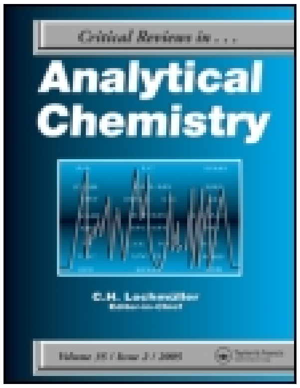

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Recent Developments of Extraction and Micro-extraction Technologies with Porous Monoliths

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Porous monoliths are widely used as stationary phase in high-performance liquid chromatography and capillary electrochromatography due to their simple preparation, versatile surface chemistries, high reproducibility, and fast mass transport. In recent years, porous monoliths were also used as sorbents in sample preparation and impressive achievements have been obtained. This review focuses on all facets of extraction technologies based on porous monoliths. Cartridge-based solid-phase extraction (SPE), CIM-based SPE, in-tube solid-phase micro extraction (SPME), fiber-based SPME, chip-based micro extraction, tip-based micro extraction, stir bar sorptive extraction, and monolithic spin column micro-extraction are presented and discussed with regards to these emerging roles.

Keywords porous monolith, solid phase extraction, micro-extraction, preconcentration

INTRODUCTION

Principles of Solid Phase Extraction and Micro-extraction

Solid phase extraction (SPE) is one of the most popular sample preparation methods. It was invented in the mid 1970s in order to reduce the use of large amounts of solvents (mandatory in the conventional liquid-liquid extraction (LLE) or Soxhlet methods) and simplify the labor intensity of LLE (Hennion et al., 1998). Presently, SPE is the most popular sample preparation technique for chromatographic analysis. Thurman and Mills (1998) defined SPE as "a method of sample preparation that concentrates and purifies analytes from solution by sorption onto a disposable solid-phase cartridge, followed by elution of the analyte with solvent appropriate for instrumental analysis." According to this definition, SPE is a binary on-off separation method. That is different from chromatographic separation, which depends on continuous cycles of sorption and desorption with an emphasis on maximizing separation efficiency. Along with SPE, micro-extraction techniques such as solid-phase micro extraction (SPME) (Guillot et al., 2006), stir bar sorptive extraction (SBSE) (Huang and Yuan, 2007), and in tube micro-extraction (Wen et al., 2007) have also experienced increasing acceptance in routine analytical procedures. In contrast to SPE and the classic procedures, micro-extraction techniques rely on an equilibrium partitioning of the sample onto sorbents. The major advantages of the micro-extraction approach are the easy miniaturization and automation of devices, as well as the inherent speediness and relative straightforward adaptation of the methods for on-site analysis.

The first and obviously common challenge for extraction and micro-extraction is the need for effective sorbents. According to the format of the extraction technique as well as the target analytes and samples, the main goals of the development of novel sorbents are variable: achievement of better selectivity (or even specificity towards definite target species), improvement of the sorptive capacity (therefore, high sensitivity and detectability), and provision og extractive media with enhanced thermal, chemical, or mechanical stability. Until now, there are many kinds of sorbents that can be used in extraction and micro-extraction. Several recent reviews have summarized their preparation, characters, and application (Fontanals et al., 2007; Abdel-Rehim, 2010; Augusto et al., 2010). The popular sorbents in extraction are silica-based (C2, C8, C18), carbons, or polymeric with particulate format (typically, several tens of micrometers large). The inherent problem of all particulate separation media is their inability to completely fill the available space, due to the effect of the irregular interparticle voids. This problem is easily solved by a monolith, which is a single large "porous particle" that entirely fills the column volume. Therefore, there is no interparticle void. Because of this and other advantages such as ease of preparation, high reproducibility, versatile surface chemistries, and fast mass

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transport, the role of monoliths in extraction has come into public notice.

Brief History and Merits of Porous Monoliths

In 1989, Hjertén and coworkers prepared a highly swollen cross-linked gel by polymerization of aqueous solutions of N, N′-methylenebisacrylamide and acrylic acid in the presence of a salt (Hjertén et al., 1989). The polymer exhibited very good permeability to flow; they called this separation medium a continuous bed. The gel obtained by polymerization does not appear to be a real monolith, but it is still called the beginning of monolithic materials. Nearly at the same time, Svec et al. used glycidyl methacrylate (GMA) to copolymerize in situ with ethylene dimethacrylate (EDMA) to obtain monolithic blocks of porous polymers with high permeability. Then, they modified the polymers with diethylamine and realized very fast separations of proteins. From then on, monolithic separation column prepared in situ within the confines of standard stainless steel columns was investigated (Tennikova et al., 1990). In the mid 1990s, Tanaka's group designed the preparation process of silica-based monoliths with controlled porous properties. The in situ preparation of typical analytical-size columns based on silica-based monoliths is difficult due to the significant shrinkage of silica occurring during the polycondensation reaction, aging, and heat treatment. Therefore, they used a plastic holder to encase the preformed monolith to get chromatographic column (Hiroyoshi et al., 1996). Tanaka's work enlarged the class of monoliths and made a great contribution to the family of different monolithic technologies. Based on the above results achieved by the three research groups, a large number of worldwide researchers have been inspired to put a great deal of effort into pursuing innovative research of monoliths. There are more and more preparation technologies for porous monoliths, as can be found in recent reviews (Zhang et al., 2010; Buchmeiser, 2007).

Compared with particle-based stationary phase, the shape of monolith can be compared to a single large "particle" of porous material that does not contain interparticle voids typical of packed beds. Consequently, all the mobile phase can flow through the stationary phase. Furthermore, monolithic columns can be prepared by polymerization in situ, which makes the preparation of capillary columns easily (Zhang et al., 2010). Monoliths also possess other advantages such as ease of preparation, high reproducibility, versatile surface chemistries, and fast mass transport. Based on these outstanding characteristics, monoliths have been accepted in chromatography as a member of the large family of stationary phases. There are several excellent reviews (Bedair and El Rassi, 2004; Zou et al., 2002; Vegvari, 2005) that describe the wide application of monoliths in a variety of chromatographic modes including gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis chromatography (CEC). In fact, monolithic materials not only can be used as separation columns, but also have other less-known functions, including carriers for immobilization of enzymes (Josic and Buchacher, 2001), supports for solid-phase and combinatorial synthesis (Pflegerl et al., 2002), and solid-phase extractors (Svec, 2006a). Svec has summarized these less-common applications of monoliths in a series of reviews (Svec, 2006a, 2006b; Svec and Kurganov, 2008; Krenkova and Svec, 2009).

Numerous advantages of porous monoliths are also applied in extraction techniques. One obvious merit is that convection dominates the mass transport of porous monoliths. Namely, the sorption of analytes onto monoliths is less affected by diffusion. Therefore, higher linear flow velocities can be used to realize high-throughput extractions from large sample volumes. Furthermore, monolithic stationary phase entirely fills the column volume and does not contain interparticle voids typical of packed beds. As a result, all the fluid of a sample can flow through the stationary phase and all the monoliths take part in the sorption of targets. Another significant advantage of porous monoliths is their ease of synthesis. Monoliths can be formed in situ, within a mold such as capillary (Zheng et al., 2010a), stainless steel tube (Zhu and Row, 2009), pipette tip (Altun et al., 2010), or channel on a chip (Yu et al., 2001). This capability is very important for micro- and nanoscale devices where the incorporation of particulate sorbent materials is difficult and plagued by poor reproducibility (Zhang et al., 2010). In addition to thermally initiated polymerization, some polymer monoliths can also be synthesized using UV light, which allows for precision control over the location of monolith within a capillary of microfluidic channel (Baryla and Toltl, 2003).

The significant drawback of monoliths is that they do not have as large a surface area as other sorbents. This is disadvantageous for monoliths as extraction materials because binding capacity increases with surface area. In research done by Xie et al. (1998), poly (divinylbenzene-ethylstyrene) monolith with high surface area was developed. They increased the surface area to an impressive 400 m^2/g by increasing the ratio of divinylbenzene to ethylstyrene without changing the porogen composition. The increase of surface area by increasing the usage of cross-linker will produce more small pores in monoliths. As a result, the high permeability of monoliths will be discounted. Furthermore, increasing the ratio of cross-linker to monomer means less monomer will be used, and thereby the extraction performance might decrease when the monomer in monoliths plays a key role in extraction. Based on this, more studies should be done on how to increase the surface area and keep high extraction capacity without sacrificing other advantages of monoliths.

With all of these intrinsic merits, porous monoliths are playing a more and more important role in extraction and microextraction. In 2006, Svec reviewed the use of monoliths in SPE and preconcentration (2006a). Later, Potter and Hilder (2008) summarized the diverse application and platforms of porous polymer monoliths in extraction. The roles of monoliths in extraction and application in pharmaceutical and biological analysis were summarized by Yang and Liu (2010) and Xu et al. (2011), respectively. The growing importance of monoliths in extraction and micro-extraction is now highlighted by the fact that more and more original articles have been published in the past two years; the application areas of monoliths also includes environmental monitoring, food inspection, pharmaceutical analysis, and others. This review therefore serves as an update to previous reviews as well as extends a wider discussion of porous monoliths in extraction and micro-extraction. Attention is paid to different extraction formats such as cartridge-based SPE, CIM-based SPE, in-tube solid-phase micro-extraction (SPME) (ITSPME), fiber-based SPME (FB-SPME), chip-based micro extraction (CBME), tip-based micro extraction (TBME), SBSE, and monolithic spin column micro-extraction (MSCME). We do not intend to provide all the references, but we have selected typical research to show up-to-date achievements and discuss the existing questions and possible solutions. At the same time, the potential development direction is also included.

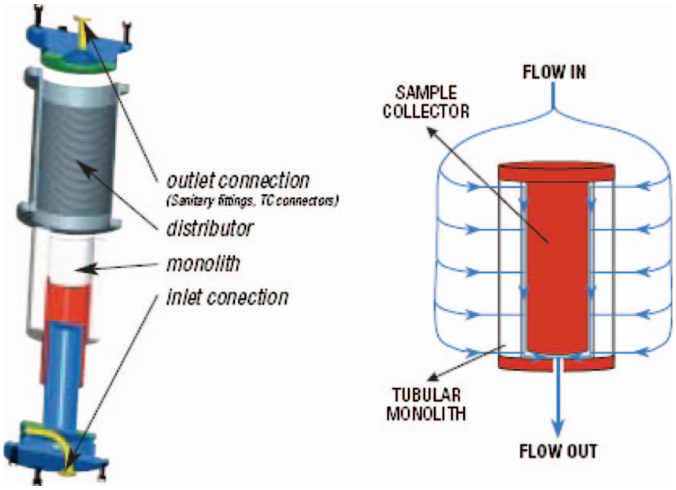

FIG. 1. The structure (left) and the flow distribution and collection (right) of CIM Tube Monolithic Column. Reproduced from the Manual of CIM Convective Interactive Media (color figure available online).

EXTRACTION AND MICRO-EXTRACTION WITH POROUS MONOLITHS

Cartridge-Based SPE and CIM-Based SPE

Normally, the holders for SPE based on particulate sorbents are syringe barrels or disposable cartridges. But monoliths are seldom held in barrels or cartridges. Recently, Nema et al. (2010) prepared a silica monolith with ionizable silanol groups and large surface area. The monolith was able to function as an off-line cation exchange SPE cartridge for extracting polar analytes such as epinephrine, normetanephrine, and metanephrine from urine samples. The preparation of monolithic cartridge seems somewhat laborious. They used sol-gel technology to synthesize the monolith, and then 2 cm length of monolith was cut and fixed in a 2 mL syringe to form the monolithic cartridge. Stainless steel cartridges are also used as the holders of monoliths. Liang et al. (2010) prepared poly (GMA-EDMA) monolith in a 50 mm × 4.6 mm i.d. stainless steel cartridge. Antibodies against pyrethroids were covalently immobilized onto the monolithic support via Schiff base reaction to obtain an immunoaffinity monolith (IAM). Under the optimum loading, washing, and eluting conditions, the IAM specifically retained deltamethrin, flumethrin, flucythrinate, and cis/trans permethrin. The IAM can be connected on-line with HPLC for high-throughput analysis of pyrethroids in various samples. The application of monoliths in the extraction of metal ions was also exploited. Wang and Zhang (2006) used epoxy resin, ethylenediamine, and pore-forming reagent (polyethylene glycol, PEG-1000) to prepared macroporous polymer monolith in a glass-tube (10 mm × 10 mm i.d.). The experimental results showed that trace Pb(II) ions could be quantitatively preconcentrated by the monolithic SPE with chelation. The maximum static sorption capacity of the monolith sorbent was 106.8 mg g^{-1} and the monolith could be reused at least 20 times. However, the abundance of similar metal ions (such as Hg^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}) has a strong influence on Pb(II) ion sorption. Chemistry modification or ion-imprinted technology will improve ion-selectivity of monoliths.

In the late 1990s, the BIA Separations company in Ljubljana, Slovenia, introduced a commercial range of short polymer monolith columns called CIM, which stands for convective interactive media. There are two families of CIM: CIM Disk and CIM Tube Monolithic Column. The CIM Disk consists of a methacrylate-based porous polymer matrix and a non-porous, self-sealing fitting ring that ensures only axial flow through the disk and prevents any sample and mobile phase leakage or bypass. The CIM Tube Monolithic Column enables a reproducible and uniform inward radial flow profile through the monolith and has the advantage that the achieved resolution is comparable to the axial flow disk columns (Figure 1). CIM are primarily poly (GMA-EDMA) and poly (styrene-divinylbenzene) monoliths, and modification can be further performed on the matrix to produce all kinds of functional groups.

CIM is capable of preparation and purification of proteins. Móster et al. (2011) established CIM chromatography for the purification of blood group antigens. The proteins employed in their study were derived from blood group antigens Knops, JMH, and Scianna, equipped with both a His-tag and a V5-tag by which they can be purified. In first step, a monoclonal antibody directed against the V5-tag was immobilized on a CIM Disk (dimensions were 12 mm × 3 mm, corresponding to a bed volume of 0.34 mL and a mean pore size of 675 nm) with epoxy chemistry. After this, the immobilized CIM Disk was used in immuno-affinity chromatography to purify the three blood group antigens from cell culture supernatant. The purity obtained on the CIM Disk was equal to conventional affinity chromatography using a HiTrap metal-affinity column. But flow rates using the CIM Tube were16 mL/min and thus six times faster than flow rates employing the HiTrap column (2.5 mL/min). Due to this fact, isolation could be carried out rapidly, which resulted in higher productivity. Up to now, there have been several modes of interaction such as ion-exchange, affinity, hydrophobic, and hydrophilic interaction that can be utilized to prepare and separate larger molecules. More details about CIM and its application can be found at www.monoliths.com. Despite an emphasis on larger molecules, CIM has also been used for extracting small inorganic species. Tyrrell et al. (2006) coated poly (styrene-divinylbenzene) CIM disks with three different organic acids to form suitable copper-chelating sorbents. The monoliths were then tested as part of a flow injection analysis (FIA) system designed to detect copper in estuarine and coastal seawater. Dipicolinic acid was found to be the most effect ligand for copper capture. With this system, they were able to detect Cu(II) in simulated seawater with a linear response ($R^2 = 0.998$) over a range of 0–125 μg/L. This study also indicates that porous monoliths are suited to extract metal ions.

CIMs are prepared from polymer rather than silica monoliths. The reason may be that the silica monoliths will shrink away from the walls of larger volume containers during formation. However, silica monoliths possess unique advantages over polymer monoliths such as high mechanical strength, large surface area, and dual pore sizes. Thus, if silica monoliths can be utilized in CIM, the application field of CIM will be expanded.

ITSPME

When a syringe barrel, disposable cartridge, or stainless steel tube is used as a monolith holder, there is no connected interaction between the inner walls and monolithic material. Thus, monolith may be flushed out from the holder, especially when the permeability of the monolith is not as good as expected. Further, the eluent will flow through the void between the monolith and the wall. In order to avoid the above problems, fused-silica capillaries are selected as the holders of monoliths, called ITSPME. During the preparation process, the inner surface of capillaries is activated by 3-(triethoxysilyl) propyl methacrylate (γ-MAPS) to create double bonds, which can take part in the following polymerization of monoliths. Therefore, the walls of capillaries closely connect with monolithic materials by chemical bonds, and the ITSPME shows higher stability. At the same time, ITSPME is easily coupled on-line to instruments to achieve automatic analysis.

Recently, Zheng et al. (2010b) prepared a hybrid organic-inorganic silica monolith with cyanoethyl functional groups and used it as a sorbent for in-tube SPME. Then, a rapid, sensitive, and automated ITSPME-LC-MS method was developed for the analysis of 10 antidepressants in urine and plasma (Figure 2). The integration of the sample extraction, LC separation, and mass spectrometry (MS) detection into a single system permitted direct injection of the diluted urine or plasma after filtration. Automation of the sampling, extraction, and separation procedures was realized under the control of a program in their study. The total process time was 30 min, and only 30 μL of urine or

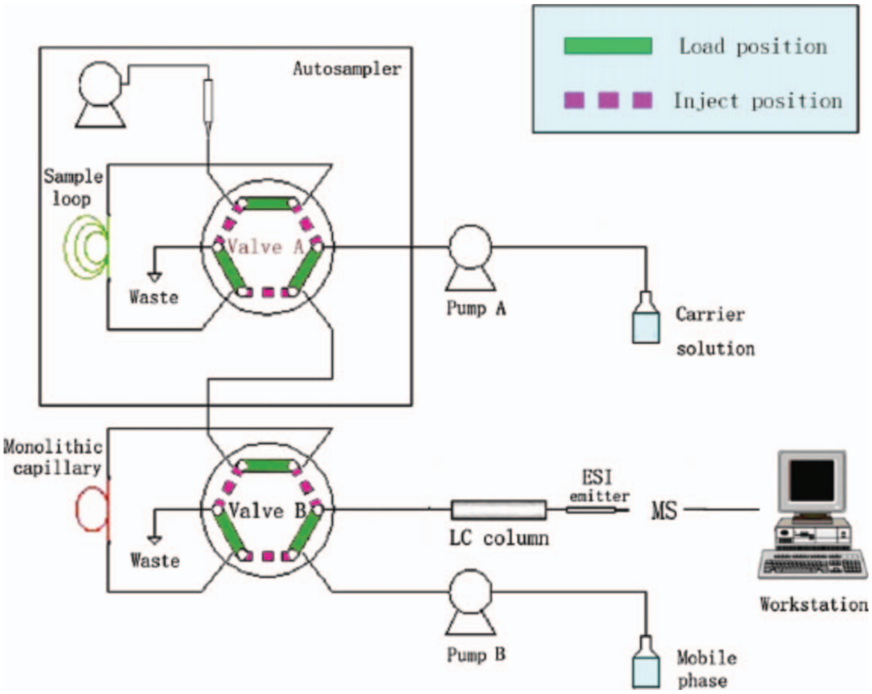

FIG. 2. Construction of automated in-tube SPME-HPLC/MS system (Zheng et al., 2010a) (color figure available online).

plasma was required in one analysis cycle. The limits of detection (S/N = 3) for 10 antidepressants were found to be 0.06–2.84 ng/mL in urine and 0.07–2.95 ng/mL in plasma. The recoveries of antidepressants spiked in urine and plasma were from 75.2% to 113.0%. Poly (methacrylic acid-EDMA) monolith (Zheng et al., 2010b) and poly (N-isopropylacrylamide-EDMA) monolith (Ma et al., 2009) were also used as the sorbents for ITSPME. Although the automated method offers advantages such as minimizing laborious, repetitive work, improving precision of the method, and eliminating the analyst's exposure to toxic solvents, the on-line system is complex and difficult to fabricate. At the same time, the system also shows inflexibility and weakness.

Breadmore's group developed a novel approach for on-line ITSPME capillary electrophoresis (CE) for basic analytes. In their method, a weak cation-exchange monolith was synthesised in situ in the front end of the CE capillary (75 μm i.d.) via photoinitiated polymerization to form poly (methacrylic acid-EDMA), which was used to create the micro-extraction phase on-line with the CE separation capillary (Thabano et al., 2007). Due to the discontinuous electrolyte combination, analytes were simultaneously eluted and focused as the electrophoretically mobilized pH step boundary moved through the monolith, after which the analytes were separated by conventional capillary zone electrophoresis (CZE) in the remainder of the capillary (Figure 3). This method provided a sensitivity enhancement for dopamine 462 times that of CZE using hydrodynamic injection. In this approach, the ITSPME and separation column combine in one capillary, and there is no interface between extraction phase and separation column. Therefore, the whole system seems very simple, but skilled preparation technologies are needed for the synthesis of monoliths in this format. Armenta et al. (2007) also set up similar system, but they used poly (butyl methacrylate-EDMA) monolith as sorbent to on-line remove immunoglobulin G and preconcentrate low-abundance proteins.

Increasing the extraction selectivity will further reduce the matrix interference and simplify chromatographic separation. Zhang et al. (2009) synthesized a molecularly imprinted polymer (MIP) monolith with guanosine as a dummy template for urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), which has been widely used as a biomarker of oxidative DNA damage. The MIP was used as the medium for ITSPME and coupled with HPLC/UV to determine urinary 8-OHdG. The enrichment factor for 8-OHdG was found to be 76, and the limits of detection and quantification of the method for urinary samples were 3.2 nmol/L (signal-to-noise ratio 3) and 11 nmol/L (signal-to-noise ratio 10), respectively.

FBSPME

FBSPME, which was first introduced by Arthur and Pawliszyn's group (1990), is considered as one of the most promising sample preparation methods because it combines extraction, concentration, and sample introduction in one simple step. At the same time, it is portable, simple to use, and relatively fast and can be automated and coupled on-line to analytical instrumentation. The fiber coating is the key factor in the FBSPME system. In order to achieve higher applicability for various compounds, many materials have been utilized as the coating (Balasubramanian and Panigrahi, 2011). There are several studies using porous monoliths as sorbents for FBSPME. Qiu et al. (2010) used testosterone as template to prepare a MIP on commercial silica fiber. The thickness of MIP coating was 3.1 μm, and it showed thermogravimetric and chemical stability, but there was no data about mechanical stability. The MIP-FBSPME was coupled with GC-MS for simultaneous analysis of four anabolic steroids in spiked human urine with satisfactory recoveries. Developing monolithic coatings on silica fibers is not a simple task because of the fragility of silica fibers. Turiel et al. (2007) synthesized MIPs in silica capillaries as molds. After polymerization, the capillaries were etched to obtain MIP fibers (monoliths). This method does not need extra materials as support, and different sizes of monolithic fibers can be easily prepared by selection of different sizes of silica capillaries. The fibers were evaluated by determination of triazines from environmental and food samples and showed good performance. Prasad et al. (2010) also prepared a self-supported cylindrical fiber based on MIP using folic acid as template. The MIP fiber showed high extraction capacity because the thickness of MIP was as large as 1.2 mm. When it was coupled off-line with the differential pulse cathodic stripping voltammetry technique, the detection limit of folic acid in human blood serum was as low as 0.0036 ngmL^{-1}. Liquid desorption was used in the above studies; the monolithic fibers do not need to be directly introduced into the GC injector for thermal desorption, which is the typical desorption mode of FBSPME. A new carbon monolith was synthesized via a polymerization-carbonization method, styrene and divinylbenzene being adopted as precursors and dodecanol as a porogen during polymerization (Shi et al., 2009). The carbon monolith was directly used as an extracting fiber, taking the place of coated silica fibers in a commercially available SPME device for the extraction of phenols, followed by gas chromatography-mass spectrometry.

Compared with monolithic coatings on silica fibers, the monolith-based fibers show high extraction capacity because the latter possess more monolithic materials. However, the lack of mechanical strength will hamper the development of monolith-based fibers. Increasing the cross-linking degree of monolithic materials will enhance the mechanical strength of monoliths. But the sorptive ability of monoliths should not be counteracted when increasing the strength.

CBME

The interest in micro total analytical systems based on microfluidic chips is growing rapidly. Due to many advantages, such as low consumption of both sample and reagent and fast analysis, microfluidic chips have been developed into a robust analytical platform and are broadly applied in chemical,

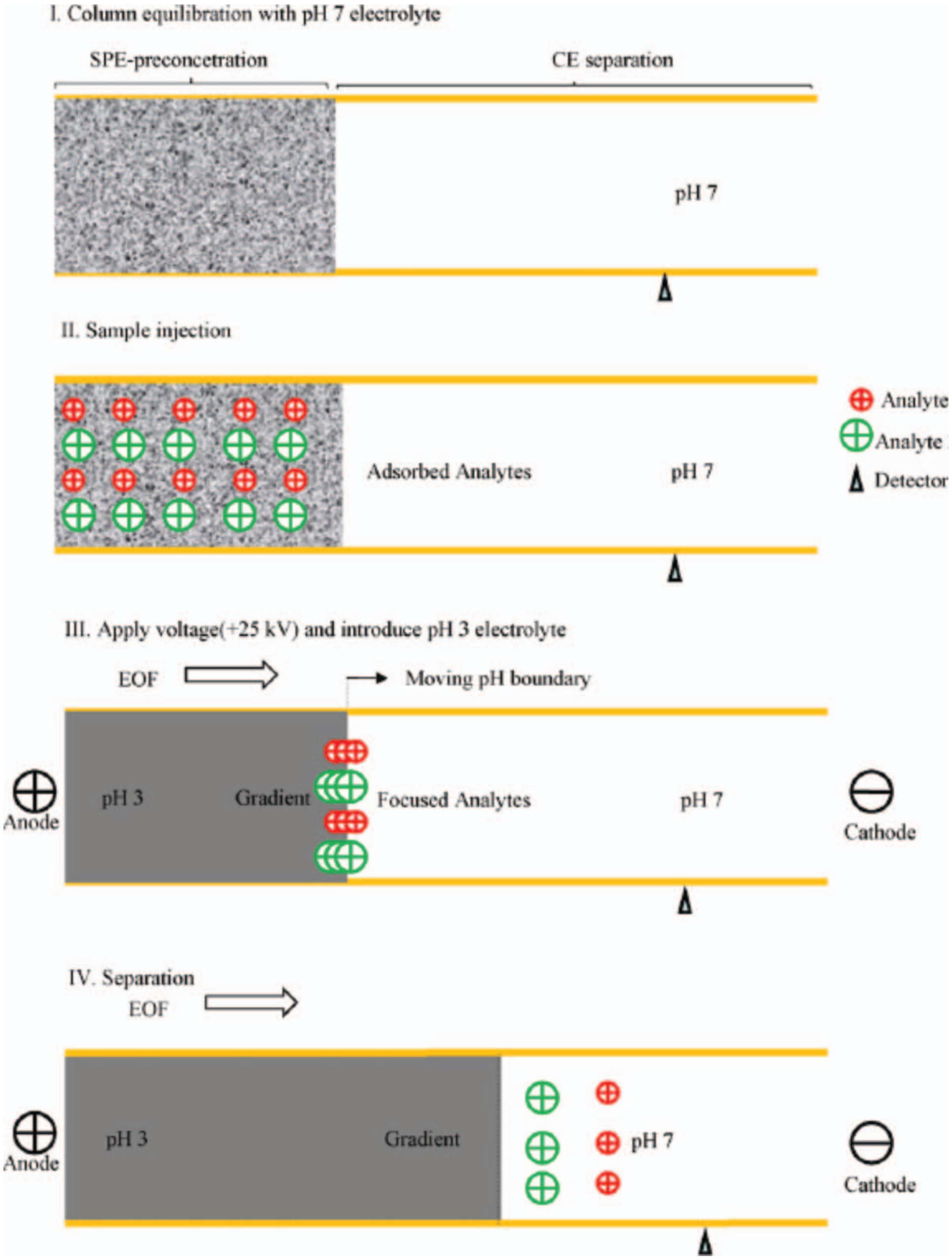

FIG. 3. Schematic diagram of the proposed ITSPME-CE technique. (I) Column is equilibrated with 6 mM phosphate electrolyte buffered at pH 7 by flushing with 1 MPa pressure for 7.2 min, which fills the whole capillary and deprotonates the caboxylate groups. (II) Sample or standards dissolved in the 6 mM phosphate electrolyte buffered at pH 7 are injected at 1 MPa for 10 min and the neurotransmitters, which are positively charged, interact with the negatively charged methacrylic acid monolith, leading to extraction. (III) Once the injection is accomplished, the capillary is placed in the 12 mM phosphate electrolyte buffered at pH 3, which is below the p*K*a of the methacrylic acid monolith, and voltage of 25 kV is applied, creating a pH step gradient as the low pH buffer enters the monolith and protonates the methacrylic acid groups. The analytes are desorbed and migrate ahead of the buffer into the high pH buffer, where they readsorb back onto the monolithic column due to electrostatic interactions. This cycle continues until the focused analytes are eluted from the monolithic column. (IV) The analytes enter into the CE section of the capillary and are separated according to their electrophoretic mobilities (as illustrated by the two positively charged different sized circles denoting two different analytes) (Thabano et al., 2007) (color figure available online).

biochemical, and biomedical fields. The challenges of microfluidic chips are design of the chips and incorporation of several analytical processes in a small device. At the same time, researchers also focus on how to integrate solid-phase functional materials into tiny channels on chips. An important advantage for monoliths is ease of preparation; they can be formed in situ in a mold, including channels. Furthermore, for some types of monoliths such as methacrylate esters radical polymerization can be initiated by UV, so monoliths easily form in specific areas of micro channels within a microchip (Dulay et al., 2001). Consequently, monolithic materials can offer multiple analytical components with various functionalities in microchips.

Since the introduction of monolithic separation columns in microchips (Ericson et al., 2000), organic polymer- and silica-based monolithic separation columns have been successfully prepared in microchips for capillary electrophoresis chromatography (CEC) and HPLC. Monolith-based extraction has also been demonstrated on microfluidic chips (Stachowiak et al., 2004). Extraction by a porous monolith on a microfluidic chip was first demonstrated by Yu et al. in 2001 (Yu et al., 2001). Using the photoinitiated polymerization methods, monoliths were synthesized in surface-modified glass chips with channels of dimensions 40 μm × 100 μm × 8 cm. Anion-exchange and hydrophobic monoliths using [2-(methacryloyloxy) ethyl]trimethylammonium chloride and butyl methacrylate as monomers, respectively, were prepared. Both types of monoliths were successfully demonstrated for the preconcentration of small organic acid Coumarin 519 with an enrichment factor of 1650. The monoliths were also used to enrich hydrophobic tetrapeptide and green fluorescent protein, for which an increase in concentration by a factor as high as 103 was achieved.

At present, most research on microfluidic devices has focused on biological analysis. This is an excellent review that highlighted the monolithic media in microfluidic devices for proteomics (Ro et al., 2006). Using porous monoliths in microfluidic device ensures large surface area, which provides abundant interaction sites for large-molecule extraction. There are a few of examples of extraction/cleanup for biomacromolecules on porous monoliths (Wen et al., 2007a; Kulinski et al., 2009; Yang et al., 2008; Kang et al., 2010). Wen et al. (2007a) developed a two-stage, dual-phase chip for the extraction of whole blood DNA. In the first stage, initial protein-capture precolumns based on four parallel chambers packed with C18 beads were used to remove undesired interferences from whole blood; the second monolithic DNA extraction channel, based on a tetramethyl orthosilicate-modified 3-(trimethoxysilyl)propyl methacrylate monolith, was used to recover target DNA. With this design, the extraction capability of the monolith for whole blood DNA was improved 100-fold; the extraction efficiency was as high as 69 ± 1%. Using polymer monoliths incorporating silica particles to purify DNA under a microfluidic module was proposed by Kulinski et al. (2009). They synthesized a silica particle-impregnated poly (butyl methacrylate-EDMA) monolith and used it to extract DNA from human urine. Better recovery of DNA at low concentration than a commercial kit was achieved. This study also demonstrates the easy preparation of polymeric monoliths with no shrinkage and the surface property of silica for extraction. In order to enhance extraction selectivity, Yang et al. (2008) prepared poly (GMA-EDMA) monolithic columns within microchannels by photopolymerization. Afterwards, antibodies were immobilized on monoliths, and subsequent lysozyme treatment blocked nonspecific adsorption. The affinity monolith had high selectivity for fluorescently tagged amino acids. Twenty-fold enrichment and 91% recovery were achieved.

Although the research on microfluidic chips focuses on large molecules, applications of this technology to small molecules are also exploited. Recently, Huang's group reported the use of porous polymer monolith columns in an integrated multilayer polydimethylsiloxane (PDMS)/glass microchip for microvalve-assisted on-line micro-extraction and microchip electrophoresis. Under the control of PDMS microvalves, the grafting of the microchannel surface and in situ photopolymerization of poly (methacrylic acid-EDMA) monolith in a defined zone were successfully achieved. Dopamine was micro-extracted on-line, eluted, electrophoresized, and electrochemically detected in the microchip, with a mean concentration enrichment factor of 80 (Kang et al., 2010).

TBME

Micropipette tip-based micro-extraction is based on sorbent phase fixed in a pipette tip. The whole extraction process is achieved by repeated aspirating/dispensing cycles utilizing a manual micropipetter or commercially available systems with 96-well extraction plates and a robot. Compared with conventional SPE, the operation of TBME is easier and faster and is suitable for disposable usage. This micro-extraction format has been used to purify and concentrate proteins and peptides in the study of genomics and been also used to extract pesticides and heavy metal ions. Commercial extraction tips can be obtained from Millipore (Bedford, Mass., USA), Varian (Palo Alto, Calof., USA), and GL Sciences (Tokyo, Japan). There are two reviews summarizing the applications of bonded monoliths as the sorbents in biological analysis (Altun et al., 2010; Blomberg, 2009).

If particles are used as sorbents in tips, filters should be used to stabilize the bed. However, frits may be a source of endogenous adsorption (Souverain et al., 2004). Using monoliths as sorbents can circumvent the question easily. Abdel-Rehim's group (Abdel-Rehim et al., 2008) prepared 96-well pipette tips with a chemically bonded monolithic methacrylate sorbent plug and used them for micro-extraction of pindolol and metoprolol in human plasma samples. The sorbent based on poly (butyl methacrylate-EDMA) monolith was formed by in situ photoinitiated polymerization. The whole extraction and desorption procedure can be automated. When it was coupled on-line to LC-MS, the methodology showed that the accuracy values of quality control samples were between 101% and 103% for metoprolol and between 94% and 114% for pindolol. The standard calibration curves were obtained within the concentration range 5–5000 nM in plasma samples. Liang and Chen (2009) demonstrated that emulsion techniques such as oil-in-water can be used to form monoliths with large through pores ($> 20\ \mu$m) and with rigid structures on small (10 μL) and large (200 μL) pipette tips by photopolymerization. In order to increase functionality of monoliths, they used this technique to encapsulate versatile functional beads such as C18 and phospho-extraction beads for qualitative and quantitative micro-extractions for diverse applications. Due to high permeability and large surface area, quick equilibration can be achieved. Using tryptic digests of ovalbumin as the standard, a recovery yields of 90–109% (RSD: 10–16%) with a loading capacity of 3 μg were obtained.

MIP as sorbent of TBME was demonstrated by Zhou et al. (2010). In their study, a pirimicarb MIP monolith was synthesized in a micropipette tip using methacrylic acid as functional monomer, EDMA as cross-linker, and a mixture of toluene-dodecanol as porogenic solvent. The MIP monolith showed highly specific recognition for the template pirimicarb. When it was coupled off-line with HPLC-DAD, the linear ranges were 2.0–1400 μg/kg for pirimicarb in tomato and pear with the correlation coefficient of above 0.999, and the detection limits (s/n = 3) for both were 0.6 μg/kg. It is noteworthy that the application of TBME in metal ions is also exploited. Rahmi et al. (2010) prepared chelating monoliths in a syringe filter tip by a two-step process: (1) in situ polymerization of GMA and EDMA and (2) its subsequent modification with iminodiacetate solution via ring-opening reaction of epoxide. The extraction performance of the chelating monolith was evaluated through the analysis of two river water certified reference materials (CRMs: JSAC 0301-1 and NMIJ 7201-a). The results showed expected extraction performance; 22 trace elements were quantitatively determined by inductively coupled plasma mass spectrometry. The analytical detection limits were in the range from 0.000003 μgL^{-1} for Ho to 0.18 μgL^{-1} for Fe. The detected values agree well with the certified or reference values, but the extraction does not possess selectivity.

Because of the limited amount of sorbents in tips, the extraction capacity of TBME may not be as high as expected. Although the 96-tip robotic device will increase the capacity to a certain extent, the related device is not all-pervading. Therefore, developing high extraction capacity, monolith-based sorbents is the main task of TBME.

SBSE

Stir bar sorptive extraction, similar to SPME, is a solventless sample preparation technique based upon sorptive extraction. But the coated amount on the stir bar is 50–250 times higher than that on SPME fiber, which results in a significant increase in recovery and extraction capacity. SBSE was developed at the Research Institute of Chromatography (Kortrijk, Belgium) in 1999 by Baltussen and coworkers (Baltussen et al., 1999) and commercialized by Gerstel (Mülheim, Germany) under the name "Twister." At present, SBSE has been developed rapidly and widely applied to the trace enrichment of various target analytes in environmental, food, and biological samples; extremely low detection limits were obtained by combining with other separation instruments such as HPLC, GC, and capillary electrophoresis (Prieto et al., 2010).

Without doubt, the extractive medium (coating) of SBSE determines the extraction performance that can be achieved. At present, SBSE coated with PDMS is the only kind of stir bar that is commercially available. Because of the apolar character of PDMS, SBSE has been mainly applied to extract nonpolar and weakly polar compounds, and failed in the extraction of strong polar compounds unless they have been previously derivatized to produce more hydrophobic species. However, sample derivatization will involve more reaction steps and effect recovery, and requires toxic derivatization reagents (Montero et al., 2005). In order to solve the above predicament, we prepared a series of monolith-based SBSE (SBSEM) (Huang et al., 2008, 2009a, 2009b, 2010, 2011). A polar monomer-vinylpyridine was copolymerized with EDMA to form a polar coating for SBSE (Huang et al., 2008). Because of the existence of pyridyl groups in the monoliths, the new coating can directly extract polar phenols through hydrogen-bond and hydrophobic interaction. Vinylpyrrolididone and divinylbenzene are the precursors for the preparation of resins of Oasis HLB SPE (www.water.com), which is famous for the extraction of strong polar compounds. Based on it, we synthesized hydrophilic poly (vinylpyrrolididone-divinylbenzene) (VPDB) monolithic material and used it as the SBSE coating (Huang et al., 2009a). The SBSE-VPDB shows high affinity to strong polar compounds. For example, the $\log K_{\mathrm{VPDB/W}}$ for phenol is 2.48, however, the corresponding $\log K_{\mathrm{O/W}}$ value is 1.46. The results give us a hint that the preparation methods for resins can be used for references in the preparation of monoliths. It is noteworthy that using monoliths as sorbents can simplify the sample preparation for some complex samples. We prepared a new SBSE based on poly (vinylimidazole-divinylbenzene) (VIDB) monolithic material (Huang et al., 2009b). When the SBSE-VIDB was used to extract sulfonamide veterinary residues in milk samples, the milk was diluted by Milli-Q water, then the SBSE-VIDB was directly put into the solution to extract analytes. There is no step of elimination of fats and protein in samples. Poly (VIDB) monolithic material is a kind of polymer sorbent that possesses a polar group, an imidazole group, in the hydrophobic bone structure and is biocompatible. As a result, when the target compounds are adsorbed onto the extraction phase through hydrophobic interaction and ion exchange, irreversible adsorption of proteins and fats does not occur due to the presence of a hydrophilic pendant group (imidazole group) and the special pore structure in the monolithic material. Therefore, no additional steps to eliminate the fats and proteins of the milk sample prior to extraction were required. The same procedure was also used to simply extract

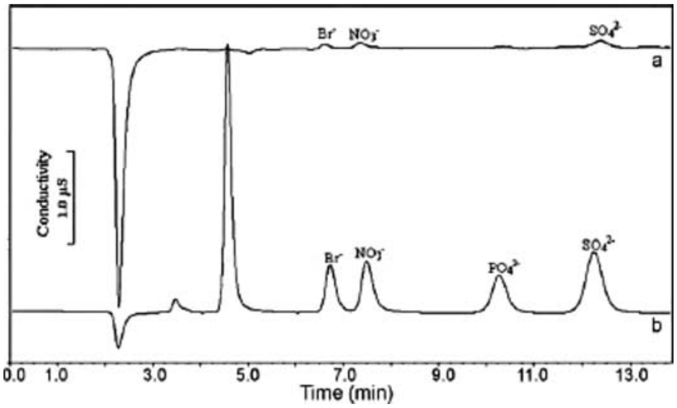

FIG. 4. IC chromatograms of four inorganic anions. Direct injection of spiked water sample with each anion at 100 ng/mL. Spiked water sample with each anion at 100 ng/mL and treated with SBSE-AE (Huang et al., 2010).

nitroimidazole residues in honey (Huang et al., 2011). MIP is also used as the coating of SBSE. Hu's group (Hu et al., 2010) has prepared a MIP coating using terbuthylazine as template. The coating showed stronger affinity to template and structural-related compounds of nine triazines. The SBSE-MIP was used for selective extraction of triazine herbicides in rice, apple, lettuce, and soil samples. When it was coupled off-line to HPLC/DAD, the limits of detection obtained were in the range of 0.04–0.12 μgL^{-1}.

At present, SBSE is mainly used for the extraction of organic compounds. There are few studies related to inorganic ions. In order to explore the possibility of using SBSE to extract inorganic ions, we prepared a novel anion-exchange (AE) SBSE coating based on poly (2-(methacryloyloxy)ethyltrimethylammonium chloride-divinylbenzene) monolithic material for the extraction of inorganic anion (Huang et al., 2010). The new SBSE-AE can effectively enrich trace concentration of inorganic anions in samples by anion-exchange interaction between coating and analytes (Figure 4). The results demonstrate that SBSE can also play a role in inorganic ions extraction if suitable coatings are synthesized.

Although impressive results were obtained, there are still two problems preventing the wide application of monoliths in SBSE. First of all, there is a friction loss for monolith-based coatings during the stirring process. Typically, the SBSEM can be reused just 20∼60 times (Huang et al., 2008, 2009a, 2009b, 2010, 2011). Thus, the preparation conditions should be investigated in detail to ensure the rigidity and extraction capacity of monoliths. Besides, only liquid desorption is used for SBSEM and it is coupled off-line to analytical instruments. The whole analytical procedure is quite inconvenient, and the samples are easily contaminated. Therefore, designing new on-line equipment is a pressing need.

MSCME

Namera et al. (2008) designed a new micro-extraction format based on monolithic spin column. The device comprises a spin column packed with octadecyl silane-bonded monolithic silica. Preparation of a monolithic spin column is relatively simple: the monolithic material is fixed inside the spin column by supersonic adhesion. Compared with other extraction formats, the operation steps, including preconditioning, loading, washing, and elution, are performed with the assistance of centrifugation (Figure 5). Now, spin columns with monolithic silica C18 can be obtained from GL Sciences (Tokyo, Japan). A series of research studies about MSCME has been done by the same group. They used C18 monolithic silica spin column coupled off-line with GC-MS to detect amphetamines and 3,4-methylenedioxyamphetamines in urine (Nakamoto et al., 2010) and nine cold medication compounds in human serum (Saito et al., 2009). In a recent study, they used cation-exchange and anion-exchange spin columns to extract basic drugs and acidic drugs, respectively, from serum and urine (Miyazaki et al., 2010).

The authors claimed that this approach afforded easy operation, needed only a low volume of extraction solvent, reduced operator labor, ensured high sample throughput, and avoided solvent evaporation. But the extraction sorbents are silica-based

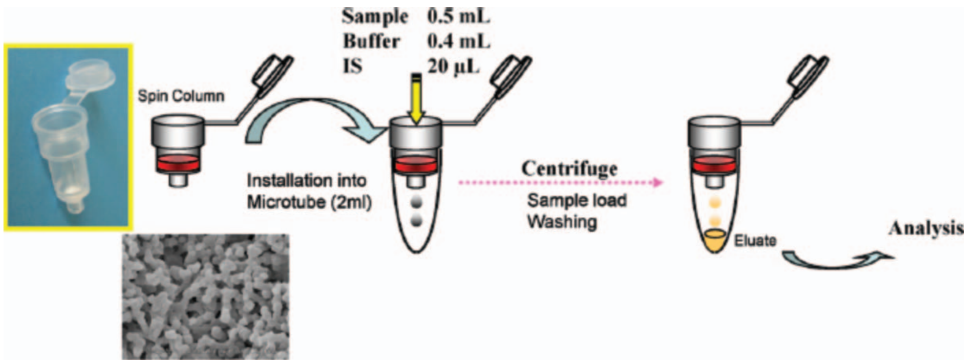

FIG. 5. The operation procedures of MSCME (Namera et al., 2008) (color figure available online).

monoliths, which show some disadvantages, such as limited usable pH range. Therefore, using polymer-based monoliths as sorbents will promote the development of MSCME.

CONCLUSIONS AND FUTURE PROSPECTS

Because of numerous advantages, porous monoliths play a more and more important role in sample preparation, especially, as sorbents in extraction and micro-extraction with all kinds of formats. However, in our opinion, the following points are worthy of being noted in future studies of monoliths as sorbents:

(1) The main classes of porous monoliths, silica-based and organic polymer-based, possess respectively unique characteristics and weakness. It is well known that silica-based monoliths are readily derivatized by silylation and have high mechanical strength. At the same time, they have ideal resistance to organic solvents and elevated temperatures. The limited usable pH range and shrinkage are the disadvantages of this kind of material. For organic polymer-based monoliths, there are numerous monomers and cross-linkers that can be used to prepare organic polymer materials with diverse properties. At the same time, the usable pH range of organic polymers is wider than that of silica, but they have weak pressure resistance. Thus, developing new polymers that combine the advantages of silica-based and organic polymer-based monoliths will provide multifunctional sorbents for extraction and micro-extraction. Hybrid organic-inorganic materials are promising to take on these roles.

(2) Miniaturization and micromation are general trends of sample preparation. Accordingly, the amount of sorbents used is decreased. Therefore, in order to keep the ability for enrichment, increasing the extraction capacity of porous monoliths still needs to be developed.

(3) Most of the extractive applications of porous monoliths is focused on organic compounds. Only a few of studies are related to inorganic species. Consequently, more attention is needed to study the extraction of inorganic ions.

(4) So far, most monolithic materials have been homemade, so precision and accuracy among different laboratories are not guaranteed. Setting up relatively uniform evaluation standards will ensure the quality of porous monoliths as sorbents.

In a word, even if the merits of monoliths in sorptive extraction have been recognized, there is still some way to go before monolithic materials are accepted as routine sorbents for sample preparation.

ABBREVIATIONS

CBME	chip-based micro-extraction
CE	capillary electrophoresis
CEC	capillary electrophoresis chromatography
CIM	convective interactive media
CZE	capillary zone electrophoresis
EDMA	ethylene dimethacrylate
FBSPME	fiber-based SPME
FIA	flow injection analysis
GMA	glycidyl methacrylate
ITSPME	in-tube SPME
LLE	liquid-liquid extraction
γ-MAPS	3 (triethoxysilyl) propyl methacrylate
MIP	molecularly imprinted polymer
MSCME	monolithic spin column micro extraction
8-OHdG	urinary 8-hydroxy-2′-deoxyguanosine
PDMS	polydimethylsiloxane
SBSE	stir bar sorptive extraction
SBSEM	monolith-based SBSE
SPE	solid-phase extraction
SPME	solid-phase micro-extraction
TBME	tip-based micro-extraction
VIDB	vinylimidazole-divinylbenzene
VPDB	vinylpyrrolididone-divinylbenzene

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