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Review

# Less common applications of monoliths: Preconcentration and solid-phase extraction☆

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**Abstract**

Monolithic materials are finding their place in a variety of fields. While liquid chromatography is the most emphasized use of this new category of porous media, some other just as important applications are eclipsed by the success of monolithic columns. This review article describes all current facets of use of monoliths in preconcentration and solid-phase extraction. In addition to the typical off line use that does not seem to be the main stream application for the monolithic materials, in-line connection of the preconcentration with HPLC, electrochromatography, electrophoresis, enzymatic digestion, as well as its applications in microfluidics are presented.


*Keywords:* Monolith; Solid-phase extraction; Silica; Polymer; Capillary; Microchip

## Contents



## 1. Introduction

The modern monoliths emerged in the late 1980s and early 1990s to be used first as stationary phases in HPLC [1–3]. In a very simplified way, their shape can be compared to a single large "particle" of porous material. It fills entirely the column volume and does not contain interparticular voids typical of packed beds. As a result, all the mobile phase must flow through the stationary phase. This "single particle" also does not need to be packed in a column since it can be prepared by polymerization in situ. These and many other advantages of monoliths together with a large number of studies caused that this format was eventually accepted in chromatography as the legitimate member of the large family of stationary phases.

☆ This paper is part of a special volume entitled "Analysis of proteins, peptides and glycanes by capillary (electromigration) techniques", dedicated to Zdenek Deyl, guest edited by I. Miksik.

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Their applications in a variety of chromatographic modes including GC, HPLC, and CEC has recently been described in several reviews [4–14] and books [15,16]. Since the first monolithic structures were used in chromatography, monoliths are for many people synonymous with columns. However, less is known about a wide variety of other applications that are simply eclipsed by HPLC as a result of massive advertisement funded by manufacturers of commercial monolithic columns. The less common functions of monolithic materials include supports for solid-phase and combinatorial synthesis [17–19], scavengers [20,21], carriers for immobilization of enzymes [22–24], static mixers [25], thermally responsive gates and valves [26–28], as well as solid-phase extractors and pre-concentrators. Present contribution to the recently started [24] series of review articles detailing achievements in these non-chromatographic applications focuses on use of monolithic materials in solid-phase extraction (SPE) and pre-concentration. In contrast to the former that will be defined in the next section, the later process is merely used to increase concentration of the entire sample without any purification. Despite some relevancy of immunoadsorption using monolithic discs, current overview does not describe this topic since it was recently detailed in excellent reviews [22,29].

## 2. The art of SPE

SPE emerged in the mid 1970s to avoid use of environmentally unfriendly chlorinated hydrocarbons and to simplify the labor-intensive liquid–liquid extraction [30]. A recent book defines 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" [31]. According to this definition, SPE is a binary on–off separation method. The first SPE devices contained porous poly(styrene-*co*-divinylbenzene) beads several tens of micrometers large packed in stainless steel tubes that were used as pre-columns. These expensive columns were then replaced with disposable column-like containers manufactured from polyethylene or polypropylene. The inherent problem of all particulate separation media is their inability to completely fill the available space. This may not be critical for applications in column-like tubular formats, where the length of the packed bed partly compensates for the effect of the irregular interparticular voids. However, it is very difficult to avoid channeling between particles packed in a thin layer that has a different aspect ratio. This has led to the development of disk formats that include disks with sorbent particles or HPLC type beads embedded in a mesh of a polymer membrane tightly retained between two screens [30]. This approach offered both an improved performance due to use of smaller beads and higher flow rates even at applied low pressure. In addition, the SPE devices could be shaped to a variety of formats such as specifically designed holders, pipette tips, and microtiter plates. The last are currently very popular, since they allow an easy integration into robotic systems for high-throughput screening protocols. Quest for further miniaturization resulted in the development of micro-SPE using a fiber as the solid-phase [32]. An interesting twist is the use of magnetic stirrers coated with a layer of adsorbent for adsorption of compounds from solutions without their "filtering" through the adsorption bed [33,34]. For detailed description of traditional SPE, the reader is advised to look into comprehensive reviews published recently [35–37].

The disks used for SPE are typically punched from larger plates and then placed in the desired location of the device. However, direct preparation of the SPE structure within the device would be more convenient in numerous applications. While the common technologies do not enable this approach, the in situ preparation can be easily accomplished with the monoliths.

## 3. Early birds

History of SPE with monolithic materials is not very long. The first paper describing this entirely new approach was published in 1998 [38]. Since SPE is based on physisorption, the adsorbent should possess as large surface area as possible. However, typical monoliths we used earlier for the rapid separations of proteins exhibited surface areas in the range of only 5–20 $m^2/g$, which were way too small to be useful in SPE. Therefore, we had to design monoliths with a different internal structure. To achieve the desired increase in surface area, we used commercial 80% divinylbenzene (remaining 20% being ethylstyrene) as the only monomer and dodecanol with toluene as porogens. This mixture was thermally polymerized in 20 mm × 1 mm i.d. PEEK tube to afford monolith with a surface area of 400 $m^2/g$ resulting from presence of mesopores and micropores. Despite this very high surface area, such monoliths had excellent hydrodynamic properties thanks to the 6 μm large through pores.

High flow rates are generally desirable for the design of high-throughput devices. Fig. 1 shows the effect of flow velocity on capacity for 2-nitrophenol at 1% breakthrough. As expected [31], the curve shows that sorption capacity at the highest flow velocity is only about 1/10 of the initial value. Obviously, shallower breakthrough curves result from the increase in flow velocity, reflecting the increasing effect of mass transfer resistance within the sorbent [31]. Consequently, this leads to both earlier elution of nitrophenol and lower capacity. However, the capacity of 2.6 mg/g achieved at a remarkably high flow velocity of 300 cm/min (150 bed volumes/min or 2.4 mL/min) is still rather good and documents the excellent mass transfer properties of the monolithic adsorbent. In comparison, the typical flow velocities used with current thin disk-format SPE media are less than 10 cm/min.

Following similar scheme Huck and Bonn prepared a porous monolithic rod from poly(styrene-*co*-divinylbenzene) using 1-octanol as porogen [37]. They did not use the monolith directly for SPE but disintegrated it first to 3–5 μm irregular particles. Excellent properties of this sorbent were demonstrated with extraction of organochlorine and organophosphorous pesticides. Average recovery for 13 different herbicides was 77% significantly surpassing 69% observed for cartridge packed with C18 silica beads.

Owing to their high hydrophobicity, monolithic sorbents prepared from aromatic monomers are particularly well-suited for

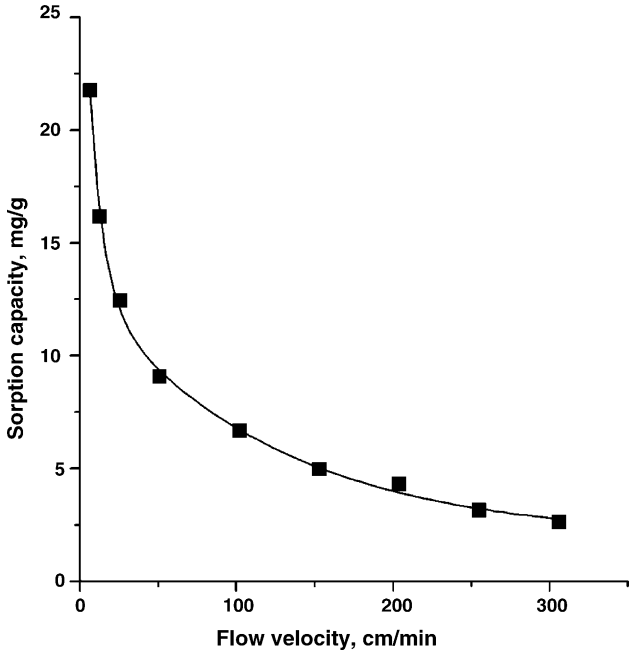


Fig. 1. Effect of flow velocity on sorption capacity of porous poly(ethylstyrene-*co*-divinylbenzene) monolith in a 20 mm × 1 mm i.d. PEEK cartridge for 2-nitrophenol from a 10 μg/mL solution (reprinted with permission from ref. [38]. Copyright 1998 American Chemical Society). Monolith prepared from commercial 80% divinylbenzene in presence of 8% toluene and 52% dodecanol.

the extraction of non-polar compounds. In contrast, more polar compounds are less retained and may even break through during the sorption step. This leads to a decrease in recovery of polar compounds and errors in the quantization of results. An increase in polarity and wettability of the internal surface of the divinylbenzene monolith was achieved by the addition of a more polar monomer 2-hydroxyethyl methacrylate to 91% grade divinylbenzene and help of porogen comprising a higher aliphatic alcohol tetradecanol with only a small amount of toluene [31]. Comparative study of recovery for a whole range of different phenols carried out with both monolithic devices is summarized in Table 1. The results clearly demonstrate the higher recoveries obtained with the more polar sorbent. This is particularly true for phenol. The average recovery calculated for all 11 phenols is close to the theoretical value and reaches a remarkable value of 95%.

Table 1
Recovery of phenols from porous poly(ethylstyrene-*co*-divinylbenzene) (EST-DVB) and poly(2-hydroxylethyl methacrylate-*co*-ethylstyrene-*co*-divinylbenzene) (HEMA-EST-DVB) monoliths determined at a flow velocity of 102 cm/min [38]

| | Recovery (%) | |
|---|---|---|
| | EST-DVB | HEMA-EST-DVB |
| Phenol | 58 | 92 |
| 4-Nitrophenol | 77 | 90 |
| 2-Chlorophenol | 82 | 97 |
| 2-Nitrophenol | 88 | 96 |
| 2,4-Dinitrophenol | 76 | 91 |
| 2,4-Dimethylphenol | 85 | 95 |
| 4-Chloro-3-methylphenol | 88 | 99 |
| 2,4-Dichlorophenol | 79 | 97 |
| 4,6-Dinitro-2-methylphenol | 80 | 94 |
| 2,4,6-Trichlorophenol | 82 | 96 |
| Pentachlorophenol | 91 | 97 |
| Average | 80 | 95 |

## 4. Preconcentration in capillary electrochromatography

As defined above, the target of "classical" SPE is to concentrate compounds from dilute solutions and to prepare sample for the analysis. However, as the size of analytical devices, and specifically chromatographic column, is decreasing, sensitivity of detection of peaks starts to play an important role. Unfortunately, the injected plug of sample cannot be too long to maintain good column efficiency. Therefore, high concentration of the analytes in separated sample is required to enable their good detection.

Following this paradigm, Zare's group developed monolithic sol–gel capillary column for separation in capillary electrochromatographic (CEC) mode using photopolymerization of a mixture of (methacryloyloxypropy)-trimethoxysilane and HCl in toluene [39]. They found that the monolithic structure could also be used for the preconcentration of a variety of compounds including thiourea, naphthalene, anthracene, alkylphenones, and peptides [40]. Their approach was simple: first, they injected a plug of the sample solution using pressurized flow. Then, the voltage was applied and the separation achieved in the CEC mode. They demonstrated a significant increase in detection sensitivity with an increase in injected plug length. For example, Fig. 2 illustrates the isocratic preconcentration and separation of five peptides in a monolithic sol–gel column, which surface was modified using (pentafluorophenylpropyl)-trichlorosilane to increase the retention. About 20-fold increase in peak heights

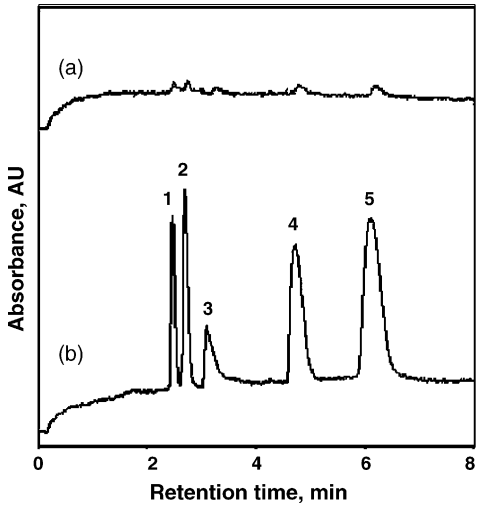


Fig. 2. Electrochromatograms showing separation of bradykinin (peak 1), angiotensin II (peak 2), tripeptide I (Gly-Gly-Gly; peak 3), tripeptide II (Val-Tyr-Val; peak 4), and methionine enkephalin (peak 5) after injection of 0.1 (a) and 12 mm long sample plug (b) (reprinted with permission from ref. [40]. Copyright 2001 American Chemical Society). Conditions: Pressure for injection 3.5 kPa; peptide concentrations, 16.7 μg/mL each; mobile phase 50 mmol/L phosphoric acid/water/acetonitrile (1/5/4); applied voltage, 15 kV; detection, 214 nm, 20 °C.

clearly demonstrates the positive effect of the longer injected plug. The observed effect was related to the high mass-transfer rates possible in the porous monolithic structures.

The same group then extended the preconcentration/separation approach and used elution in a gradient of the mobile phase and sample stacking, respectively [41]. The preconcentration effect for neutral analytes was vastly improved by using a solvent gradient. However, limited solubility of hydrophobic compounds in the solvent featuring a low concentration of the organic solvent and high percentage of water was the limiting factor for using broad gradients. Unfortunately, a rapid deterioration of peak shape was observed in gradient preconcentration/separation of ionized analytes resulting from the effects of the organic solvent on the conductivity of the mobile phase. To eliminate this problem, they used field-enhanced sample injection. The sample is dissolved in a low-conductivity mobile phase and injected using voltage. As the voltage is applied, electroosmotic flow (EOF) drives the low-conductivity pH 2 sample matrix in the capillary while both EOF and electrophoretic flow force the cationic peptides to enter the column. Only a very small plug of solvent is introduced because the EOF at the low pH of the separation solution is slow due to restricted dissociation of silanol groups of the capillary walls. The electric field in the sample matrix zone introduced into the column is much higher than the separation zone. As a result, the cationic peptides rapidly enter the column thanks to their high electrophoretic mobility. Thus, a larger amount of peptides can be introduced. Their high electrophoretic mobility also leads to focusing (preconcentration) or sample stacking of peptides at the concentration boundary between the sample matrix and separation solution. This electrokinetic preconcentration in the polymerized sol–gel column using sample stacking by hydrodynamic injection or electrokinetic injection was demonstrated with positively charged analytes. Similar approach is imaginable also for negatively charged analytes. However, a change in column chemistry that would have to afford anodic EOF is required.

Ping et al. [42] observed analogous effects using polymer-based monolith in CEC and called it self-concentration. They claim that a solute in a CEC column does not distribute after injection uniformly due to its absorption interaction with the stationary phase. In comparison with CZE, sample zone is narrower owing to the stacking resulting from this absorption. The poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith, which was prepared using procedure developed by our group [43,44,45], enabled improvement in sensitivity of detection by up to 22,000 times and worked well for both neutral and ionized compounds. However, the effect of preconcentration was demonstrated only on a single analyte with no subsequent separation.

Oguri et al. adopted the early Zare's work [46] and prepared monolithic CEC column using C18 silica beads embedded in a typical tetraethyl orthosilicate gel [47]. They targeted the separation of biogenic amines. Since these compounds do not include any chromophore, their UV detection is only possible after functionalization with *o*-phthalaldehyde that can be achieved on column. A mixture of five amines in the mobile phase that in addition to a buffer and acetonitrile also contained sodium chloride was electrokinetically injected on the column for various times from 5 to 20 s. The peak height of the separated amines grew appropriately. Although this demonstration of preconcentration ability is interesting, the following CEC separations were not very impressive affording only 40,000–50,000 plates/m. This is in accord with previous observations that the "monolithized" packed CEC columns have never matched the efficiency of both packed and true monolithic columns [46,48,49].

Novotny's group prepared a monolithic capillary column via in situ free radical polymerization of a mixture prepared by dissolution of acrylamide, methylene bisacrylamide, lauryl acrylate, vinylsulfonic acid, and poly(ethylene glycol) in *N*-methylformamide/Tris buffer/aqueous boric acid [50]. This column afforded an excellent separation of isoflavone phytoestrogens in CEC mode. Use of highly aqueous mobile phase enabled introducing a large volume of dilute sample that was adsorbed on the top of the column. A significant in-line preconcentration could be achieved with little or no effect on separation efficiency. This approach decreased the limit of detection (LOD) for the isoflavone standards to 100 ppb after 90 s electromigration injection as opposed to 700 ppb without the on-line preconcentration and a 10 s injection representing a seven-fold increase in sensitivity [51].

Since changing the injection solvent for the mobile phase is not convenient, this group designed a different approach to further enhance the on-column preconcentration and detectability. Their new method involved introducing a water plug prior to sample injection into a column that was equilibrated with a mobile phase containing 30% acetonitrile. This plug of water changed the surface characteristics of the monolithic stationary phase and made it more retentive toward the sample components. While an average column efficiency of 245,000 plates/m was observed using a 10 s injection, it was found to be 210,000 plates/m for a 90 s sample injection preceded by a short plug of water. They also found that the exact length of both water and sample plugs is essential to attain high column efficiency [51].

## 5. Preconcentration in capillary electrophoresis

Similar to CEC, on column detection of compounds in capillary electrophoresis (CE) using UV detector also suffers from the short optical path and small injection volume making preconcentration the obvious solution to these problems. The effect of preconcentration was first demonstrated in 2003 by Baryla and Toltl [52]. They prepared a 1 cm long monolithic plug by UV initiated polymerization of a mixture of methacrylic acid, ethylene dimethacrylate, and azobisisobutyronitrile in toluene and used it for preconcentration of propanolol (**1**, Fig. 3), which may interact with the surface of the monolith via Coulombic forces, hydrogen bonding, and/or hydrophobic interactions. They also developed a procedure enabling preconcentration and CE separation. First the sample is pumped through the capillary. Unbound sample is removed from the capillary by rinsing with water. The capillary is then filled with separation/elution buffer from the outlet end by applying a negative voltage and

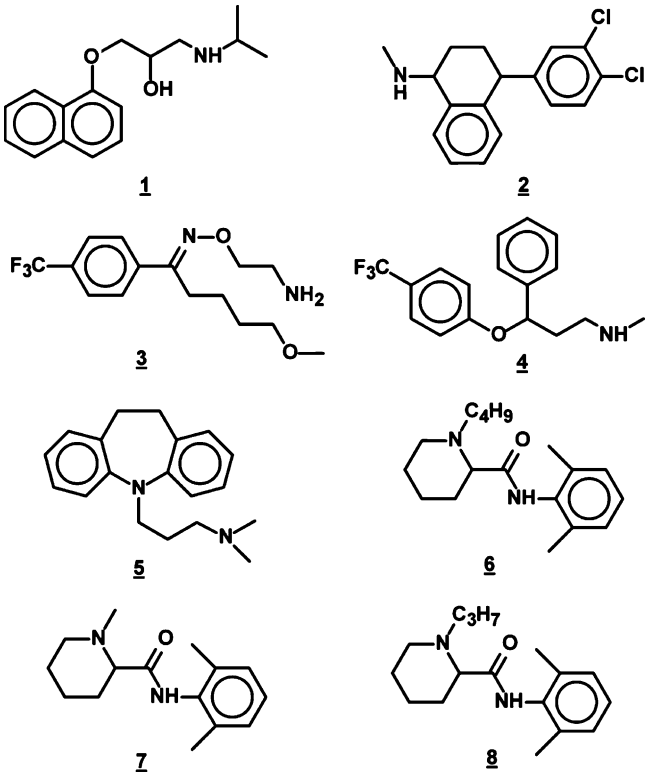


Fig. 3. Structures of used compounds. Propanolol **1**, sertraline **2**, fluoxetine **3**, fluvoxamine **4**, imipramine **5**, bupivacaine **6**, mepivacaine **7**, and ropivacaine **8**.

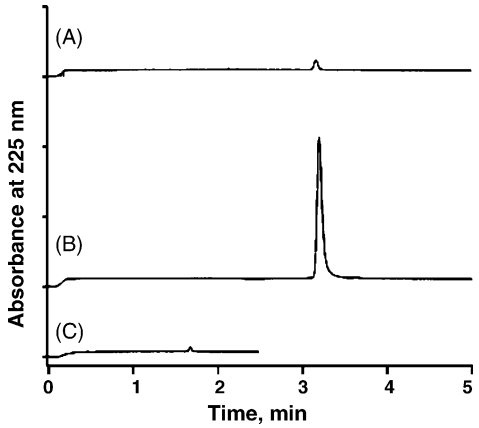


Fig. 4. Peaks obtained by electrophoretic elution of *S*-propranolol preconcentrated from the 5 (A) and 500 nmol/L (B) solutions and from 500 nmol/L without on-line preconcentration (C) (reprinted with permission from ref. [52]. Copyright 2003 The Royal Society of Chemistry). Conditions: Capillary, 27 cm × 75 μm i.d. (20 cm to detector); voltage +10 kV; buffer 80:20 2 mol/L acetate–acetonitrile pH 3.0; UV detection at 225 nm; temperature, 25 °C. On-line preconcentration: 2 min rinse with 500 nmol/L or 10 min rinse with 5 nmol/L *S*-propranolol solution in water, current = 12 μA. No preconcentration: 3 s injection (3.5 kPa) with 500 nmol/L *S*-propranolol solution in water, current = 12.5 μA.

using the EOF as a pump. The applied negative voltage generates an EOF through the capillary in direction from the outlet end to the inlet end. It is not clear though how this EOF is generated since the whole column is filled with water that does not contain any conductive electrolyte. However, they claim that it is very important to fill the capillary with just a single one column volume of separation/elution buffer to avoid release of the sample and its flushing out through the inlet end. The exact volume at which the separation/elution buffer completely fills the capillary is determined from the measurement of the current through the capillary. Initially the current is very low since the capillary is filled with water. However, it increases rapidly to a constant current once the capillary is completely filled with buffer. At that point, the voltage is switched such that a positive voltage is applied and the preconcentrated and released analyte migrates through the capillary to be subsequently detected.

Fig. 4 clearly demonstrates the positive effect of preconcentration on the peak size. While using a bare silica capillary with no preconcentration, the limit of detection for *S*-propranolol was about 500 nmol/L at a signal-to-noise ratio of 3 (trace C), the compound could be detected from 5 nmol solution after 2 min preconcentration (trace A). Use of the higher concentrated *S*-propranolol solution together with preconcentration then yields a large peak shown as trace B. The authors claim: "Achieving detection limits in the low nanomolar range using this preconcentration method is a great improvement in sensitivity over traditional UV detection in CE (low-micromolar range)" [52].

The slower elution of *S*-propranolol from the capillary with preconcentration unit compared to that in a bare capillary is due to suppressed EOF resulting from modification of the wall with (methacryloyloxypropy)-trimethoxysilane prior to the preparation of the monolith. Since the total adsorption capacity was not exhausted, pumping the solution through the capillary for a longer period of time can further increase the enrichment. Experimentally determined capacity of the 1 cm long monolith for *S*-propranolol was 59 ng. Although a combination of preconcentration with CE is the topic of this study, no actual separation is presented.

Hilder et al. extended this approach by demonstrating both preconcentration and separation of clinically important antidepressant drugs sertraline **2**, fluoxetine **3**, fluvoxamine **4** (Fig. 3) [53]. Detection limits of current CE for these compounds are ca. 1 mg/L while their therapeutic levels in plasma are 2–3 orders of magnitude lower. Clearly, preconcentration is required. They implemented two approached. First, they prepared a monolithic poly(butyl methacrylate-*co*-ethylene dimethacrylate) plug using UV initiated polymerization and tried to reproduce result published by Baryla and Toltl [52]. However, neither the original method nor a combination of both voltage and pressurized flow afforded consistent EOF. Therefore, they prepared a poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith also containing 3-sulfopropyl methacrylate. Although an increase in EOF could be observed, only combination of voltage and pressure driven filling of capillary with the separating buffer provided for reproducible results. A significant improvement in the peak shape was then achieved by replacing phosphate buffer with acetate. Fig. 5 shows the effect of percentage of 3-sulfopropyl methacrylate in the monolith. At 1% content, the Coulombic interactions are too strong and separation of the three drugs could not be achieved. However, reducing the content of

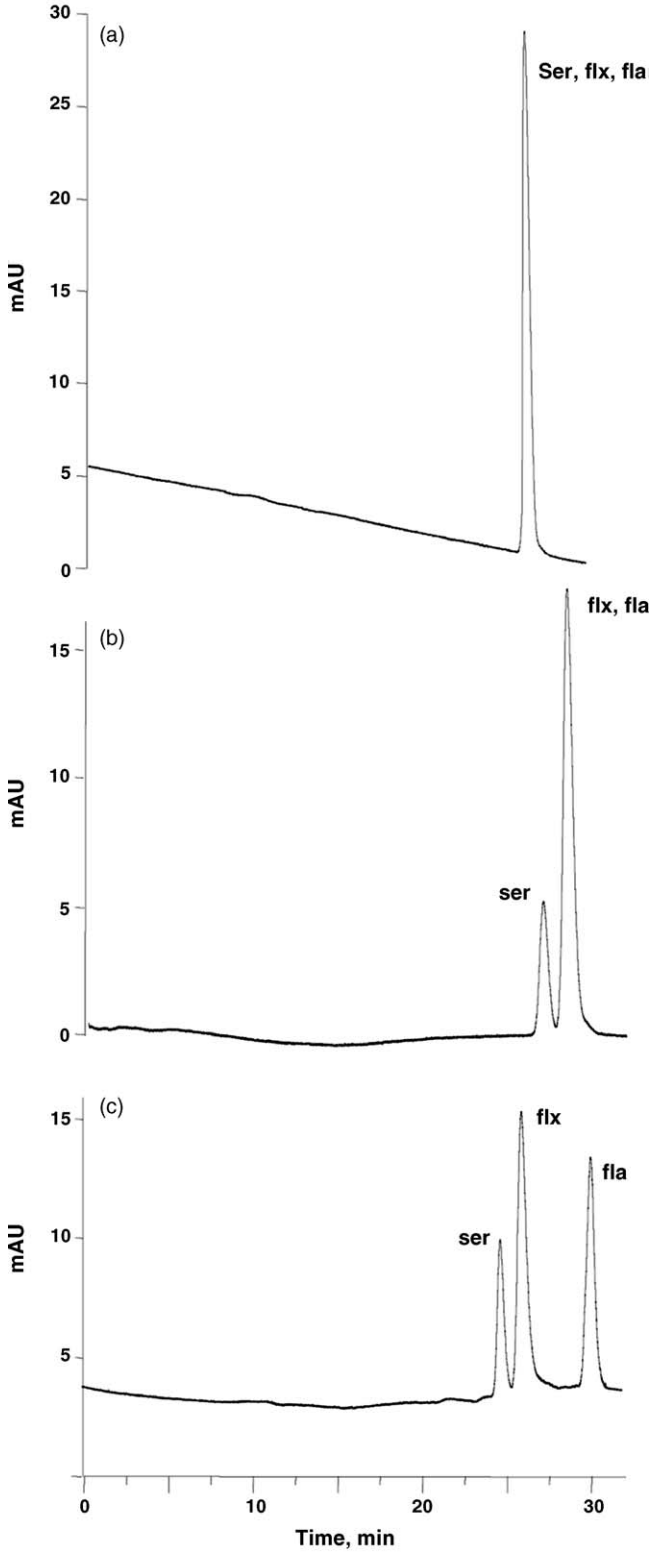


Fig. 5. Comparison of the elutio/separation of sertraline (ser), fluoxetine (flx), and fluvoxamine (fla) by SPE-CE using monolithic preconcentrators containing various percentage of sulfopropyl methacrylate (SPMA) (reprinted with permission from ref. [53]. Copyright 2006 Elsevier). Conditions: (a) 1% SPMA, elution with a 10:90% mixture of 2 mol/L acetate buffer pH 3.5 and acetonitrile; (b) 0.5% SPMA, elution with a 10%:90% mixture of 1 mol/L acetate buffer pH 3.5 and acetonitrile; (c) 0.1% SPMA, elution with a 10%:90% mixture of 0.2 mol/L acetate buffer pH 3.5 and acetonitrile.

the sulfonic acid units to only 0.1% resulted in an excellent baseline separation. With an enrichment factor of 500, detection limits reaching the desired microgram range could be achieved [53].

The arsenal of modes used for in-line preconcentration followed by electrophoresis has recently been expanded to immunoextraction [54]. Lee's group prepared a 1.5 cm long poly(glycidyl methacrylate-*co*-trimethylolpropane trimethacrylate) monolith using photoinitiated polymerization. The epoxide functionalities were then used for immobilization of Protein G and used for the preconcentration of immunoglobulin G (IgG). Since the CE system available in their laboratory enabled only 0.1 MPa pressure to push the sample through, large pore monolith was desirable. Therefore, a new porogenic system consisting of cyclohexanol, methanol, and hexane had to be used affording a highly porous monolith (82% porosity) with a pore size of 3.3 μm and a specific surface area of 4.8 $m^2$/g. The preconcentration of IgG was achieved from its solution in binding ammonium formate–formic acid buffer pumped through the capillary followed by washing away the unbound protein with the same buffer. Then, the capillary was conditioned with a lower molarity ammonium formate–formic acid. IgG was desorbed from the protein G monolith by injecting a 6 cm long plug of aqueous formic acid. Finally, a 6 cm plug of separation buffer was injected. This step is important since failure to pump separation buffer resulted in re-adsorption of the desorbed IgG on the protein G monolith. The charge of IgG depends on the pH of the medium. Thus, IgG bears a positive charge when dissolved in elution buffer. As a result, the negatively charged protein G monolith and the positively charged IgG may easily interact. Once eluted, voltage was switched on and IgG migrated along the separation capillary and was detected by UV absorption. The most impressive application of this concept is preconcentration of IgG from human serum. Fig. 6 shows preconcentration and detection of IgG at about 1 nmol level. Although this concept was demonstrated using the pair Protein G-IgG, it can be extended to preconcentration of biomarkers provided antibodies specific to these proteins are available.

Quite novel approach to functionalization of monoliths for preconcentration of metal ions followed by their EC separation has been developed by Hutchinson et al. [55]. In contrast to the "classical" copolymerization of functional monoliths and/or functionalization of reactive monolithic polymers, they prepared a poly(butyl methacrylate-*co*-ethylene dimethacrylate-*co*-2-acrylamido-2-methyl-1-propanesulfonic acid) monolith bearing cation-exchange sites. Pore surface of the polymer monolith was then coated with monodisperse latex particles bearing quaternary ammonium groups strongly interacting with the sulfonic acid functionalities of the monolith. Fig. 7 shows electron micrograph of the monolith which surface was partly covered with the latex particles. This monolithic preconcentrator was attached to open capillary serving as the CE separation unit.

Using monolith with attached nanoparticles, several capillary volumes of sample containing bromide, nitrate, iodide, iodate, bromate, thiocyanate, and chromate could be loaded onto the monolithic preconcentrator using pressurized flow. The monolithic device was then filled with a weak electrolyte. Transition to

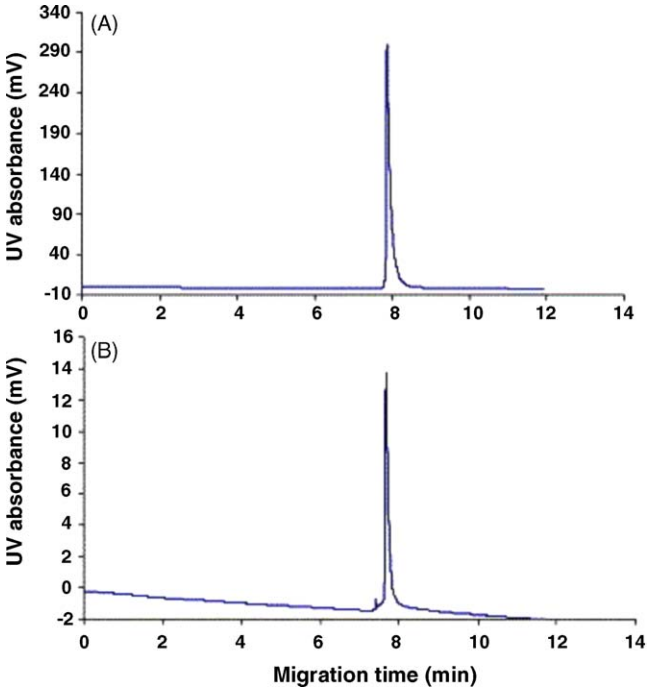


Fig. 6. Electropherograms demonstrating in-line preconcentration and CE of IgG from human serum (reprinted with permission from ref. [54]. Copyright 2005 Elsevier). Conditions: fused silica capillary 64 cm (53 cm to detector) × 75 μm i.d.; protein G monolithic preconcentrator 1.5 cm; elution buffer 50 mol/L formic acid (0.1 MPa, 0.3 min); separation buffer 12.5 mmol/L ammonium formate–formic acid (pH 7.6); separation voltage +15 kV; UV detection at 214 nm. (A) 500 times diluted human serum (∼6 μL volume sampled) and (B) 65,000 times diluted human serum (∼28 μL volume sampled).

a strong electrolyte created a transient isotachophoretic gradient affording elution of the inorganic anions as a short plug of concentrated anions at the front of the gradient. Finally, all anions were readily separated using CE mode in less than 90 s [55].

Very recently, poly(methacrylic acid-*co*-ethylene dimethacrylate) monolith containing 90% of the crosslinker placed in a 2 cm long 530 μm i.d. capillary was used for off-line preconcentration of angiotensin II receptor antagonists from human urine [56]. More specifically, several milliliters of the dilute urine were mechanically pushed from a syringe through the monolith at a flow rate of 0.2 mL/min. Washing with a phosphate buffer, and release with 0.05 mL of acetonitrile in a vial followed the sorption step. This preconcentrated solution was then electrophoretically injected and the components separated in CE mode. Recovery found for all studied drugs was around 80% and limit of detection 15–20 ng/mL [56].

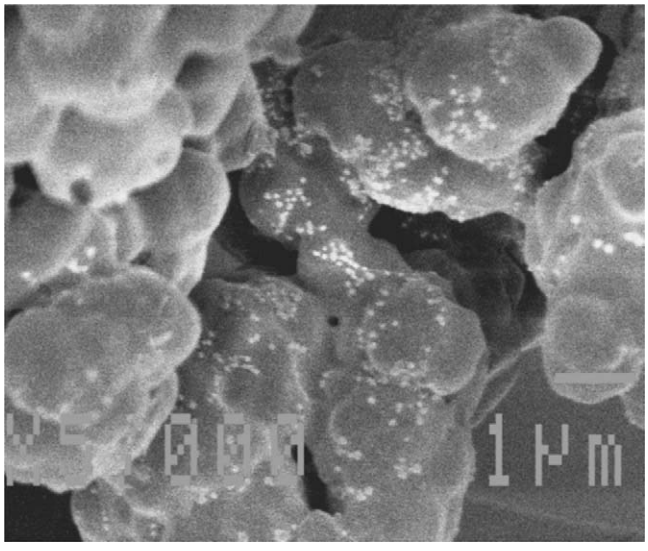

Fig. 7. SEM of the latex-coated polymer monolith (reprinted with permission from ref. [55]. Copyright 2005 American Chemical Society).

## 6. SPE and preconcentration in microfluidic chips

Similar to the standard SPE, our group also pioneered on-chip preconcentration using glass chips with etched channels [57]. Following our success with the microscale preparation of porous polymer monoliths that combined well-controlled porous properties with appropriate surface chemistry using UV-initiated polymerization [58], we realized that this approach was well-suited for the in situ fabrication of SPE microdevices. Using this simple and straightforward in situ preparation we obtained porous polymer monoliths with two different surface chemistries, hydrophobic poly(butyl methacrylate-*co*-ethylene dimethacrylate) and ionizable hydrophilic poly(2-hydroxyethyl methacrylate-*co*-[2-(methacryloyloxy)ethyl]-trimethylammonium chloride-*co*-ethylene dimethacrylate). Using irradiation through a mask, 7 mm long plug of the monolithic material was placed in straight 100 μm wide and 40 μm deep channel fabricated in glass substrate. Then the dilute solutions of fluorescent compounds were pumped through the device at a flow rate of 3 μL/min. This flow rate represents a rather high flow velocity of 12 mm/s. Performance of the monolithic preconcentrator was first tested with a small molecule of Coumarin 519 in both ion exchange and hydrophobic modes. We found that several 500 nL pulses of the releasing solution of sodium salicylate were required to achieve a complete desorption from the ion exchange monolith. In contrast, only a single 500 nL plug of acetonitrile was sufficient to completely elute the fluorescent compound from the hydrophobic monolith. Enrichment by a factor of 190 was achieved with the former while a remarkable concentration enhancement of up to 1650-fold was demonstrated with the latter. The adsorption capacities of the monolith exceeded by 3–5 orders of magnitude those observed with the open channels [59] and channels packed with C18 silica [60]. Our hydrophobic concentrator was also used with success for adsorption/release of tetrapeptide Phe-Gly-Phe-Gly labeled with Coumarin 519 and green fluorescent protein (GFP). Fig. 8 shows elution of GFP with aqueous acetonitrile at three different flow rates. An enrichment of up to 1000 times was observed [57].

Tan et al. adopted technology we have developed earlier [57] and prepared large pore monoliths in a chip containing eight parallel channels fabricated by hot embossing of standard fused silica capillaries in cyclic olefin copolymer (Zeonor) plates [61]. About 1% sulfonic acid monomer was added to the polymerization mixture to support electroosmotic flow. In contrast to our experience with monolith in plastic devices, they used

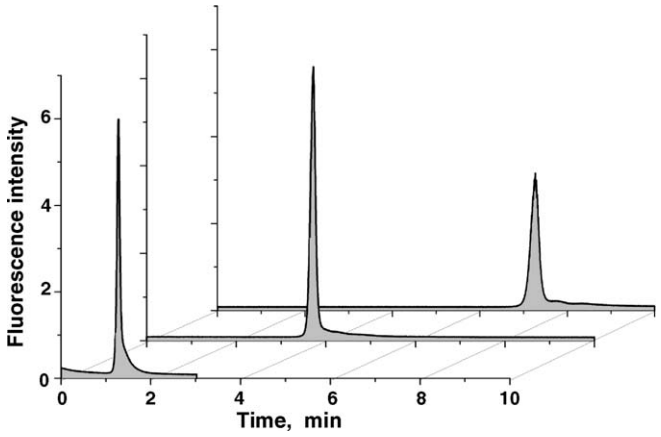


Fig. 8. Elution of green fluorescent protein from hydrophobic monolithic concentrator (reprinted with permission from ref. [57]. Copyright 2001 American Chemical Society). Conditions: loading: 200 μL of 18.5 nmol/L protein solution in 8 mmol/L Tris–HCl buffer (pH 8) containing 0.95 mol/L ammonium sulfate, flow rate 3 μL/min; elution with 1:1 acetonitrile–water at a flow rate of 3 (1), 1.03 (2), and 0.53 μL/min (3).

the chip without any further treatment yet did not observe any shrinkage during polymerization and creation of a void at the interface monolith-wall. They found that presence of the monolith improved retention of the studied compound imipramine **5** (Fig. 3) which could be expected due to the basic character of the drug containing amine groups. The elution of imipramine was achieved using 0.1% solution of formic acid in acetonitrile and the eluent analyzed by electrospray ionization and mass spectrometry. An adsorption capacity of 300 ng was estimated from frontal experiments shown in Fig. 9. Although the batch-to-batch R.S.D. found for the capacity was only 26.3%, this value for the recovery was much better reaching 7.9%. The monolithic SPE device exhibited a rather broad dynamic range of 25–10,000 ng. These monoliths were also used for cleanup of biological samples. Experiments with both human urine and P450 drug metabolism incubation mixture demonstrated that imipramine could be selectively separated, eluted, and detected.

Craighead's group used a silicon master to emboss a simple structure in chip made again from the cyclic olefin copolymer. The chip also included an integrated gold electrode and an electrospray emitter [62]. A 5 mm long poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith was prepared inside the 150 μm × 150 μm channel by in situ polymerization initiated by UV irradiation through a simple mask. Although this monolith was again fabricated without any surface treatment of the channel thus precluding the covalent attachment of the monolith to the walls, no irregularity of the flow through was observed. Inspired probably by Henion's work described above [61], they tested compatibility of the monolith with electrospray using imipramine. Good mass spectra were obtained.

In continuation of their efforts, they updated the procedure and eventually included the wall modification most likely to avoid dislodging of the monolith within the channel and flow through the void between the monolith and wall [63]. They used our approach [64] and photografted first a thin layer of 1:1 methyl methacrylate–ethylene dimethacrylate copolymer that contained numerous pendant double bonds. The poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith was then prepared in situ using standard photoinitiated polymerization. Using setup shown in Fig. 10, imipramine was extracted from

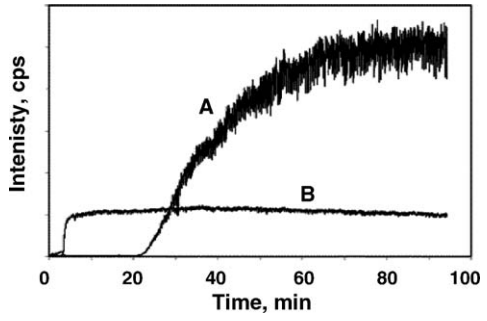


Fig. 9. Breakthrough curve for sample capacity measurement (reprinted with permission from ref. [61]. Copyright 2001 American Chemical Society). Conditions: test analyte standard solution, 5 μg/mL imipramine and 0.5 mmol/L $K^+$ (pH 9.3); flow rate, 2 μL/min. (A) SIM at *m/z* 281 for protonated molecule of imipramine; (B) SIM at *m/z* 39 for potassium.

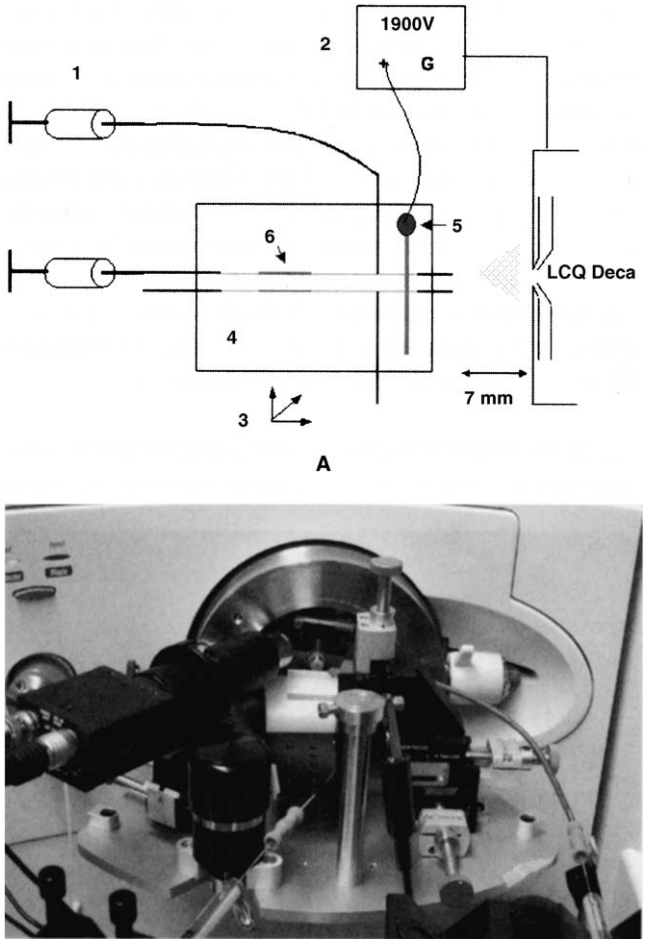


Fig. 10. Experimental setup for coupling SPE microchip to an LCQ Deca IT mass spectrometer (reprinted with permission from ref. [63]. Copyright 2005 Wiley-VCH). Upper panel: schematic drawing of the setup. (1) Syringe infusion pump; (2) power supply; (3) X, Y, Z stage; (4) microchip; (5) metal screw for voltage application; (6) monolith column. Lower panel: picture of the experimental setup modified from PicoView system.

its 10 ng/μL solution in 10 mmol/L ammonium acetate buffer at a flow rate of 150 nL/min and the breakthrough monitored by mass spectrometry. The sorption capacity of the 5 mm long monolith was 81 ng or 0.3 nmol. The elution was achieved by pumping pure acetonitrile through the SPE monolith. They also demonstrated the usefulness of their approach with imipramine-spiked urine. The monolithic preconcentrator enabled a 208-fold increase in imipramine concentration and a clean peak of the drug was obtained after elution.

Landers' group demonstrated in several papers efficient isolation of DNA from whole blood using microdevices containing unmodified silica [65–68]. Since packing microfluidic manifolds with beads was difficult, they prepared tetramethoxysilane sol–gel monolith in a microchannel [66]. Lysed whole blood diluted with aqueous quanidine hydrochloride solution was then pumped through the device, followed by washing with 80% propanol in water to remove the adsorbed proteins, and finally, the genomic DNA was released using tris(hydroxymethyl)aminomethane/ethylenediaminetetraacetic acid solution. However, more thorough investigation of this apparently simple approach revealed its weaknesses [68]. The matrix tended to crack on drying and, more importantly, the extraction efficiency was very low (Table 2).

To avoid these problems, they used the already mentioned approach relaying on silica beads embedded in sol–gel. While base catalyzed gelation process afforded monolith with properties even less favorable than the sol–gels alone, acid-catalyzed matrices extracted DNA comparably with the simply packed silica beads. However, Table 2 shows that fabrication of this monolithic device was significantly more reproducible [68]. The best results were achieved using a two-step process. A frit was first fabricated in the channel from beads/tetraethoxysilane/nitric acid slurry. Then, 15 μm bare silica beads were packed in the channel and finally, the bed was solidified using the tetraethoxysilane/nitric acid sol solution. This approach, despite its enhanced complexity afforded the best extraction efficiency and reproducibility. Since this device was used for the on–off solid-phase extraction only, reduced column efficiency observed for this type of monolith does not play any role.

The performance of these microfluidic devices was then studied in detail and the extraction process optimized [67]. For example, they observed that DNA loaded at pH 6.1 resulted in a higher recovery than at pH 7.6. The lower pH also enabled use of higher flow rate, resulting in a decrease in extraction time to less than 15 min. Using this procedure, template genomic DNA from whole human blood was purified on the microchip platform with the only sample preparation being mixing of the blood with a buffer prior to loading on the microchip device. They also compared the microchip SPE procedure with a commercial microcentrifuge method and found that comparable amounts of PCR-amplifiable DNA could be isolated from cultures of *Salmonella typhimurium*. The great potential of their device was illustrated by purifying DNA from spores of the vaccine strain of *Bacillus anthracis*. They hypothesized that an integration of SPE, PCR, and separation on a single microdevice could potentially enable complete detection of the infectious agent in less than 30 min [67].

Karwa et al. copied Landers' approach and prepared a 2 cm long monolithic SPE device for extraction of DNA via packing channel in a poly(dimethylsiloxane) (PDMS) microchip with 3 μm bare silica or 5 μm octadecyl silica beads and their "monolithization" using a mixture of propyltrimethoxysilane, methyltrimethoxysilane, and trifluoroacetic acid in water [69]. Alternatively, they used slurry of silica nanoparticles (fumed silica) mixed with tetraethylorthosilicate and trifluoroacetic acid in methanol–water mixture. With extraction efficiencies from crude cell lysate claimed to range from 70 to 80%, this approach appears to be a viable alternative for extraction of highly polar compounds. However, small non-polar molecules tended to penetrate PDMS and were difficult to recover.

Table 2
DNA extraction efficiency and reproducibility for various silica-based solid-phase materials [68]

| SPE material[a] | Extraction efficiency (%) | Standard deviation (%) | Coefficient of variance (%) |
|---|---|---|---|
| Silica beads | 57.1 | 43.1 | 75.5 |
| TEOS, PTS sol–gel | 19.2 | 15.0 | 78.1 |
| TEOS, sol–gel | 33.2 | 20.2 | 60.8 |
| Acid catalyzed sol–bead slurry | 60.9 | 12.4 | 20.4 |
| Base catalyzed sol–bead slurry | 8.7 | 5.5 | 63.2 |
| Two-step bead–sol system | 70.6 | 2.2 | 3.0 |

[a] *Abbreviations*: TEOS, tetraethoxysilane; PTS, propyltrichlorosilane.

## 7. Solid-phase extraction coupled to HPLC

In-tube solid-phase extraction is becoming an important part of automated miniaturized HPLC systems designed for both detection and separation of trace compounds in biomedical and environmental samples. Although the volume of the sample is often large, the typical HPLC columns can only accommodate a certain sample volume without loosing too much on efficiency. On the other hand, even in HPLC a higher quantity of the sample components is required to achieve sufficient detection sensitivity. These apparently conflicting requirements can again be solved by using solid-phase preconcentration.

In the typical setup for the SPE-HPLC, both extraction and separation columns are connected to a six-port injection valve. First, this implementation enables pumping the desired volume of the sample using a syringe or mechanical pump through the extraction unit. Then, the valve is switched to position "inject", the HPLC mobile phase releases the preconcentrated compounds into the separation column where they are separated.

Shintani et al. were the first using 15 cm × 200 μm i.d. C18 bonded monolithic silica microcolumn for preconcentration in front of a HPLC column [70]. Results of the breakthrough experiments carried out with biphenyl with on-column UV detection at different flow rates are summarized in Table 3. They clearly indicate that the monolith did not loose its sorption capacity even at very high flow rate of 50 μL/min, which enabled very fast runs. A significant 50-fold increase in sensitivity of the in-tube

Table 3
Breakthrough of 7.7 ppm biphenyl solution on 150 mm × 200 μm i.d. C18 silica monolith used as in-tube solid-phase microextraction device at different flow rates [70]

| Flow rate (μL/min) | Breakthrough time (min) | Biphenyl capacity (ng) | Back pressure (MPa) |
|---|---|---|---|
| 5 | 28.5 | 1097 | 0.2 |
| 10 | 13.8 | 1063 | 0.4 |
| 15 | 9.0 | 1040 | 0.7 |
| 50 | 2.6 | 1001 | 2.4 |

SPE/HPLC was demonstrated with the separation of a mixture of pesticides that are rather common compounds present in water in trace amounts.

Lim et al. prepared a series of 20 mm long monolithic C18 silica preconcentrators in capillaries varying in i.d. from 0.1 to 0.32 mm [71]. They observed a significant increase in the UV signal intensity in HPLC separation of phthalates after their enrichment from 20 ng/mL solution. A side-by-side comparison of C18 monolith and a unit packed with C30 silica beads clearly demonstrated advantages of the former. Typical recoveries of all studied phthalates from the monolith were close to 100%. Fig. 11 shows an impressive separation of phthalates in tap water finding 5.6 and 15.3 ng/mL of benzyl-*n*-butyl phthalate and di-*n*-butyl phthalate, respectively.

A similar system including a C18 monolithic silica-based 25 mm × 4.6 mm i.d. preconcentration column and a 150 mm × 2.1 mm i.d. separation column packed with 3 μm phenyl bonded silica beads was implemented by Kato et al. [72]. This group developed an automated system for the determination of 16 phthalate metabolites in urine including sample preparation followed by in-line preconcentration, HPLC, and tandem mass spectrometry. Use of mass spectrometry enabled to achieve subnanogram per milliliter limit of detection for the metabolites with both high accuracy and reproducibility.

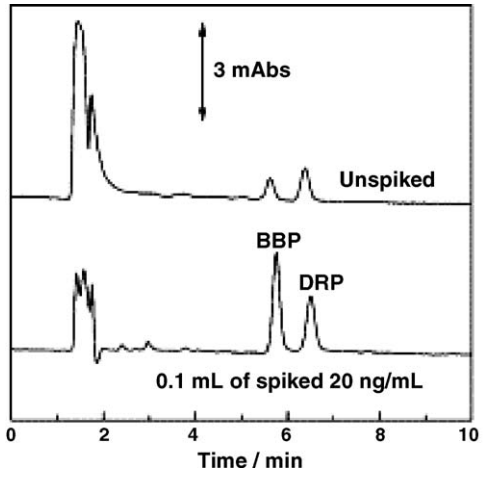


Fig. 11. Determination of phthalates in tap water (reprinted with permission from ref. [71]. Copyright 2004 Elsevier). Conditions: Separation column 100 mm × 0.32 mm i.d. C18 silica; precolumn 20 mm × 0.15 mm i.d. C18 monolith; mobile phase: 75:25 acetonitrile–water; flow-rate: 4.2 μL/min; sample: 1.0 mL of tap water (upper trace), and 0.1 mL of water spiked with 20 ng/mL of benzyl-*n*-butyl phthalate (BBP) and di-*n*-butyl phthalate (DBP) (lower trace).

In contrast to silica based SPE devices described previously, Feng's group prepared polymer-based preconcentration units in a 15 cm long 0.25 mm i.d. capillary by thermally initiated free radical copolymerization of a 10:90 mixture of methacrylic acid and ethylene dimethacrylate in the presence of binary toluene–dodecanol porogen [73]. They used their SPE column in conjunction with a standard analytical size C18 silica packed column for preconcentration and separation of methylxanthines—theobromine, theophylline, and caffeine from blood serum. They obtained high extraction efficiency for all three compounds with the detection limits of 12.0, 8.0, and 6.5 ng/mL, respectively, using UV detection. Quite good reproducibility with a R.S.D. of less than 2.9% was found over a dynamic range of 0.05–2 μg/mL. In the following paper, this monolithic preconcentrator was applied to the determination of amphetamine, methamphetamine and their methylenedioxy derivatives in urine decreasing the detection limits to 1.4–4.0 ng/mL [74]. Although the monolithic capillary column were claimed to be reusable for several times, it is unlikely that its recycling demonstrated by the authors would be accepted in clinical diagnostics.

While the poly(methacrylic acid-*co*-ethylene dimethacrylate) monolith proved useful for the preconcentration of basic compounds, extraction of acids requires monoliths with basic functionalities. Therefore, monolithic capillary columns were prepared from a monomer mixture comprising equal amounts of acrylamide, methylenebisacrylamide, and 4-vinylpyridine in the presence of dimethylsulfoxide and dodecanol as porogens [75]. This SPE monolith remarkably well adsorbed acidic compounds such as non-steroidal anti-inflammatory drugs and phenols.

The common problem of both of these monolithic devices is the missing optimization of the monoliths and complete lack of characterization of their porous structures. These data would be very useful for repetition of the results in different laboratories.

Another approach to monolithic SPE unit with sol–gel entrapped packed C18 silica particles was mimicking again that one developed by Zare's group [46] and mentioned several times throughout the text. A 250, 320, and 530 μm i.d. capillaries were packed with 5 μm C18 particles and "monolithized" using methyltriethoxysilane in methylene chloride, trifluoroacetic acid, and water mixture [76]. Then, 5 mm long plugs were cut and used as SPE units for the preconcentration of peptides obtained by tryptic digestion of bovine serum albumin. This procedure enabled a 60-fold increase in detection limit to an average of 1 ng/μL.

## 8. Solid-phase extraction and protein digestion

In most of the previous applications the preconcentration preceded separation. Both ease to exactly locate the monolith within the microfluidic channel using UV initiated polymerization through a mask as well as to fine tune the pore surface chemistry via photografting that we have developed [64,77–79] enabled fabrication of more complex microfluidic devices involving monolith serving multiple functions. For example, Peterson et al. developed a dual function microanalytical device for protein mapping [80]. The device included a 25 mm long porous

poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith prepared within a 50 μm i.d. capillary. This capillary with a pulled 9–12 μm needle tip was used as a nanoelectrospray emitter coupling the device to a mass spectrometer. Photografting with irradiation through a mask was then used to selectively functionalize a 20 mm long portion of the monolith with reactive poly(2-vinyl-4,4-dimethylazlactone) chains to enable the subsequent attachment of trypsin thereby creating an enzymatic microreactor with high proteolytic activity. The other 5 mm of unmodified hydrophobic monolith served as micro solid-phase extractor. This dual function device was demonstrated with concentration of myoglobin that was absorbed from its dilute 19 pmol/μL solution followed by its elution with 50 nL plug of acetonitrile and digestion. Alternatively, the device was used in the opposite direction, i.e. the protein was digested first and the peptides were collected in the concentration unit from which they were released in the mass spectrometer. Table 4 presents effect of sample loading on sequence coverage. Clearly, the larger the volume of the dilute protein solution, the higher the coverage. Interestingly, little difference has been observed based on the sequence of events. Practically equal peptide coverage was obtained after both preconcentration of the protein followed by digestion and digestion followed by extraction of peptides.

Table 4
Effect of sample loading and percentage of acetonitrile in the eluent on sequence coverage of digested myoglobin for dual function device operated in different flow directions [80]

| Volume loaded (μL) | SPE → digestion[a] | Digestion → SPE[a] |
|---|---|---|
| | Sequence coverage (%) | Sequence coverage (%) |
| No. SPE | 41 | 41 |
| 2 | 58 | 60 |
| 5 | 61 | 61 |
| 10 | 62 | 67 |
| 15 | 65 | 75 |
| 20 | 74 | 79 |

[a] The arrow indicates the direction of flow through the device.

## 9. Solid-phase extraction with molecularly imprinted monoliths

Vast majority of molecularly imprinted polymers (MIP) is prepared as a monolith. Most often however, the monolithic structure is then disintegrated, sieved, and packed in a column. Thus, only limited number of reports concerns application of MIPs in their "native" monolithic form. For example, MIP monolith was prepared in a 150 mm × 4.6 mm i.d. tube by copolymerization of styrene, glycidyl methacrylate, and methacrylic acid (monovinyl monomers) with divinylbenzene and triallyl isocyanurate (crosslinking monomers) in the presence of ceramide III as the imprinted molecule [81]. Although the texture, pore size distribution, hydrodynamic characteristic, and chromatographic performance of the monolith were determined, no optimization of these properties has been pursuit. Addition of ceramide III as print molecule in the polymerization mixture significantly affected porous structure of the monolith, and increased the retention of the imprint and its analogues. Application of the ceramide III imprinted monolith was

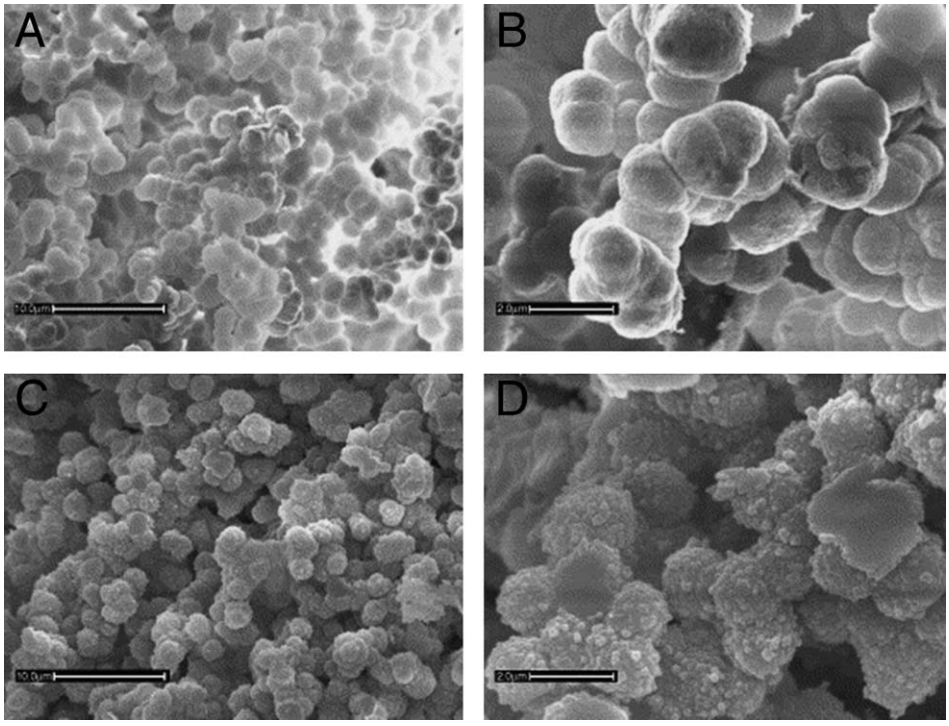


Fig. 12. Scanning electron micrographs of non-grafted core monolith at magnification 3000× (A) and 10,000× (B), as well as grafted bupivacaine monolithic MIP at magnification 3000× (C), and 10,000× (D) (reprinted with permission from ref. [82]. Copyright 2006 Elsevier).

demonstrated with the isolation of ceramides from yeast lipid extracts.

Courtois et al. used a novel approach for synthesized of monolithic molecularly imprinted polymers with selectivity towards local anesthetic drugs bupivacaine **6**, mepivacaine **7**, and ropivacaine **8** (Fig. 3) [82]. First, they prepared poly(trimethylolpropane trimethacrylate) "generic" monolith via photopolymerization in a 100 μm i.d. UV-transparent capillary that had properties they previously optimized [83]. The pores of this monolith were then filled with secondary polymerization mixture consisting of the imprinting compound, methacrylic acid, ethylene dimethacrylate, and initiated a photografting to create the thing layer of grafted MIP. Fig. 12 shows electron micrographs of the parent and grafted monolith. This two-step technique allowed the imprinted cavities to be directly created on the pore surface of the monolith using only a minimum amount of template. An additional benefit of this technique is the ease of removing the template from MIP after the secondary polymerization is completed. Long lasting "bleeding" of imprint molecules from the MIPs prepared in a single step is a serious problem currently preventing MIPs from a broad use in solid-phase extraction. Three different MIPs were prepared and evaluated to test the retention properties and cross-selectivity and compared with the non-imprinted reference column. They also imprinted one column with an equimolar mixture of all three anesthetics to assess the possibility of using the material for sample enrichment.

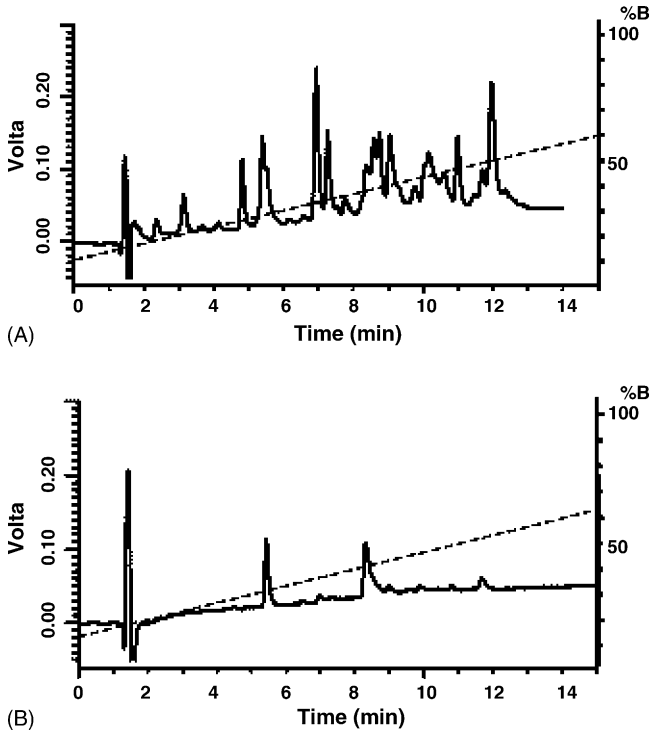


Fig. 13. Purification digest. Chromatogram of tryptic digest of β-casein without purification (A) and after using the titania-coated tip (B) (reprinted with permission from ref. [84]. Copyright 2004 Elsevier). Conditions: Sample: 50 μg tryptic digest of 0.5 mg/mL β-casein. Gradient: A–B (90:10) in 15 min to A–B (40:60). Detection: UV at 210 nm. Sample volume: 10 μL.

## 10. Extraction in pipette tips

Myiazaki et al. prepared 1.0 mm × 2.8 mm i.d. monolithic silica plug in 200 μL pipette tips and demonstrated its use for purification of peptides and proteins [84]. The 20 μm large pores allowed for pumping through the monoliths at a low drawing pressure provided by a typical mechanical pipette. The monolithic silica structure was modified with either C18 phase or coated with titania. While both native silica and its C18 bonded counterpart were used for sample concentration, desalting and removal of detergents from proteomic samples, titania-coated tip proved to be useful for purification and concentration of phosphorylated peptides. Fig. 13 shows separation of tryptic digest of β-casein injected in HPLC column after treatment in the titania tip and compares it with the separation of the original non-treated sample. While the original mixture exhibited a number of peaks, the isolation in the tip reduced the number of peaks to only two phosphorylated peptides, which were then identified in mass spectrometer.

## 11. Conclusions

This review clearly confirms that monoliths are finding their way in preconcentration and solid-phase extraction field. When I started editing the book "Monolithic Materials" in the year 2001 [16], I asked Dr. Shaofeng Xie to write a chapter concerning the use of monoliths in SPE. Only three relevant references were found at that time. Today, several tens of papers have been published on this topic as demonstrated in the presented text. Although the monolithic materials can hardly compete with the well-established concepts of solid-phase extraction in the typical off-line applications, they appear to be very promising in small size devices such as capillaries and microfluidic chips, which would be difficult to pack with particulates. They are also indispensable for in-line or in-tube applications and complex multifunctional systems.

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