations, clearly demonstrating that they are excellent alternatives to current sample-preparation methods. Important aspects in the future evolution and application of SPME are the development of new extraction devices and systems to facilitate on-site analyses and automated sample introduction to analytical instruments. Miniaturization and automation of sample preparation techniques are essential for effective high-throughput analysis, and allow for coupling to a variety of analytical micro-instruments, including capillary and microfluidic systems. Full automation of sequential delivery, extraction and introduction is possible for GC using a fiber SPME format and for LC using an in-tube SPME format. Furthermore, the use of coated fibers or thin films arranged in a 96-well plate format facilitates parallel high-throughput sample processing. Highly integrated chip-based microfluidic systems using machined microchannels have attracted much attention due to the many benefits of miniaturized platforms to researchers, including small sample volume, low device production costs, parallel processing of samples, fast sampling times, accurate and precise control of samples reducing the need for pipetting, low power consumption, and a versatile format for the integration of various detection schemes.

The development of SPME coatings with great sorption capacity or good selectivity is of great important to efficiently extract analytes at low concentrations in a complicated matrix. Various coating materials have been developed for effective sample preparation, including polypyrrole, polythiophene, immunosorbent, MIPs, RAMs, sol-gel porous silica, ion liquid, and monolithic polymers. SPME-based sensors[29,109] can be tailored for the target analyte by selecting a sorbent with appropriate affinity for the analyte. Furthermore, temperature-response[151] and light-response polymers and polymer-coated magnetic particles[152–154] may be useful as an intelligent polymer device for selective sample preparation. One of the main practical obstacles to SPME implementation to date has been the lack of commercial devices with appropriate characteristics, such as biocompatibility and good inter-fiber precision, for this type of application. This has limited the use of this technology to laboratories willing and capable of producing their own in-house devices. However, the recent design of biocompatible *in vivo* SPME devices with smaller dimensions and segmented coating has facilitated tissue studies with improved spatial resolution. Moreover, these devices further extend the adoption of SPME for future studies (kidney dialysis and cerebrospinal fluid reflux). The anticipated new coating chemistries can further increase the scope and type of applications of *in vivo* SPME.[155] *In vivo* SPME sampling has been used in monitoring biogases from human breath and skin, and in studies of drug pharmacokinetics. In contrast, the non-exhaustive features of SPME techniques allow the monitoring of chemical changes, partitioning equilibria and speciation, since sampling results in the minimum perturbation of the system. Non-exhaustive SPME strategies result in signal magnitudes proportional to the free concentration of the target analyte, defining the fraction of the analyte that is bioavailable, and allowing binding constants to be measured in complex matrices. The development of *in vivo* SPME will likely be a very important tool, especially in studies of ligand-receptor binding.

In summary, SPME techniques are very useful for sample preparation in pharmaceutical and biomedical analysis. However, there are numerous untapped opportunities available for exploration, particularly considering the unique features of SPME, making future research in this area vital and scientifically interesting. The scope of potential applications of SPME is expected to expand in the near future through the development of intelligent materials as microextraction devices, and better integration of high-throughput sampling/sample preparation and instrumental analysis.

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