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Monolithic Porous Polymer for On-Chip Solid-Phase Extraction and Preconcentration Prepared by Photoinitiated in Situ Polymerization within a Microfluidic Device

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Monolithic porous polymers have been prepared by photoinitiated polymerization within the channels of a microfluidic device and used for on-chip solid-phase extraction and preconcentration. The preparation of the monolithic material with hydrophobic and ionizable surface chemistries is easily achieved by copolymerization of butyl methacrylate with ethylene dimethacrylate, or 2-hydroxyethyl methacrylate and [2-(methacryloyloxy)ethyl]trimethylammonium chloride with ethylene dimethacrylate, respectively. The porous properties, and consequently the flow resistance, of the monolithic device are controlled by the use of a mixture of hexane and methanol as a porogenic mixture. This mixture was designed to meet the specific requirements for pore formation within macroporous monoliths useful in the microfluidic formats. The low flow resistance enables high flow rates of up to 10 μL/min, which corresponds to a linear flow velocity of 50 mm/s and far exceeds the flow velocities typical of the common analytical microchips. The function of the monolithic concentration device was first demonstrated using very dilute solutions of Coumarin 519. The performance in a more realistic application was then demonstrated with the enrichment of a hydrophobic tetrapeptide and also of green fluorescent protein for which an increase in concentration by a factor as high as 10^3 was achieved.

The interest in microfabricated devices designed for micro total analytical systems (μ-TAS) is growing rapidly. A number of applications of analytical microchips in areas such as enzymatic analysis,[1−3] polymerase chain reaction (PCR),[4,5] immunoassay,[6−8] DNA sequencing, hybridization, and mapping,[9−12] isoelectric focusing, capillary zone electrophoresis, and capillary electrochromatography[13−20] have already been reported. The first products involving microfluidic chip designed for the gel electrophoretic analysis of biopolymers are now commercially available from Agilent Technologies and compete successfully with classical polyacrylamide gel electrophoresis (PAGE). Although a few microanalytical systems with more complex architectures have already been demonstrated,[2−4,9,14,15] the vast majority of reports concerns individual devices.

Despite the undeniable success of microfluidic chip technologies in a variety of applications, some problems persist. For example, almost all of today's reported microfluidic chips feature open channel architecture. Hence, the surface-to-volume ratio of these channels is rather small. This is a serious problem in applications such as chromatographic separations, heterogeneous catalysis, and solid-phase extraction that rely on interactions with a solid surface. Since only the channel walls are used for the desired interaction, the microdevice can handle only minute amounts of compounds.

The issue of surface area in the macroscopic devices can be solved by packing them with porous particles that significantly increase the available surface area and also enable the introduction

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of specific chemistries into the device. However, only a few reports have dealt with attempts to enhance the limited surface area in channels of a microchip. For example, Regnier fabricated microchip channels containing arrays of ordered tetragonal posts.[21−23] Ramsey incorporated a porous silicate membrane into the microchip. The pore size was chosen to support electric current but prevented passage of large molecules. Using this device, electrokinetic preconcentration of DNA from dilute samples was demonstrated.[24] Early attempts to pack a microchip channel with beads were less successful due to technical difficulties.[25] Using a different approach, Harrison recently succeeded in packing octadecyl silica beads from a side channel into a specifically designed chamber of the microchip and used this device for solid-phase extraction.[26]

Sample preconcentration is a critical operation generally required for the determination of trace amounts of compounds of interest for which the concentration in the original solution exceeds the detection limits of the instrumentation. This is even more important in the case of microfluidic chip-related applications given the very small volumes of samples that can be handled within the microchip. One of the techniques enabling an increase in the concentration of desired compounds involves sample stacking, a technique successfully used in capillary electrophoresis.[27−30] However, this approach is only practical for electrodriven systems. In contrast, solid-phase extraction (SPE) is a more general method since it enables handling of large sample volumes regardless of the method used to ensure sample flow.[31−38] In SPE, samples are adsorbed on porous materials with appropriate chemistry to effect preconcentration and they are later released using a stronger eluent. In addition to the significant increase in concentration of the sample, the use of selective SPE devices may also eliminate interfering compounds.

Since analytical systems are a common target of microfluidic devices, they are likely to contain a variety of functional elements with specific functions that may not be compatible. For example, the continuous mode of operation typical of a microreactor may prevent its direct coupling with a chromatographic separation unit with its typical discontinuous operational mode. However, introduction of a SPE device between the reactor and the column provides for accumulation of the products flowing from the reactor in the SPE unit from which they may be released at the desired instant by applying a small volume of an eluent.

In the early 1990s, we introduced macroscopic rigid porous monoliths prepared in situ by a thermally initiated polymerization process. We have since demonstrated their use in a number of applications including HPLC and CEC of small molecules, chiral compounds, proteins, peptides, and nucleic acids.[39−45] The monolithic technology has also been successfully applied to the preparation of devices for scavenging undesired compounds from solutions and for SPE.[46,47] Our previous studies have also shown that good control may be exerted over both porous properties and surface chemistry of the monolithic polymers.[43,48] However, the free-radical polymerization initiated by heat we used originally is not ideal for the preparation of monolithic structures within a microchip. Precise positioning of various components of a microchip system is necessary to ensure its efficient function. While this could be achieved by locally heating selected areas of the chip to effect polymerization of the monolithic material, we prefer to use a UV-initiated polymerization process similar to the standard photolithography used for patterning in microelectronics. This enables the formation of monoliths only within a specified portion of the microfluidic device.[43] Using a mask, the polymerization may be strictly confined within areas exposed to radiation while no polymerization is observed in dark areas.

Following our recent success with the microscale preparation of porous polymer monoliths that combined well-controlled porous properties with appropriate surface chemistry using UV-initiated polymerization,[43] we had anticipated that this approach would be well-suited for the in situ fabrication of SPE microdevices. This report describes the simple and straightforward preparation of microfluidic devices for SPE that involves porous monoliths with two different surface chemistries. The function of these devices is demonstrated on adsorption and release of small molecules, peptides, and proteins.

EXPERIMENTAL SECTION

Materials. Coumarin 519 was purchased from Exciton (Dayton, OH). The hydrophobic peptide Phe-Gly-Phe-Gly was obtained from Sigma (St. Louis, MO) and recombinant green fluorescent protein (GFP) from Clontech Laboratories, Inc. (Palo Alto, CA). Ethylene dimethacrylate (EDMA) was obtained from Sartomer (Exton, PA). 2-Hydroxyethyl methacrylate (HEMA), butyl methacrylate (BMA), [2-(methacryloyloxy)ethyl]trimethylammonium chloride (META), azobisisobutyronitrile (AIBN), and 3-(trimethoxysilyl)propyl methacrylate were purchased from Aldrich (Milwau-

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Table 1. Synthesis of Ion Exchange (IE) and Hydrophobic (HI) Concentrator[a]

polymer	EDMA,[b] g	HEMA,[c] g	META,[d] g	BMA,[e] g	AIBN,[f] mg	MeOH, g	hexane, g	pore size, μm	surface area, m^2/g
IE	0.48	0.5857	0.1541		12	2.52	1.08	13.2	1.3
HI	0.48			0.7191	12	2.52	1.08	19.5	0.7

[a] Reaction condition: UV irradiation at room temperature for 3 h. [b] Ethylene dimethacrylate. [c] 2-Hydroxyethyl methacrylate. [d] [2-(Methacryloyloxy)ethyl]trimethylammonium chloride. [e] Butyl methacrylate. [f] Azobisisobutyronitrile.

kee, WI). EDMA, BMA, and HEMA were purified by passing them through a column with basic alumina followed by distillation under reduced pressure. AIBN was recrystallized from methanol. Solvents of analytical or HPLC grade (Fisher Scientific, Pittsburgh, PA) were dried over 4-Å molecular sieve. Glass wafers were obtained from Precision Glass & Optics (Santa Ana, CA).

The Coumarin 519−peptide conjugate was prepared by reaction of 4.5 mg of Coumarin 519 acid chloride with 2.5 mg of Phe-Gly-Phe-Gly in the presence of 10 μL of triethylamine in 3 mL of DMF. This mixture was stirred at room temperature overnight, and the desired product was recovered by column chromatography. The identity of the conjugate was verified by mass spectrometry.

Caution: Several methacrylates and solvents are known sensitizing agents. Coumarin is an irritant. Proper precautions should be taken during the physical handling of these materials.

Instrumentation. Laser-induced fluorescence using a He−Cd laser (OmNichrome Series 56, Melles Griot, Carlsbad, CA, wavelength 442 nm, 38 mW) was employed for the detection of compounds on the microchip. In our implementation, the laser beam passes an adjustable iris, a neutral density filter, directed by a set of mirrors, and two additional adjustable irises and is reflected by a dichroic mirror into a 40× microscopic objective (New Focus, Santa Clara, CA), which focuses the laser beam onto the microchip channel. The fluorescence was collected by the same objective and detected by a photomultiplier (model HC-120-05, Hamamatsu Corp., Bridgewater, NJ), and the signal was processed by a computer.

Microfluidic Chip. A simple straight microchannel 100 μm wide, 40 μm deep, and 6 cm long was used in this study. This microchannel occupies a portion of a more complex microfluidic system designed to enable the future incorporation of multiple elements. The microchips were fabricated in the microfabrication laboratory of the University of California at Berkeley. Briefly, Borofloat glass wafers (10 cm diameter and 1.1 mm thick) were cleaned in the piranha solution (3:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) for 10 min and coated with a 1500-Å layer of amorphous silicon using low-pressure chemical vapor deposition (LPCVD). The wafers were then dehydrated at 120 °C for 30 min, primed with hexamethyldisilazane (HMDS) for 5 min, spin-coated with photoresist (Shipley 1818, Marlborough, MA), and soft-baked. The mask pattern of the desired channel network was transferred to the wafer by exposing the photoresist to UV irradiation in a Quintel UV contact mask aligner for a few seconds. The photoresist was developed in Microposit developer concentrate (Shipley). The exposed sacrificial amorphous silicon layer was removed by CF_4 plasma etching in a plasma-enhanced chemical vapor deposition reactor (PIII-A, Technics West, San Jose, CA), and the glass substrate was exposed. The wafers were then etched in a 47% aqueous HF solution to generate the microchannels. The remaining photoresist was removed with PRS-3000 photoresist stripper, and the amorphous silicon was removed by CF_4 plasma etching. Access holes (1.4 mm diameter) were drilled through the etched wafers using diamond-tipped drill bits (Crystalite, Westerville, OH). Finally, an unetched Borofloat wafer was thermally bonded to the etched wafer in a programmable vacuum furnace (Centurion VPM, J. M. Ney, Yucaipa, CA) heated at 623 °C for 3.5 h.

Monolithic Concentrators. The walls of the microchannel were vinylized to enable covalent attachment of the monolith.[49] The channel was washed with acetone and water, filled with 0.2 mol/L NaOH for 30 min, washed with water, filled with 0.2 mol/L HCl for 30 min, washed again with water and acetone, and dried in an oven at a temperature of 120 °C for 1 h. The channel was then filled with 30% of 3-(trimethoxysilyl)propyl methacrylate in acetone and allowed to react at room temperature in the dark for 24 h. The vinylized channel was then washed with acetone and dried using a stream of nitrogen.

The monolithic ion-exchange and hydrophobic concentrators were prepared from the polymerization mixtures shown in Table 1 containing META and BMA, respectively. The mixture was first purged with nitrogen for 10 min to remove dissolved oxygen. The microchip channel was then completely filled with the polymerization mixture using a pipet and sealed with tape. The surface of the chip was then covered with a mask that had an open window allowing a specific section of the channel to be exposed to the UV light. The microchip was then covered with foil except for the 7-mm-long window and exposed to UV light in a reactor equipped with two 365-nm, 8-W UV tubes with an overall intensity of 1150 $\mu W/cm^2$ at a distance of 7.6 cm (VWR Scientific Products, West Chester, PA). The reactor was kept at room temperature by a continuous flow of pressurized air to avoid undesired thermal polymerization. The polymerization reaction was allowed to proceed for 3 h. Good reproducibility is easily achieved if monomers, solvents, and initiators of the same quality are used in the preparation of multiple batches of monoliths under the same fixed set of experimental conditions.

A 25-μm-i.d. and 50-cm-long fused-silica capillary (Polymicro Technologies, Phoenix, AZ) was attached to the microchip inlet access hole using epoxy glue. The other end of this capillary was connected to a programmable micropump (Micro-Tech Scientific, Sunnyvale, CA). Prior to its use, the monolith was washed with methanol to remove all unreacted components from its pores.

Bulk polymers for the determination of the porous properties were prepared from the remaining polymerization mixture in glass vials using identical conditions. Once the polymerization process

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was completed, the vials were carefully broken and the polymer was collected, extracted with MeOH for 16 h in a Soxhlet, and dried overnight at 60 °C in vacuo. The pore size distribution and specific surface area of these monolithic materials were determined in the dry state using mercury intrusion porosimetry (Autopore III) and nitrogen adsorption/desorption, respectively (ASAP 2010, both from Micromeritics, Norcross, GA). Microscopic images were obtained with an optical microscope (Eclipse TE 200, Nikon, Melville, NY), while scanning electron microscopy (SEM) micrographs were obtained with an electron microscope (JSM-6300, JEOL, Peabody, MA).

Adsorption and Elution of Probes. (a) Small Molecule. Coumarin 519 was used as the low molecular weight probe since this dye affords substantial fluorescence emission in aqueous solutions and the maximum of its absorption spectrum (446 nm) is a good match for the wavelength of the He−Cd laser (442 nm) that was used for these experiments. A 600 μmol/L stock solution was prepared by dissolving 8.6 mg of Coumarin 519 in 50 mL of acetone.

The stock solution was diluted with deionized water to give a final concentration of 10 nmol/L. A 200-μL injection loop was filled with this solution and this solution pumped through the ion-exchange concentrator at a flow rate of 3 μL/min. The adsorbed Coumarin 519 was eluted using 10-s-long pulses of 0.5 or 1.0 mol/L aqueous sodium salicylate at a flow rate of 1000 or 203 nL/min, respectively. The concentrating function was regenerated by washing with a 1:1 mixture of 0.1 mol/L HCl and acetonitrile.

For adsorption using the hydrophobic concentrator, the stock solution was diluted with water to 100 nmol/L. This solution (100 μL) was mixed with 100 μL of 0.1 mol/L HCl and 800 μL of 1 mol/L ammonium sulfate to afford a 10 nmol/L Coumarin 519 solution. The 200-μL loop was used again for application of this solution at a flow rate of 3 μL/min. Elution was achieved with acetonitrile at various flow rates.

(b) Peptide. Coumarin 519−peptide conjugate was diluted with deionized water to a concentration of 10 nmol/L. Using the loop, 200 μL of this solution was pumped through the hydrophobic concentrator at a flow rate of 3 μL/min. Elution was achieved with continuous flow or a pulse of acetonitrile.

(c) Protein. The concentrating ability of the hydrophobic monolith for a protein was tested using a 0.5 μg/mL (18.5 nmol/L) solution of the recombinant GFP in 0.95 mol/L ammonium sulfate/8 mmol/L Tris-HCl buffer, pH 8.0. In a typical experiment, 200 μL of the protein solution was pumped at a flow rate of 3 μL/min through the monolith. Elution was achieved using acetonitrile/water mixtures.

Frontal Analysis. The total capacity of the concentrator was determined using a 30 μM solution of Coumarin 519 in 0.8 mol/L ammonium sulfate that was pumped through the hydrophobic monolith. Detection points at which the fluorescence was continuously monitored were set immediately before and after the concentrator.

RESULTS AND DISCUSSION

Preparation of Monolithic Concentrator. Our UV-initiated polymerizations afforded 7-mm-long monoliths within the longer channels of our experimental multifunction microchips. Monoliths were prepared with two different surface chemistries at the desired location in the channel of the microchip (Figure 1). All of these

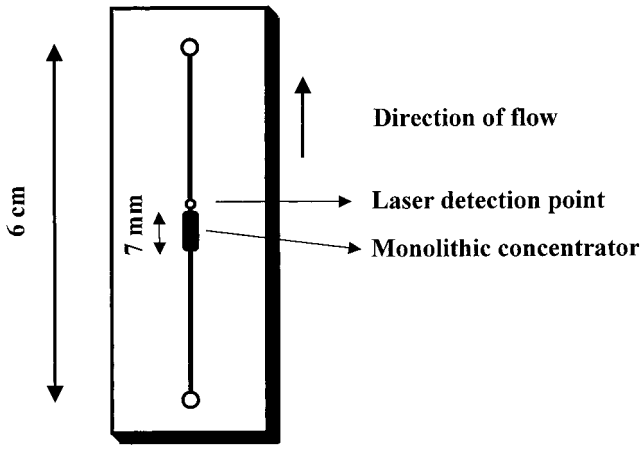

Figure 1. Microfluidic chip layout.

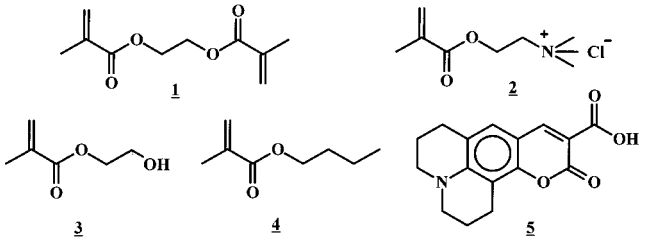

Figure 2. Structures of compounds used in this study: ethylene dimethacrylate (**1**), [2-(methacryloyloxy)ethyl]trimethylammonium chloride (**2**), 2-hydroxyethyl methacrylate (**3**), butyl methacrylate (**4**), and Coumarin 519 (**5**).

monoliths were cross-linked with 40% of the divinyl monomer, EDMA (**1**; Figure 2), a cross-linker that provides the material with high mechanical stability and contributes to the formation of the macroporous structure.

Compositions of polymerization mixtures used for the preparation of the monoliths are shown in Table 1. The ion-exchange (IE) type was prepared from a polymerization mixture that, in addition to EDMA, included two monovinyl monomers META (**2**) and HEMA (**3**). Since the quaternary ammonium functionality of META is a strong base, the monolith prepared from this mixture exhibits properties typical of strong anion exchangers. The addition of HEMA has a 2-fold function: (i) it increases the hydrophilicity of the polymer, thus reducing the undesired hydrophobic interactions with the probe, and (ii) it allows control of the loading of the monolith with the ionizable repeat units. The hydrophobic monolith (HI) was obtained from a polymerization mixture with only two monomers BMA (**4**) and EDMA. Our earlier capillary electrochromatography studies have indicated that monoliths with this composition were as hydrophobic as C18 bonded silica beads.[42,43]

Porogenic solvents are another essential part of the polymerization mixtures. Their function is (i) to dissolve all monomers and the initiator to form a homogeneous solution and (ii) to control the phase separation process during polymerization in order to achieve the desired pore structure. A porogenic mixture consisting of methanol and hexane was found to be suitable for the preparation of the monolithic concentrators. This new mixture was developed in an extensive study of a large number of porogens, which will be published elsewhere. Both the IE and HI monoliths were prepared using polymerization mixtures

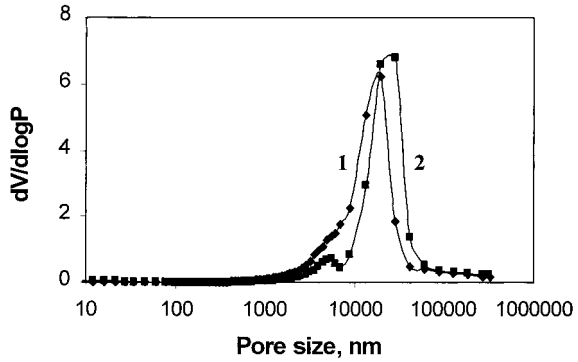

Figure 3. Pore size distribution profile of monolithic material in ion-exchange (1) and hydrophobic concentrator (2).

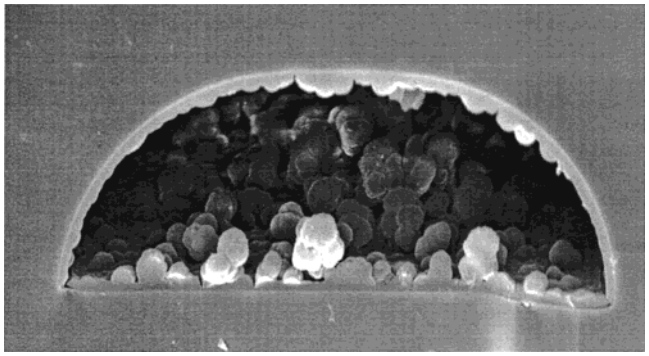

Figure 4. SEM image of the monolithic ion-exchange concentrator.

containing a large percentage of these porogenic solvents. Therefore, they are characterized by very large pores of 13.2 and 19.5 μm and pore volumes of 3.48 and 3.85 mL/g, respectively, that provide the monoliths with a low flow resistance and allow the use of high flow rates. Figure 3 shows the pore size distribution profiles for both types of monoliths used in this study. A surface area as large as possible to endow the monolith with a high capacity is another desirable property. Although the specific surface areas shown in Table 1 are relatively small, amounting to $\sim$1 m^2/g, it must be emphasized that these values are determined using monoliths in the dry state while the monoliths are typically used in a solvated state. Since the capacity of the concentrators used in this study was found sufficient for the desired application (vide infra), no efforts were wasted in attempts to increase their dry surface areas.

Flow of Liquid through the Monoliths. Although a few reports describe the use of mechanical pumps with microfluidic chips,[50−53] electroosmotic flow (EOF) is most often used to drive liquids through these devices.[1−20] The use of EOF is very convenient since its flow profile is flat. This feature is important to achieve very efficient separations. However, EOF also has some significant limitations. For example, ionizable functionalities must be available in the system and a high voltage must be used to generate adequate flow. Transport of ionized compounds through the microchannel may also be difficult since variations in the number and sign of charged moieties endows the molecules with different electrophoretic mobility. In addition, electrically driven flow can only be achieved with a limited number of solvents and requires the use of electrolytes with low ionic strength to avoid excessive generation of the Joule heat.

Since the flat flow profile typical of EOF is irrelevant for the adsorption/desorption processes of this study, we used the pressurized flow of a micro-HPLC pump attached to the microfluidic chip through a capillary. This enables the use of any liquid within a very broad range of flow rates. In the current implementation, the system has a dead volume of $\sim$3.8 μL. Therefore, a delay between the time of switching the solvents and the actual time of contact of the new eluent with the monolith is always observed. Obviously, the length of this interval depends on the flow rate. Integration of a pumping device directly in the microchip system would eliminate this delay.

The large pore size of our monolithic materials minimizes the flow resistance, enabling use of very high flow rates of up to 10 μL/min without causing mechanical breakage of the monolith (Figure 4.). It is worth noting that this flow rate is equivalent to a linear flow velocity of $\sim$50 mm/s that far exceeds the flow velocities typical of existing analytical microchips.[1−20] The achievement of high flow rates is clearly an important target for applications in which high volumes of very dilute samples must be pushed through the concentrator in a reasonable period of time to achieve the required enrichment.

Monolithic Concentrators. The spectrum of chemistries that will be required for solid-phase extraction and preconcentration in microfluidic devices is likely to be similar to those of current macroscopic devices.[31] Their range must clearly match the variety of samples that would be processed through the devices. Since the monoliths are obtained by a direct polymerization process in which a wide variety of monomers can be used, a broad array of chemistries are obtainable by using polymerization mixtures comprising monomers that carry the required functionalities or their precursors. These include monomers such as styrenesulfonic acid, 2-acrylamido-2-methyl-3-propanesulfonic acid, and [2-(methacryloyloxy)ethyl]trimethylammonium chloride for the incorporation of ion-exchange functionalities; nonpolar monomers such as styrene and alkyl methacrylates for hydrophobic interactions; and precursor monomers such as glycidyl methacrylate and chloromethylstyrene that may be readily modified to obtain other desired functionalities. All these monomers are commercially available. To demonstrate the monolithic technology in the context of SPE, we selected two different chemistries—ion exchange and hydrophobic.

Ion Exchange. The concentrator with ion-exchange functionalities adsorbs compounds with the opposite charge through Coulombic interactions. Since our probe molecule, Coumarin 519 **5** (Figure 2), is a carboxylic acid, we fabricated a monolithic concentrator with quaternary ammonium groups by the in situ polymerization of a monomer mixture containing META. The concentrator was first conditioned using a 1:1 mixture of 0.1 mol/L HCl and acetonitrile followed by loading a specific volume of probe solution. Desorption was later achieved using a 10-s pulse of a salt solution.

A number of salts including sodium chloride, fluoride, sulfate, and carbonate were tested. We initially planned to use the ion

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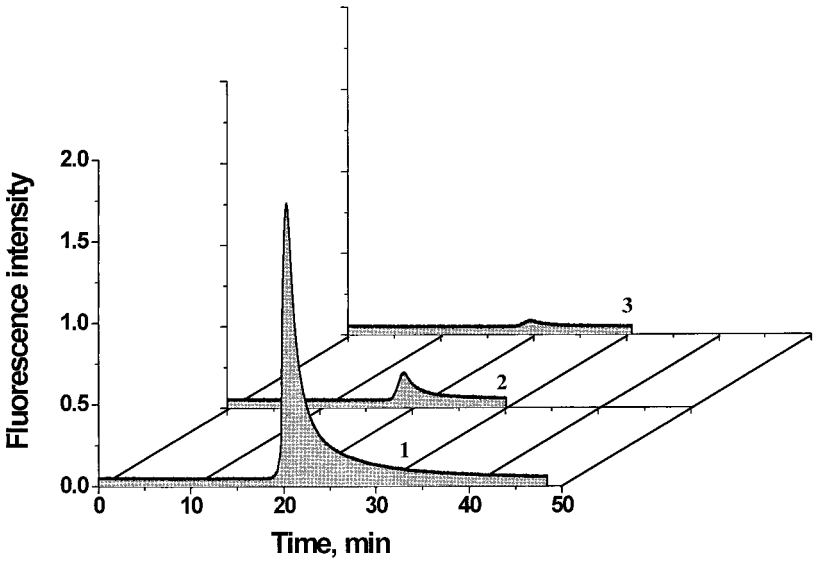

Figure 5. Elution of Coumarin 519 from ion-exchange concentrator. Conditions: loading, 200 μL of 10 nmol/L Coumarin 519, flow rate 3 μL/min; elution, 10-s pulses of 1.0 mol/L sodium salicylate, flow rate 203 nL/min. First pulse (1), second pulse (2), and third pulse (3).

exchanger in its fluoride form since this weakly bound counterion would be easily exchanged for the probe molecule. However, the interaction of this anion and the polymer is too weak and transition of the ion exchanger to its fluoride form is very slow. Sulfate and carbonate ions were also tested for desorption of the probe molecule. Although release of the probe is readily achieved with these anions, subsequent regeneration of the binding ability of the device is difficult. Best results were obtained with sodium salicylate with good elution of the probe molecule and easy regeneration using a 1:1 mixture of 0.1 mol/L HCl and acetonitrile. HCl is used as the salicylate anion is completely protonated in the strongly acidic solution, while acetonitrile increases its solubility in the mobile phase.

As a result of the mechanical robustness of the monolith and its covalent attachment to the wall of the microfluidic channel, high flow rates can be used to load the probe. This enables pumping relatively large volumes of solution through the concentrator within a short period of time. For example, the ion-exchange concentrator was loaded with 200 μL of 10 nmol/L Coumarin 519 at a flow rate of 3 μL/min.

Release of the probe from the concentrator is monitored by measuring the intensity of fluorescence and visualized as a peak. The concentration enhancement is then simply calculated by dividing the volume of probe solution used in the adsorption step by the volume of the eluted peak, which is the product of the peak width and the flow rate.[26] The elution rates may vary in a broad range. For example, elution of the probe was achieved using a 10-s pulse of 0.5 mol/L sodium salicylate at a flow rate of 1 μL/min. This affords a peak in which the dye concentration is increased 100 times. Unfortunately, the elution under these conditions is not complete and a second 10-s pulse of sodium salicylate solution releases more dye molecules from the concentrator. The second peak has an area 48% that of the first peak. In contrast, elution with a 10-s pulse of a more concentrated 1 mol/L sodium salicylate solution at a reduced flow rate of 203 nL/min (Figure 5) affords almost complete elution and a concentration enhancement by a factor of 190. From this result, the total adsorption capacity is estimated to be at least 2×10^{-12} mol representing a volumetric capacity of 8.6×10^{-5} mol/L. A further increase in capacity would likely result from the use of monoliths containing a higher percentage of META.

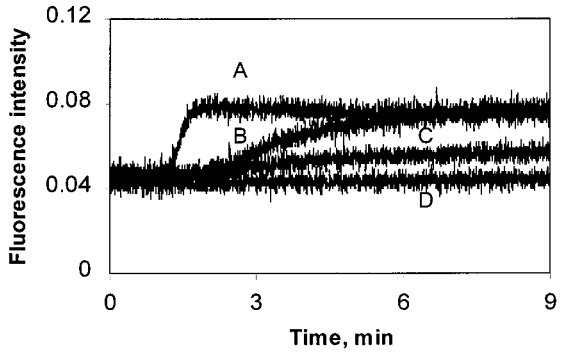

Figure 6. Effect of composition of the loading solution on adsorption of the probe. Conditions: flow rate, 3 μL/min, 10 nmol/L Coumarin 519 solution in water (A), 0.9 mmol/L HCl (B), 0.9 mol/L ammonium sulfate (C), and a mixture of 0.8 mol/L ammonium sulfate and 10 mmol/L HCl (D).

Hydrophobic Interactions. Given its chemical structure, Coumarin 519 is only slightly hydrophobic. Therefore, this probe is not adsorbed appreciably onto the surface of the butyl methacrylate-based monolith from its water solutions as demonstrated by the rapid breakthrough and steep increase in fluorescence shown in Figure 6 (trace A). In contrast the protonated form of Coumarin 519 is somewhat more hydrophobic and is therefore better retained by the monolith (Figure 6, trace B). Even better adsorption of Coumarin 519 was achieved when ammonium sulfate was added to the aqueous dye solution at neutral pH (Figure 6, trace C). Increasing the ionic strength of the solution is well known to induce adsorption of proteins onto organic supports in hydrophobic interaction chromatography.[54] Despite this improvement in the Coumarin 519 adsorption, a slow increase in the fluorescence was still monitored, indicating a limited retention.

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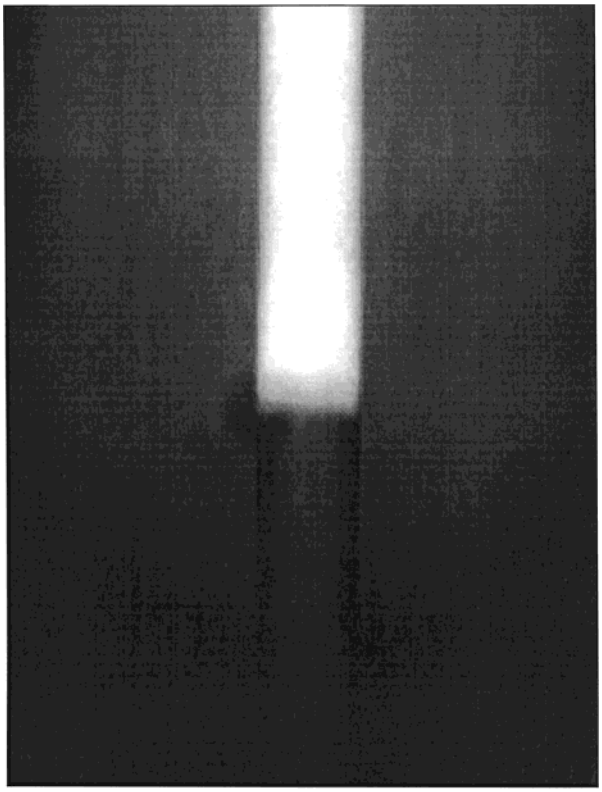

Figure 7. Microscopic image of the hydrophobic concentrator loaded with Coumarin 519 and adjacent channel filled with water.

Table 2. Effect of Flow Rate on Enrichment of Coumarin 519 Using the Hydrophobic Concentrator[a]

flow rate, nL/min	concn enhancement
3000	337
761	905
115	1650

[a] Eluent acetonitrile.

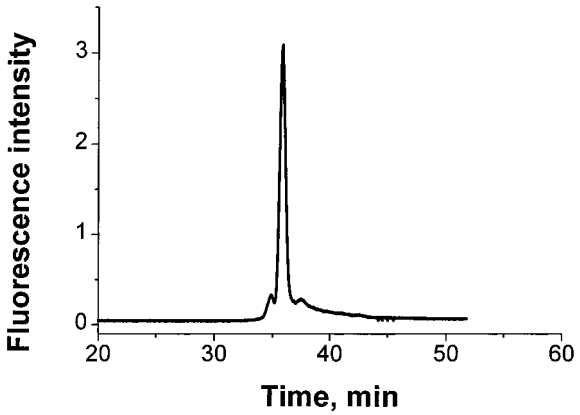

Figure 8. Elution of Coumarin 519 from hydrophobic concentrator. Conditions: loading, 200 μL of 10 nmol/L Coumarin 519, flow rate 3 μL/min; elution, acetonitrile, flow rate 115 nL/min.

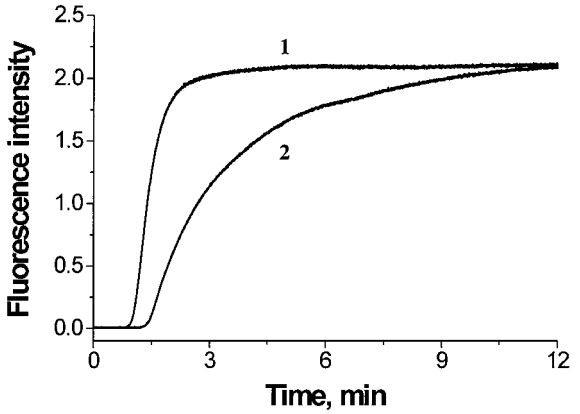

Figure 9. Capacity determination for hydrophobic monolithic concentrator using frontal analysis. Conditions: probe solution 30 μmol/L Coumarin 519 in 0.8 mol/L ammonium sulfate, flow rate 3 μL/min, fluorescence intensity measured before (1) and after the concentrator (2).

In contrast, no breakthrough was observed for adsorption from a solution that contained both HCl and ammonium sulfate in addition to the dye (Figure 6, trace D). Therefore, this solution was used in the concentration experiments. Figure 7 shows an optical micrograph of the saturation of the monolithic concentrator. No fluorescence was observed in the microchannel while the saturated monolith emits a rather strong fluorescence.

The adsorption of the Coumarin 519 probe was carried out again at a flow rate of 3 μL/min. This flow represents a velocity of 15 mm/s. As a result, adsorption from a typical sample with a volume of 0.2 mL is achieved in only 67 min. Desorption experiments performed at different flow rates are summarized in Table 2. The flow rate has a significant effect on the extent of the enrichment. For example, while a concentration enhancement of 337 times was achieved at a flow rate of 3 μL/min, a much higher enrichment of 1650 calculated from the peak shown in Figure 8 was obtained at a flow rate of 115 nL/min. The overall capacity of the hydrophobic concentrator as calculated from the results obtained by frontal analysis shown in Figure 9 is 1.93×10^{-10} mol or 8.3×10^{-3} mol/L. Table 3 compares these data with results obtained by other research groups. Although each group used different probes and sizes of the device, the adsorption capacity for the monolith is 3−5 orders of magnitude larger than that of both open channel and cavity filled with ODS beads.

Microchip SPE of Peptides and Proteins. Although fluorescent dyes are typically used for the evaluation of microfluidic concentrators,[26,55] practical applications of such concentrators are likely to involve quite different compounds. For example, a microfluidic system used in protein mapping might make use of a sequence such as protein digestion followed by separation of the resulting peptide fragments and their identification by mass spectroscopy.[56] Such a system is likely to require an element allowing preconcentration of a protein from its dilute solution prior to its enzymatic degradation and a second concentration element located in front of a separation unit for accumulation of the peptides prior to their injection in the analytical column of the device. Therefore, we have also studied the enrichment of peptide and protein probes using the hydrophobic concentrator.

First, a conjugate of Coumarin 519 and the tetrapeptide Phe-Gly-Phe-Gly was adsorbed onto the butyl methacrylate-based

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Table 3. Comparison of the Capacity of the Concentrator Prepared by Different Method

concept	compound	flow rate, nL/s	elution vol, nL	capacity, mol	ref
functionalized open channel	C-460	0.16	0.48	2.2×10^{-13}	55
channel cavity packed with beads	BODIPY	1.2	0.33	2.8×10^{-15}	26
channel with monolith	C-519	50	35	1.9×10^{-10}	this work

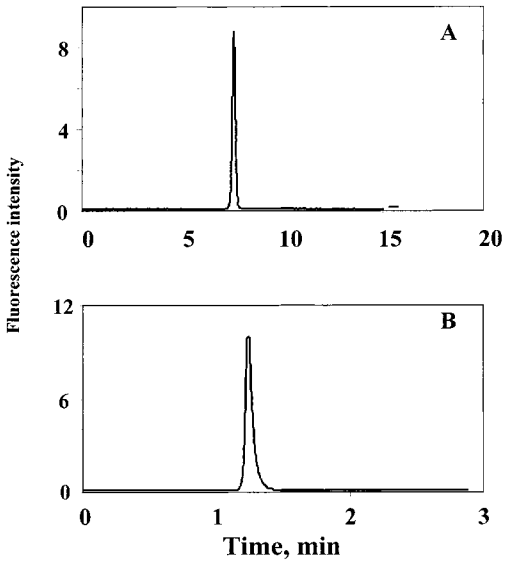

Figure 10. Elution of tetrapeptide labeled with Coumarin 519 from hydrophobic monolithic concentrator. Conditions: loading, 200 μL of 10 nmol/L peptide solution in water, flow rate 3 μL/min. Continuous elution with acetonitrile at a flow rate of 506 nL/min (A); elution using a 30-s pulse of acetonitrile at a flow rate of 3 μL/min (B).

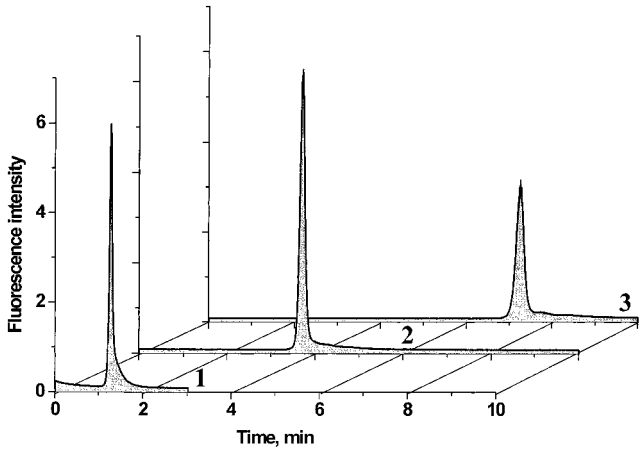

Figure 11. Elution of green fluorescent protein from hydrophobic monolithic concentrator. Conditions: loading, 200 μL of 18.5 nmol/L protein solution in 8 mmol/L TRIS−HCl buffer (pH 8) containing 0.95 mol/L ammonium sulfate, flow rate 3 μL/min; elution with 1:1 acetonitrile/water at a flow rate of 3 (1), 1.03 (2), and 0.53 μL/min (3).

monolithic concentrator. Since the conjugate is very hydrophobic, complete adsorption is achieved from its aqueous solution. Depending on the type of application, the releasing solvent can be introduced continuously by simply changing the composition of the mobile phase or as a short plug by injecting a pulse of the solvent. Using such an approach, the concentration of the dye−peptide conjugate released using acetonitrile at a flow rate of 0.5 μL/min is increased 1320 times (Figure 10 A). We also tested release of the Coumarin 519−peptide conjugate using injection of a pulse of the releasing solvent (acetonitrile). While a 10-s pulse at a flow rate of 3 μL/min is not sufficient to achieve the complete release of the conjugate, a 30-s-long pulse of acetonitrile enables recovery of all probe molecules and leads to a concentration enhancement of 520 (Figure 10B). Recombinant GFP consists of 238 amino acid residues having a molecular weight of 27 000. When illuminated with blue or UV light, GFP yields a bright green fluorescence.[57,58] Since GFP is less hydrophobic, its adsorption was carried out from solution in 0.95 mol/L ammonium sulfate in 8 mmol/L Tris-HCl buffer, pH 8.0. Once again, 200 μL of 18.5 nmol/L GFP solution was pumped through the monolith at a flow rate of 3 μL/min. The protein was released using a 1:1 water/acetonitrile mixture. While a sample enrichment of 355 times was obtained at a flow rate of 3 μl/min, this value increased to 756 at 1.03 μL/min and to 1002 at 0.53 μL/min (Figure 11.).

Stability of the Monolithic Concentrator and Recovery of Compounds. To test the long-term stability and reproducibility of the concentrators, a large number of the measurements described above were carried out using a single microfluidic chip containing monolithic polymer. For example, the hydrophobic concentrator was used over a period of ~3 months with ~300 injections of various compounds. In each run, the mobile phase—water—was a precipitant for the polymer used for the concentrator while the eluent—acetonitrile—was a good swelling agent. Although the high level of cross-linking does not allow extensive swelling of the monolithic material, even small volumetric changes of the matrix could constitute a periodic stress for the monolith. However, if such stress was indeed occurring, it had no effect on the long-term performance of the concentrator. During the course of this study, the flow rate, which is one of the most critical variables, was changed quite often, routinely reaching very high values of up to 3 μL/min. Low-flow-rate flushing with acetonitrile was the only "maintenance" carried out on the concentrator. During the entire period of study, no change in back pressure, flow, and adsorption characteristics were observed.

Special attention was also paid to the completeness of the desorption step since this is an all-important issue enabling the repeated use of the devices. With the ion-exchange concentrator, only ~50% of adsorbed Coumarin 519 was eluted using a 10-s pulse of 0.5 mol/L sodium salicylate. Therefore, the use of a second or even a third pulse was necessary to achieve complete elution. In practice, multiple pulses can be replaced by the use of a lower flow rate together with a stronger eluent or a longer pulse to complete the elution from the ion-exchange concentrator in a single step. In contrast, elution from the hydrophobic concentrator was always achieved in a single step. The eluted peaks of the

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probes were sharp with no tailing that would indicate slow elution of any residual fluorescent compound. After the elution of peaks, the eluent did not exhibit any fluorescence that could be detected by our sensitive instrumentation. Similarly, the direct detection of any residual fluorescence for the monolith itself after elution would constitute a further check on the absence of remaining absorbate. In all of our experiments, we were able to ascertain that complete elution is readily achievable and that the occurrence of carryovers leading to possible cross-contamination in the monolithic concentrator is negligible.

CONCLUSION

UV-initiated polymerization within the channels of a microfluidic device is a simple and versatile approach that enables a single-step preparation of monolithic materials with a wide variety of chemistries and porous properties at the desired location. This approach enables the design and preparation of numerous building blocks instrumental for the development of microanalytical systems. Our monoliths for solid-phase extraction and preconcentration constitute the first example of a highly efficient device that can be easily created and used in the microchip format. This work now opens a new avenue for the development of a number of other functional microelements. Although the ability to concentrate low molecular weight compounds, peptides, and proteins was only demonstrated using monoliths operating on the basis of hydrophobic or ion-exchange interactions, monolithic microconcentrators operating in other adsorption modes are easily conceivable. The high mechanical strength and the low flow resistance of the monoliths allow their use at high flow rates. This is important in situations that require passing large volumes of dilute solutions through the device in a short period of time.

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