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Rigid Macroporous Polymer Monoliths**

By Eric C. Peters, Frantisek Svec, and Jean M. J. Fréchet*

1. Introduction

The recent explosive growth of such fields as combinatorial chemistry, solid phase synthesis, catalysis, and separation science has rekindled the interest of both academia and industry in the general area of crosslinked polymer supports. First introduced over 50 years ago, these materials are primarily produced in the shape of regular beads.[1,2] Beads are easy to prepare and handle, they do not possess sharp edges that may break to form fines, and they may readily be used in packed beds for continuous flow operations. Most applications reported to date involve the use of slightly crosslinked ("gel"-type) copolymers that require swelling to become porous. Although swelling in a good solvent "opens" the polymer matrix, allowing virtually all of the copolymer sites to be accessed by reagents, the issue of swelling also limits the choice of solvents that may be used with the "gel" beads. In addition, swollen beads may be difficult to handle, and rapid changes in solvent strength often lead to the shattering of some beads.

1.1. Macroporous Polymers

A search for polymeric matrices suitable for the manufacture of ion-exchange resins with enhanced osmotic shock resistance and faster kinetics led to the discovery of macroporous polymers in the 1950s.[3–5] These polymers are characterized by fixed porous structures that persist regardless of the solvent employed, and even in the dry state. Their internal structure consists of an interconnected array of polymer microglobules separated by pores, and their structural rigidity is secured through extensive crosslinking. In addition to the manufacture of ion-exchangers, macroporous beads have found numerous other applications such as adsorbents, supports for solid phase synthesis and the immobilization of enzymes, polymeric reagents and catalysts, chromatographic packings, and as media in diagnostics.[6–8]

Just like their gel-type counterparts, macroporous beads are produced by "classical" suspension polymerization. An organic mixture containing monovinyl and divinyl monomers, an initiator, and a porogenic solvent is dispersed upon stirring in an aqueous medium.[1,2] Traditional porogens include inert diluents that may either be solvating or nonsolvating solvents for the polymer being produced, soluble linear polymers, or mixtures of the above. Thermally induced decomposition of the initiator causes polymerization to start within the individual droplets, ultimately leading to the production of spherical polymer particles. After polymerization, the inert porogen is washed away from the polymer beads, revealing the typical macroporous morphology: therefore, beads prepared with 50 % porogen have 50 % internal pore volume. Both the mechanism of pore formation and methods for its control during this process have been investigated extensively, and are described elsewhere.[9–11]

1.2. Preparation of Macroporous Beads

The efficient production of polymer beads by suspension polymerization requires optimization of numerous parameters, including reactor geometry, the shape and speed of the mechanical stirrer, and the nature and amount of suspension stabilizing agent used among others. This technology has already been developed to such a degree that excellent control over bead size and porosity is routinely achieved for a number of different monomer systems during industrial scale production. However, polymer beads containing certain reactive functional groups might be difficult to prepare directly by a classical suspension polymerization process due to the solubility or reactivity of the functional monomer in water. Therefore, either multistep post-polymerization functionalizations are often performed on precursor beads, or non-traditional suspending media such as saturated aqueous salt solutions,[12] hydrophobic liquids,[13] or perfluorocarbons[14] are employed, making the overall process far less predictable and convenient.

1.3. Diffusion Versus Convection

Porous polymer beads are typically used either in a batch operation or as a packed bed. Despite the many advantages that led to their widespread use, columns and reactors packed with typical particulate materials also have some limitations. Chief among these are the slow diffusional mass transfer of high molecular weight solutes into the stagnant liquid present in the pores of the beads, as well as the large void volume between the packed particles.[15,16]

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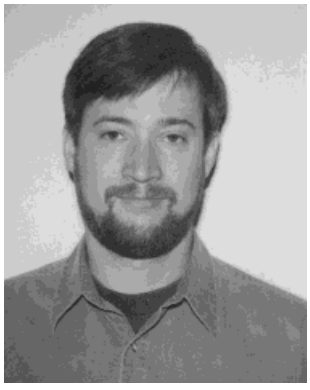
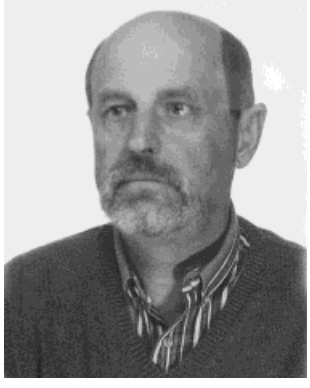
These limitations are particularly detrimental to processes where the speed of mass transfer limits the overall rate, as is the case in chromatography[17] and catalysis.[18]

In contrast to diffusion, which relies on concentration gradients as its driving force, convection uses flow to greatly accelerate the rate of mass transfer. The beneficial effects of this increase in mass transfer on the efficiency of heterogeneous catalysts has already been demonstrated in theory for inorganic supports with large flow-through pores.[19] In view of their permanent pore structure that persists regardless of the solvent employed, macroporous polymer beads appear well-suited for applications requiring high rates of mass transfer. Unfortunately, the relatively small pore size (mostly less than 100 nm) typical of traditional macroporous polymer beads is far too small to support convective flow.

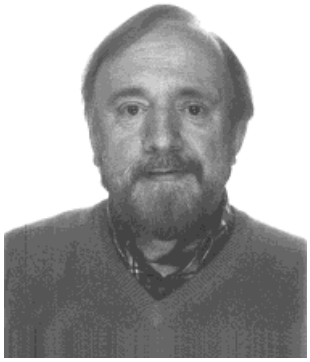
In the early 1990s,[20] Regnier et al. demonstrated that convective flow through the pores of modified macroporous beads could be achieved if they possessed pores greater than 600 nm in diameter. Despite the fact that convective flow through these beads accounted for only 2 % of total column flow,[21] these stationary phases enabled the chromatographic separation of biopolymers at substantially increased speeds compared to columns packed with "classical" smaller pore stationary phases. Although further increases in the percentage of overall convective flow should yield further improvements in performance,[22,23] the required changes in flow pattern would be difficult to effect in a traditional packed bed, since a column packed with spheres always has a large interparticular void volume of at least 26 % through which preferential flow will always occur. Clearly, a system containing little, or ideally, no inter-



Eric Peters was born in Philadelphia in 1967. After obtaining his B.S. in chemistry from Georgetown University (1989), he worked at the Rohm and Haas Company for five years on the development of new polymers for the biopharmaceutical industry. He received his M.S. from Cornell University (1996) and his Ph.D. from the University of California, Berkeley (1999) for his work on macroporous polymer monoliths under the supervision of Jean M. J. Fréchet. Eric Peters is currently a staff scientist at the Novartis Institute for Functional Genomics. His research interests include the development of novel polymer solid supports and architectures, as well as their utilization in a variety of fields.



Frantisek Svec was born in Prague, Czechoslovakia. He obtained his Ph.D. in polymer chemistry from the Institute of Chemical Technology, Prague in 1969 for his work on reactive polymers. After his graduation, he was appointed assistant professor at this Institute. From 1976 to 1992 he worked at the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences in Prague. In 1992, he joined the faculty at Cornell University in Ithaca, NY, and has been at the University of California, Berkeley since 1997. His current research interests embrace the preparation of well-defined porous beads and monoliths, the use of these materials for various applications such as HPLC separations, electrochromatography, the laboratory-on-a-chip, and molecular recognition, and the exploration of new supports and auxiliaries for combinatorial chemistry.



Jean Fréchet, born in France, studied in Lyon prior to pursuing his Ph.D. degree at SUNY Syracuse University (USA) and joining the chemistry faculty at the University of Ottawa (Canada) in 1973. In 1987 he joined Cornell University as the IBM Professor of Polymer Chemistry and subsequently the Peter J. Debye Chair of Chemistry. Since 1997 he has been Professor of Chemistry at the University of California Berkeley and Faculty Senior Scientist at the Lawrence Berkeley National Laboratory. His interests are at the interface of organic and polymer chemistry.

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particular void volume is required to fully realize the potential advantages of convective flow.

2. Monolithic Porous Polymer Materials

The ideal implementation of a medium possessing no interparticular voids would involve a single continuous piece of porous material. Monolithic open-pore polyurethane foams were investigated in the early 1970s as stationary phases for both high performance liquid and gas chromatography, but were found to suffer from excessive swelling and softening in some solvents.[24,25] Other approaches towards continuous media that emerged in the late 1980s include stacked membranes,[26] rolled woven matrices,[27,28] compressed soft poly(acrylamide) gels,[29] and macroporous disks.[30,31]

2.1. Ordered Structures

The preparation of materials with ordered macroporous structures that complement the "classical" porous polymers described above has also received a great deal of attention.[32–36] Such materials are predicted to exhibit a number of useful new properties, for example, their optical behavior enables the realization of photonic bandgaps.[37] In contrast to the polymerization methods described earlier, these materials derive their porous structure from the use of macroscopic shape templates, such as stable emulsions,[32] polymer latex,[33,34] and the interstitial volume of other porous structures[35,36] during their formation. Primarily employed to date for the production of inorganic materials, a similar shape templating approach has also been described for the preparation of organic polymers with ordered porous structures.[38] Although still in its infancy, these approaches may lead to improved macroporous materials for traditional applications such as supports, adsorbents, and chromatographic media. For example, Antonietti et al. have used two different template systems simultaneously for the preparation of silica with a bimodal pore structure.[34] This affords a material combining the increased mass transport characteristic of larger pores with the large surface area normally derived from smaller pore networks.

2.2. Polymerized High Internal Phase Emulsions

Another type of macroporous polymer may be obtained using the dispersed aqueous phase of a high internal phase emulsion (HIPE) as a template system. Such water-in-oil emulsions have monomer phase volume fractions greater than the value of 0.74 characteristic of perfectly packed uniform spheres.[39] Subsequent polymerization of the continuous organic phase results in the formation of a true monolithic structure possessing an interconnected system of huge pores and low bulk density.[40] These poly-HIPE polymers have been tested in a variety of applications,[41] including composite materials for combinatorial chemistry,[42] catalytic supports,[43] and metal chelating agents.[44] However, the multiphase nature of their preparation imposes many of the same limitations discussed earlier for suspension systems, and the resulting monolithic materials are rarely used in the flow-through mode.[45]

2.3. Rigid Polymerized Macroporous Monoliths

In the early 1990s, our research group introduced an entirely new class of continuous medium based on rigid macroporous polymer monoliths.[46,47] Produced by a very simple "molding" process, these novel materials are designed for use in a flow-through manner. The resulting increase in mass transfer, in conjunction with their unique method of preparation, allow these materials to be used to particular advantage in a broad variety of applications compared to their particulate counterparts. Inorganic silica-based analogs of these materials were later reported by several groups starting in 1996.[48,49]

These novel monolithic media possess all of the advantages of traditional macroporous polymers, such as a rigid, well-defined porous structures that persist regardless of the environment in which the polymer is used. However, unlike traditional beads, these monolithic media are produced by polymerizing the organic (monomer) phase in bulk within the confines of an unstirred mold and without the need for any suspending phase (Fig. 1). This process produces a sin-

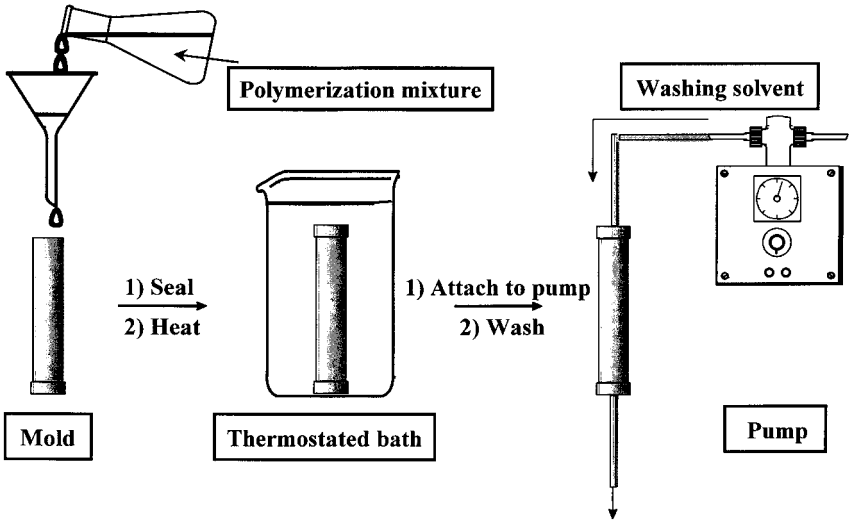

Fig. 1. Schematic for the preparation of rigid macroporous polymer monoliths.

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gle continuous macroporous object with interconnected channels that support flow at very modest pressures. Based on the pore size distribution profiles typically observed for macroporous beads, it is not intuitively apparent that the polymerization process used for the preparation of a monolith should afford a porous material with the required high permeability. However, Figure 2 shows that the porous structure of these polymer monoliths is quite different from that produced when the identical reaction mixture is subjected to a suspension polymerization process.[50] It is characterized by a bimodal pore size distribution consisting of both large micrometer-sized "channels" and much smaller pores in the 10 nm size range. This unique porosity profile and the lack of any observed wall effect (open channels along the walls of the mold due to volume shrinkage) result from the absence of both the interfacial tension between aqueous and organic phases, as well as the dynamic forces typical of stirred dispersions.

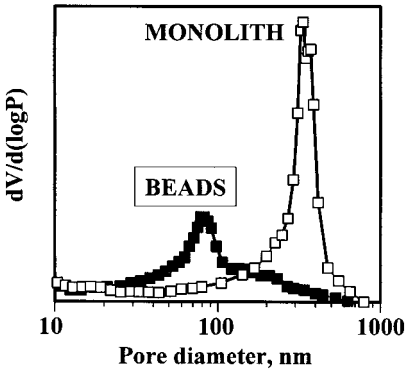

Fig. 2. Differential pore size distribution curves for poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads (■) and monolith (□) prepared using the same organic phase composition at a polymerization temperature of 70 °C [50].

2.3.1. Control of Pore Size and Hydrodynamic Properties

The network of large canal-like pores that traverse the length of the monolith allows liquids to pass through these materials under moderate pressures even at high flow rates. The hydrodynamic properties of these materials as well as their surface areas can be further refined by effecting changes in a number of variables that allow the tuning of the average pore size within a broad range spanning at least two orders of magnitude from tens of nanometers to several micrometers.[51–55] For example, changing the composition of the porogenic solvent affects the solvation of the polymer chains during the early stages of the polymerization. As a result, larger pores are generally obtained if poorer solvents are used because of an earlier onset of polymer phase separation.[51–53] In contrast, increasing the percentage of crosslinking monomer decreases the mode pore diameter of the resulting monolith due to the formation of highly crosslinked, less swellable polymer nuclei during the early stages of the polymerization.[51,53]

Although effective in controlling pore size, both of these methods involve adjustments in the composition of the polymerization mixture being used and, in the latter case, also leads to the production of monolithic materials with varying levels of chemical functionality. In contrast, temperature is an especially effective means of control, allowing the production of macroporous materials with a broad range of porosity profiles from a single polymerization mixture.[51,55] These shifts in pore size distribution as a result of changes in polymerization temperature can readily be explained by considering the number of polymer nuclei formed at different temperatures at constant initiator concentration as well as the rate of their formation.

Although easy to effect on a small scale, the accurate control of polymerization temperature during the preparation of larger size monoliths is far more problematic. The unstirred nature of the polymerization within the confines of a mold hampers the dissipation of the considerable heat produced by the exothermic polymerization reaction. In addition to an overall deviation from the desired polymerization temperature, the magnitude of the exotherm has been shown to vary radially across the contents of the mold, leading to monoliths with heterogeneous pore structures.[56] Such inhomogeneities could severely limit the effectiveness of these unique materials in larger scale applications such as catalysis or preparative chromatography. Therefore, specific techniques have been developed to obtain even larger diameter monoliths with homogeneous pore structures. These include the continuous gradual addition of the polymerization mixture to a heated reaction vessel,[56] or a batch polymerization under "living" free radical conditions.[57]

The use of porous supports in such fields as catalysis or adsorption relies on interactions with surface active sites. However, the large macropores that transverse the length of a monolith enabling liquid to flow through these materials at reasonable operational pressures possess limited surface area. Thus, the overall porous properties of the monolith must carefully be tailored for its intended application. Recently, several approaches have been reported that further increases the ability to balance the hydrodynamic and surface properties of these materials (vide infra).[57,58] For example, monolithic materials with high specific surface areas of 300 m^2/g and completely novel porosity profiles have been produced by polymerization at substantially elevated temperatures using 2,2,6,6-tetramethyl-1-pyperiodinyloxy- (TEMPO-)mediated "living" free radical process.[57] The porosity profile of the monoliths is unique since an even distribution of pores over a wide range of sizes is observed as measured by both mercury porosimetry and inverse size-exclusion chromatography (ISEC). This dramatic change in pore structure was found to result from the effect of the elevated polymerization temperature on the solvency of the porogenic solvent.[57]

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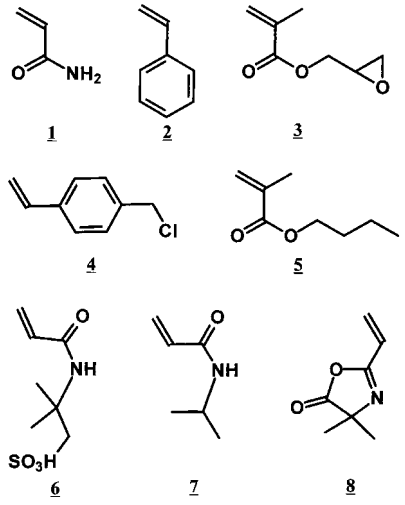
2.3.2. Surface Chemistry

Although the increased mass transfer properties of these flow-through materials have been the primary driving force behind their development, macroporous polymer monoliths possess a number of other advantages compared to their traditional bead counterparts. For example, a far greater number of surface functionalities can readily be obtained by the direct polymerization of the corresponding monomers because there is only one phase in the mold. The production of monoliths containing hydrophilic (acrylamide **1**[59]), hydrophobic (styrene **2**[51]), and reactive (glycidyl methacrylate **3**[51] (GMA) or 4-(chloromethyl)styrene **4**[60]) moieties has been demonstrated. These reactive pendant groups may further be transformed to afford functionalities for which no monomer precursor is readily available.[60] In



addition, monomers with vastly different polarities may be copolymerized directly since, in contrast to standard suspension polymerization, the occurrence of partitioning between aqueous and organic phases is not possible. For example, the hydrophobic liquid monomer butyl methacrylate **5** (BMA) and the hydrophilic solid monomer 2-acrylamido-2-methyl-1-propanesulfonic acid **6** (AMPS) may be "compatibilized" into a single homogeneous phase using a ternary porogen system. Subsequent copolymerization in a mold affords a hydrophobic monolith with controlled levels of dispersed and highly hydrophilic charged functionalities.[61]

The typical in situ polymerization approach used for monolithic media yields a single interactive site for every incorporated and accessible monomer unit. By contrast, the attachment of polymer chains to the internal surface of the pores leading to "hairy" pores can increase dramatically the density of accessible reactive groups, effectively expanding the reaction zone from the monolith's limited surface area to its internal pore volume. Grafting may also be used to impart unexpected properties to monolithic polymers, enabling the preparation of novel stationary phases for chromatography, composite materials that change their properties in response to external stimuli such as temperature, or reactive supports for solid-phase organic chemistry.[62,63] A number of grafting strategies have been investigated, including initiation from the pore surface under classical[62] or "living" free radical conditions[57] ("grafting from" approach) as well as in situ polymerization within the internal pore volume of the monolith[63] ("grafting to" approach).

3. Applications of Macroporous Polymer Monoliths

3.1. Thermally Responsive Composite Devices

Polymer of (*N*-isopropylacrylamide) **7** (PNIPAAm) is the best known member of a class of polymers that exhibits a lower critical solution temperature (LCST). While the polymer is soluble in cold water, it becomes insoluble when the temperature is raised to 32 °C.[64] PNIPAAm chains undergo a rapid and reversible phase transition from extended hydrated helices below the LCST to collapsed hydrophobic coils above this temperature.[65,66] Such dramatic changes in properties in response to an external stimulus have led to the investigation of these materials as novel sensors, drug delivery systems, immobilized biocatalyst supports, and membranes with controlled permeability.[67]

The volume transitions exhibited by PNIPAAm may also be used to regulate the flow of liquid through a polymer monolith simply by effecting changes in temperature.[63] For example, Figure 3 shows the on/off temperature-responsive back pressure behavior of a monolith with linear PNIPAAm chains grafted to its internal pore surface. At 40 °C, the grafted chains exist in their collapsed conformation, and offer little resistance to flow through the monolith. However, upon cooling below the LCST, the chains expand and fill the pores, preventing any liquid from flowing through the material. Figure 3 clearly documents that this gate effect, that can readily find an application in various

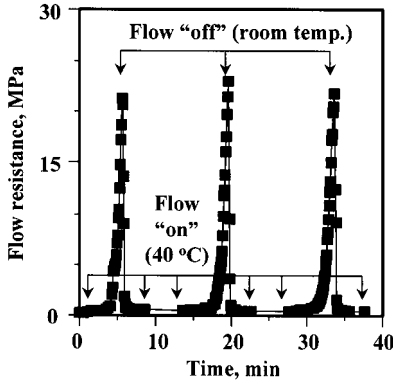


Fig. 3. Thermal gate behavior of porous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) monolith grafted with PNIPAAm. Conditions: monolithic disc 10 × 10 mm; water flow rate, 1 mL/min. Immersed or removed from 40 °C bath [63].

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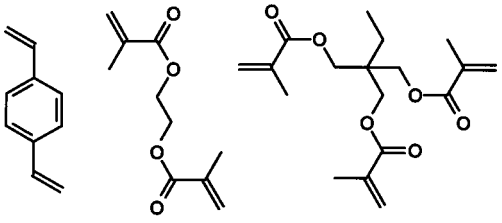
microdevices, is both rapid and reversible. It should be noted that PNIPAAm chains have also been grafted to particulate supports.[68,69] However, since flow in a packed column occurs through the interstitial voids between the packed particles, such materials may not be used to produce thermal gates.

Flow through the monoliths can be attenuated rather than completely stopped if a crosslinker is added to the NIPAAm monomer during the grafting process.[63] The addition of only 1 wt.-% of methylenebis(acrylamide) is sufficient to prevent the grafted chains from swelling to the extent necessary to entirely fill the pores. The resulting composite acts as a thermal valve, changing its flow rate in response to changes in external temperature.

3.2. Monolithic Objects as Polymeric Reagents and Scavengers

Although still very new to these applications that concern solution phase combinatorial chemistry, monolithic objects in various shapes are expected to offer new opportunities in this area. Chemical reactivity and high capacity of accessible functionalities are the basic requirements for solid-phase chemistry. Here again, grafting of functional monomers to the internal pore surface appears to be best suited for the preparation of monoliths with all of the functional groups exposed for interactions. In early work,[70] we first modified a monolith of poly(chloromethylstyrene-DVB) (DVB = divinylbenzene, **9**) with 4,4′-azobis(4-cyanovaleric acid) and then used the bound initiation sites to graft 2-vinyl-4,4-dimethylazlactone **8** affording a monolith with 1.6 mmol/g of reactive functionalities (Scheme 1). The



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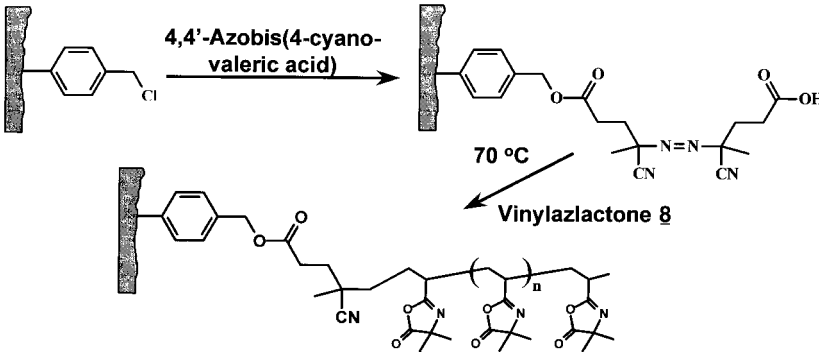
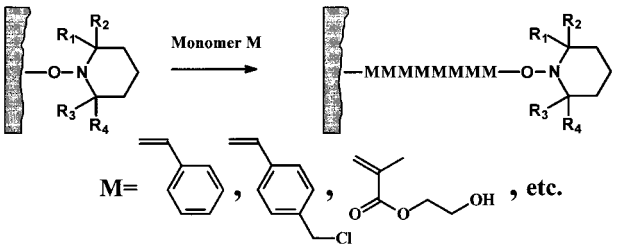


Scheme 1

product was then cut into discs and used as a scavenger[71] for the rapid removal of excess amine from reaction mixtures. Similarly, the latent radicals capped in monolithic structures prepared in the presence of TEMPO may also be used as initiating sites to grow polymer chains from the internal pore surface (Scheme 2).[57]



Scheme 2

3.3. Molecular Recognition

Molecular imprinting has attracted considerable attention recently as an approach to polymers containing recognition sites with predetermined selectivity.[72–74] Such materials, if successfully prepared, could find applications in such areas as the resolution of racemates, substrate selective catalysis, and as "artificial antibodies". The imprinting technique involves the preorganization of functional monomers around a template molecule and its subsequent copolymerization with large amounts of crosslinking monomers. Under ideal conditions and after extraction of the template, imprints possessing both a defined shape and a specific arrangement of chemically interactive functional groups that reflect those of the templated molecule remain in the polymer.

In the early pioneering days of molecular imprinting, Wulff[75] prepared imprinted materials as chunks of material that, unlike our more recently developed flow-through monoliths, could not support flow. As a result, these materials had to be powdered, size classified, and packed into standard columns in order to be tested for template recognition. Following our first publication concerning rigid monolithic flow-through media,[46] Karube's group[76] adapted this approach to molecularly imprinted media. This imprinted monolith was prepared using our original porogen system[46] and then used for the separations of both positional isomers of diaminonaphthalene and enantiomers of phenylalanine anilide. After this initial success, several other reports employing a monolithic approach have appeared in the literature.[77,78] Imprinted monoliths have also received much attention recently as stationary phases for capillary electrochromatography. These applications are discussed later in this review.

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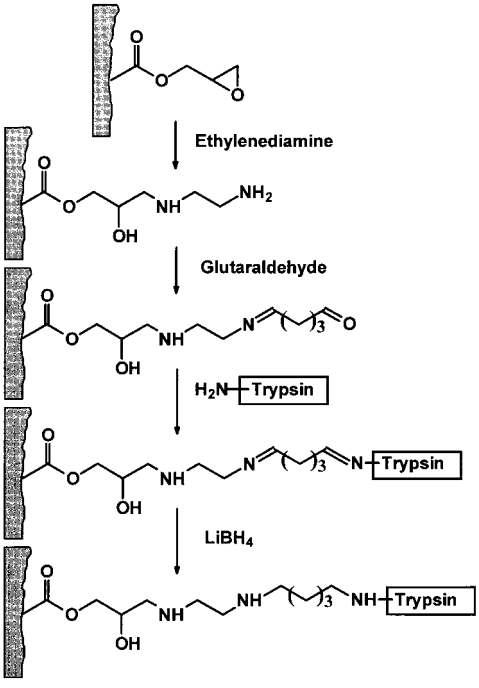
3.4. High-Throughput Bioreactors

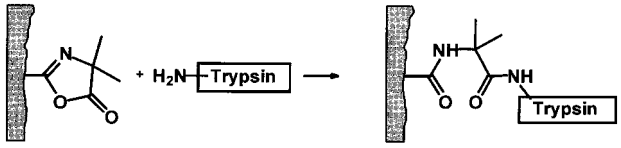
The immobilization of enzymes onto solid supports provides a number of practical advantages for biocatalytic processes. These include the ease of separation of the supported enzyme from the desired product as well as the potential for re-use in subsequent reaction cycles. However, these materials often exhibit significantly lower apparent activities than their soluble native counterparts due to the impaired transport of substrate molecules to the active sites. Therefore, several approaches have been suggested to improve mass transfer. For example, Hoffman demonstrated the positive effect of a mechanical "pumping" process achieved through the repetitive swelling and contraction of a thermoresponsive hydrogel in response to changes in temperature. The apparent activity of an enzyme immobilized within this pulsating support increased dramatically.[79]

Another option to increase the rate of mass transfer is to use convective flow to carry a substrate to the immobilized biocatalyst through the pores of a monolithic support. This has now been demonstrated in comparative studies in which trypsin was immobilized onto both macroporous GMA-EDMA (EDMA = ethylene dimethacrylate **11**) beads and onto analogous monoliths (Scheme 3).[80] Despite the relatively small size (11 μm) of the monodisperse beads used to minimize the diffusional path length, the activity of the enzyme immobilized on the monolithic material was nearly twice that of the bead-based conjugates at a linear flow velocity of 25 cm/min. Additionally, the monolithic bioreactor could be used at even higher flow rates (40 cm/min) while still maintaining its catalytic activity. In contrast, the packed bed bioreactor could not be utilized due to the prohibitive increases in column back pressure with increasing flow rate. The benefits of the increased mass transfer exhibited by these flow-through supports are even more pronounced in catalytic processes involving macromolecular substrates. This is demonstrated by the superior performance of trypsin in the digestion of cytochrome *c* when it is immobilized on a monolithic support rather than on macroporous beads packed into a column.[80]



Scheme 3

Poly(2-vinyl-4,4-dimethylazlactone-*co*-acrylamide-*co*-ethylene dimethacrylate) supports are considerably more hydrophilic than the methacrylate-based material described above, and therefore they are better suited for enzyme-based applications. In addition, preparation of the immobilized enzyme is significantly easier with the azlactone support, involving only a single step to couple amino groups of the enzyme with the azlactone moieties (Scheme 4). Figure 4 shows the effect of temperature on the apparent enzyme activity of trypsin immobilized on an azlactone-based monolith at several different flow rates.[81] The observed increase in apparent activity with increasing flow rate clearly confirms the beneficial effect of the improved convective mass transfer on the overall catalytic process. The flow rates achieved with the monolithic bioreactor are an order of magnitude greater than the recommended maximum linear flow velocities for commercially available rigid macroporous bead supports.



Scheme 4

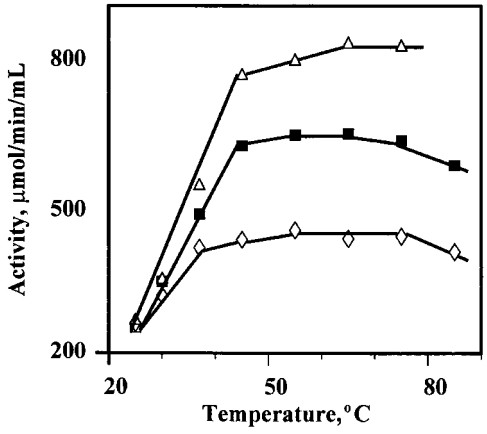


Fig. 4. Effect of temperature and flow velocity of 30 mol/L solution of L-benzoyl arginine ethyl ester on the activity of immobilized trypsin. Conditions: monolithic support, 30 wt.-% 2-vinyl azlactone, 20 wt.-% acrylamide, 50 wt.-% ethylene dimethacrylate; reactor, 20 × 1 mm i.d.; 0.05 mol/L Tris/HClbuffer (pH 8.0) containing 0.02 mol/L $CaCl_2$ and 5×10^{-4} mol/L NaN_3; temperature, 25 °C; flow rates: 51 (◇), 76 (□), and 180 (△) cm/min [81].

3.5. Solid-Phase Detection

Peroxyoxalate chemiluminescence is primarily used as a very sensitive method for the detection of hydrogen perox-

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ide,[82] although it may also be used for the indirect detection of other fluorescent compounds. Typically, these experiments are performed using reactors packed with beads that have fluorophores bound to their surface. Pontén et al.[83] have reported the use of glycidyl methacrylate-trimethylolpropane trimethacrylate (TRIM, **11**) monoliths prepared by photopolymerization within a quartz tube. Both the surface area and degree of functionality of the resulting polymers could easily be adjusted by making changes in the composition of the original polymerization mixture. This control was used to optimize the number of surface epoxide moieties available for functionalization with 3-aminofluoranthene, resulting in monolithic supports that exhibited light generation efficiencies twice as high as those of reactors packed with similarly functionalized 50 μm beads.

Sherrington et al.[84] have reported progress towards the development of an optical sensor system using molecularly imprinted anisotropic polymer monoliths. A transparent[52] imprinted polymer monolith prepared under standard conditions was irradiated with plane polarized light. Those template molecules that have transition dipole moments oriented parallel to the plane of polarization absorb the light, creating reactive species capable of insertion into the polymer backbone. Subsequent extraction of the unreacted template molecules results in an anisotropic material that contains cavities with a preferred net orientation. Monoliths prepared with Michler's ketone as the photoreactive template molecule did indeed exhibit different absorbance values when irradiated either parallel or perpendicular to the original plane of irradiation. However, the anisotropy of the polymers diminishes significantly upon extraction or heating as a result of the random rearrangements of the swollen polymer chains.

3.6. Solid-Phase Extraction

Solid-phase extraction (SPE) devices consisting of sorbent particles either embedded in a non-porous matrix or tightly retained between two screens dominate the current market since they are convenient to work with and allow easy integration into robotic systems for high-throughput screening protocols.[85] Similarly, the ability to easily produce monolithic structures in a variety of shapes should make these materials ideally suited for SPE applications, provided that their surface areas are large enough to support the required physical adsorption. Such materials can be produced by incorporating extremely high levels of crosslinking. For example, easily permeable monoliths possessing surface areas of 400 m^2/g are obtained by polymerizing high grade (80 %) DVB. SPE studies revealed that they have a very high sorption capacity of 23 mg/g for 2-nitrophenol at the flow velocity of 10 cm/min typically used with the current and less performing thin disk SPE media. However, the excellent mass transfer properties of this monolithic adsorbent afforded an acceptable capacity of 2.6 mg/g even at the remarkably high flow velocity of 300 cm/min (150 bed volumes/min).[58]

3.7. Stationary Phases for High-Performance Liquid Chromatography

The detrimental effect of slow mass transfer on the efficiency of a chromatographic separation process was first recognized by Van Deemter.[17] This effect is particularly pronounced in the separations of large molecules such as synthetic polymers and biomacromolecules, since their diffusion coefficients are several orders of magnitude smaller than those of low molecular weight compounds. As a result, the efficiency of typical packed chromatographic column deteriorates rapidly with increasing flow rate, necessitating an increase in the length of the columns or a slower flow rate to achieve the desired separation.[16] The problem of diffusion is avoided by using nonporous particulate stationary phases.[86] However, these materials only possess extremely low surface areas on which to support the required "interactive" functional group, resulting in very limited column loading capacity. In contrast, macroporous polymer monoliths tolerate higher loading levels and the convective flow dramatically enhances mass transfer, thus allowing a large increase in the speed of chromatographic separations.

3.7.1. Chromatography of Biomacromolecules

Several groups have reported significantly improved reversed-phase separations of peptides[87,88] and proteins[89,90] using monolithic stationary phases. Figure 5 shows the separation of three proteins using a ST-DVB (ST = styrene) monolith at two different flow rates while maintaining a constant gradient volume.[89] Although baseline separation

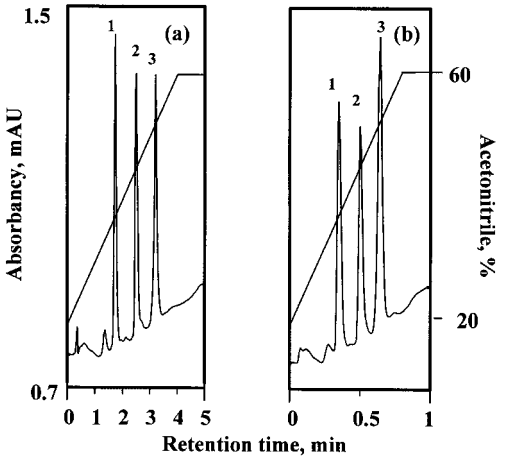

Fig. 5. Separation of cytochrome *c* (1), myoglobin (2), and chicken egg albumin (3) by reversed-phase chromatography on a poly(styrene-*co*-divinylbenzene) monolithic column at flow rates of 5 (a) and 25 (b) mL/min. Conditions: column, 50 × 8 mm i.d.; mobile phase, linear gradient from 20 to 60 % acetonitrile in water [87].

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is already achieved at the relatively high flow rate of 5 mL/min, a further five-fold increase in flow rate results in the separation being effected in less than 1 min. As expected, the quality of the separation did not change because the same gradient volume was used. Similarly, Tanaka et al. have separated a number of proteins using a octadecylsilylated silica monolith, demonstrating that the separation times could be reduced by a factor of 3 or more compared to a column packed with 5 μm silica particles.[90]

Although the enhanced mass transport properties of the monoliths have driven their rapid development, these materials possess a number of other advantages compared to their particulate counterparts. For example, the wider range of easily accessible chemistries has been used to prepare stationary phases for a variety of "classical" gradient separations.[62,91–93] Similarly, we have developed monolithic columns that change their chromatographic selectivity in response to external temperature, enabling the first reported isocratic hydrophobic interaction separation of proteins.[63] In addition, the ability to prepare monoliths within a mold of any shape was used by Lee et al.[94] to prepare monolithic ST-DVB microbeds within pulled fused silica needles for the reversed-phase separation and on-line electrospray mass spectrometry detection of proteins and peptides. As illustrated by Figure 6, these monolithic microcolumns exhibited efficiencies far better than capillaries packed with commercial C18 silica or polymeric beads.

3.7.2. Fast Chromatography of Synthetic Polymers

Size-exclusion chromatography (SEC) is currently the method of choice for the characterization of synthetic polymers. Although very effective, SEC often requires a relatively long analysis time. In contrast, extremely fast separations can be effected using monolithic materials. Figure 7 shows the separation of a series of polystyrene standards with molecular weight ranging from 519 to 2 950 000 using a gradient of THF in methanol.[95] The separation is based on the principle of precipitation-redissolution and hinges on the difference in solubility of polymer of different sizes. As a result of their higher solubility, low molecular weight fractions elute faster than their harder to dissolve higher molecular weight analogs. Faster separations are obtained at higher flow rates, and the position of the peaks in the chromatogram can be adjusted simply by changing the gradient profile. Additional increases in flow rate accelerate the separation speed even further, enabling the separation of three polystyrene standards within a few seconds.[96] Such extremely fast separations are already finding applications in the real time monitoring of industrial processes or the high throughput screening of combinatorial polymer libraries.[97]

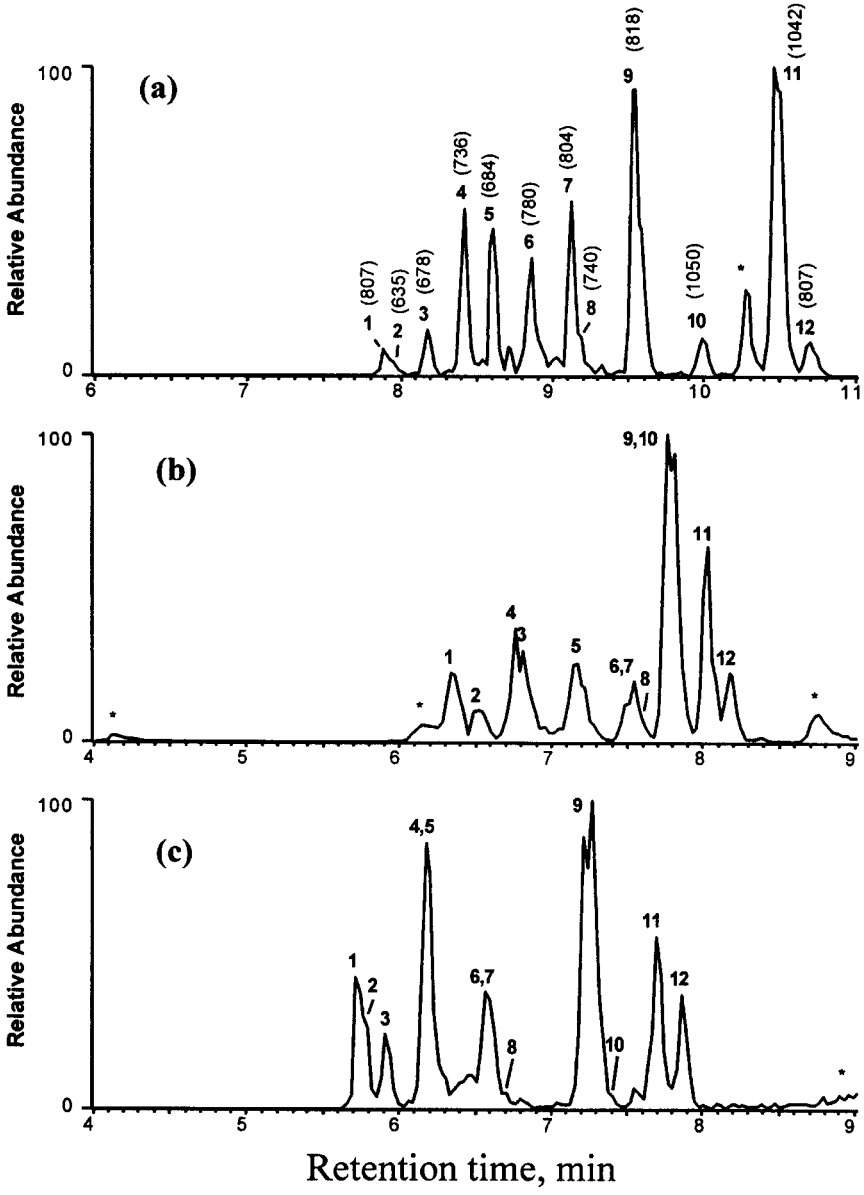

Fig. 6. Base peak chromatograms for the LC/MS analyses of a cytochrome *c* Lys-C digest (0.7 pmol injected) on a poly(styrene-*co*-divinylbenzene) monolith-filled needle (a), Vydac C18-packed needle (b), and Poros R2-packed needle (c). (Reprinted with permission from [94], copyright 1998 American Chemical Society).

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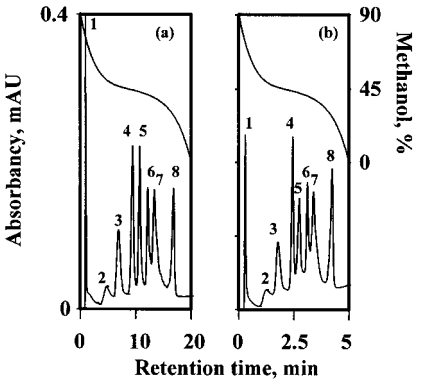

Fig. 7. Separation of polystyrene standards by precipitation-redissolution chromatography on a poly(styrene-*co*-divinylbenzene) monolithic column. Conditions: column, 50 × 8 mm i.d.; mobile phase, gradient of methanol in tetrahydrofuran, with shape of gradient shown in first chromatogram; (a) separation of polystyrene standards with molecular weights of 519 (1), 1250 (2), 9200 (3), 34 000 (4), 68 000 (5), 170 000 (6), 465 000 (7), and 2 950 000 (8) at 8 mL/min flow rate [95]. (b) Ultrafast separation of three polystyrene standards at 20 mL/min flow rate [96].

3.7.3. Chromatography of Small Molecules

The separation performance of the monolithic columns for small molecules has also been investigated. In this application, the efficiency of a ST-DVB monolith was found to be inferior to that of a column packed with macroporous beads prepared from the same monomers.[87] In contrast, Tanaka's group reported efficiencies of up to 96 000 plates/m using C18-modified silica rods.[49,88] This spectacular performance appears to arise primarily from the specific morphology[49] of these materials that have an unusually high level of pore connectivity and possess a larger volume of mesopores (Fig. 8). Presumably, refinements in the porous structure of the organic polymer-based monoliths will result in similar performance improvements. Alternatively, highly efficient small molecule separation can be performed on a monolithic medium by switching from pressure driven flow to electroosmosis employed in capillary electrochromatography (CEC).

3.8. Stationary Phases for Capillary Electrochromatography

CEC is an emerging "hybrid" separation method that employs the electrically driven flow characteristic of electrophoretic separation methods within capillary columns typically packed with solid stationary phases.[98–100] In theory, extremely high efficiencies can be obtained for CEC separations due to the plug flow profile of the mobile phase, which leads to smaller zone broadening. Although CEC was invented in the early 1970s,[101] and its potential with packed capillary columns demonstrated in the 1980s,[102–104] serious technical problems have slowed its development. These problems include the difficult fabrication of frits within a capillary, the packing of beads into a tube with a very small diameter, and the limited stability of packed beds.[105,106]

The use of monolithic capillary columns can eliminate many of these technical problems. First demonstrated using continuous hydrogel beds,[107,108] numerous groups are currently investigating a variety of approaches to macroporous monolithic capillary columns. For example, silica-based monolithic structures have been prepared by sintering silica particles,[109,110] embedding them within a silica xerogel matrix,[111,112] or directly via in situ formation of the monolithic structure.[48,113] Although effective, these C18 silica-based materials are often complicated to prepare. They are also known to be unstable at both low and high pH values,[114] and afford limited control over the level of charged functionalities that support the electroosmotic flow (EOF).

Several other groups including ours have reported the preparation of macroporous organic polymer monoliths for CEC.[61,115–121] These materials are produced by the in situ polymerization of a homogeneous liquid mixture containing monomers with widely varying polarities that support EOF generation and/or chromatographic selectivity. The scanning electron micrograph (SEM) shown in Figure 9 clearly documents that the resulting polymeric material is truly a single continuous object. Their simple and versatile preparation method enables the production of monolithic capillaries of essentially any size with efficiencies directly proportional to their length.[118]

In addition to their simplicity of preparation, some of the published approaches also afford excellent control over the properties of the resulting polymer, including its chemistry, nature and level of charged functionality, and porous properties. For example, the accurate control that may be exerted over the size of the flow-through pores was used to rapidly optimize the efficiency of the overall chromatographic system.[119] In addition, the ability to deconvolute the effects of simultaneously changing variables such as pore size and level of charged groups enabled the preparation of a CEC column that afforded equally efficient separations in only half the original run time, as demonstrated in Figure 10.[119]

Although the majority of reports of CEC using monolithic columns describe reversed-phase separations, other chromatographic modes have also been investigated, includ-

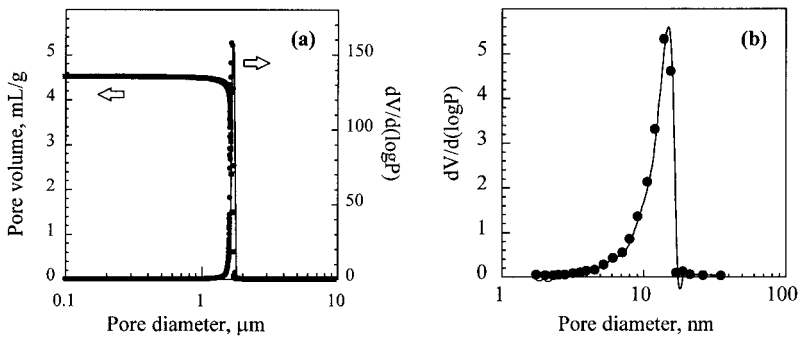

Fig. 8. Pore size distribution of a silica-based monolith as measured by mercury intrusion (a), and nitrogen adsorption (b) methods. (Reprinted with permission from [49], copyright 1996 American Chemical Society).

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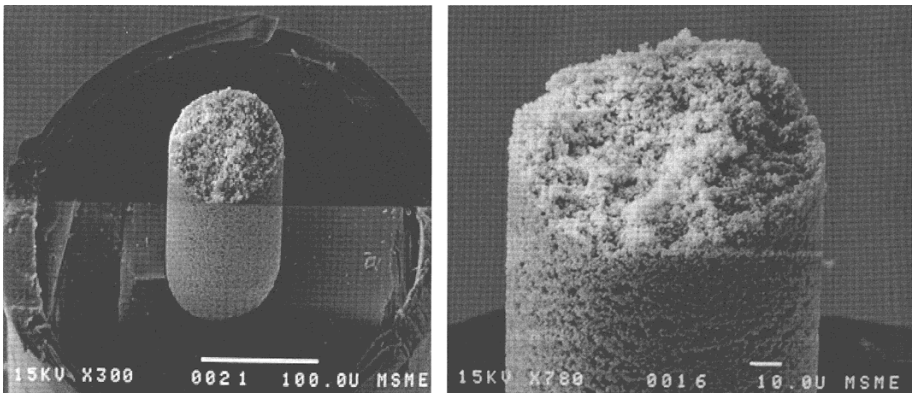

Fig. 9. SEM micrograph of a monolith prepared within the confines of a 370 μm o.d. × 100 μm i.d. polyimide-coated fused silica capillary. Conditions: monolithic support, 59.7 wt.-% butyl methacrylate, 0.3 wt.-% 2-acrylamido-2-methyl-1-propanesulfonic acid, 40 wt.-% ethylene dimethacrylate; mode pore size, 255 nm by mercury intrusion porosimetry.

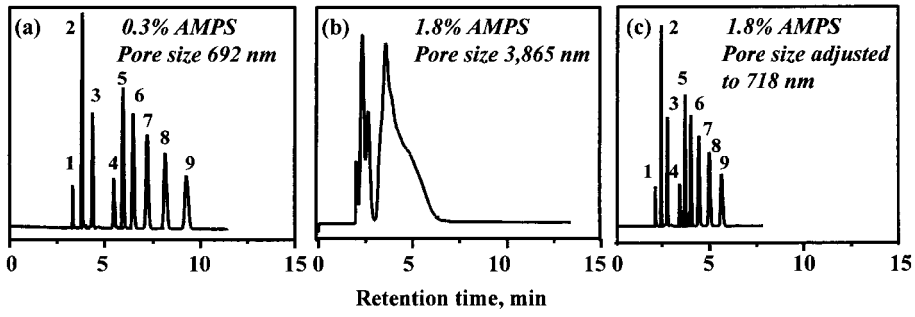

Fig. 10. Electrochromatographic separation of benzene derivatives on monolithic capillary column. Conditions: capillary column, 100 μm i.d. × 25 cm active length; stationary phase with 0.3 (a) and 1.8 (b) wt.-% 2-acrylamido-2-methyl-1-propanesulfonic acid from otherwise identical mixtures, and 1.8 wt.-% (c) where pore size is adjusted to be similar to (a); mobile phase, 80:20 vol./vol. mixture of acetonitrile and 5 mmol/L phosphate buffer pH 7; Peaks: thiourea (1), benzyl alcohol (2), benzaldehyde (3), benzene (4), toluene (5), ethylbenzene (6), propylbenzene (7), butylbenzene (8), and amylbenzene (9) [119].

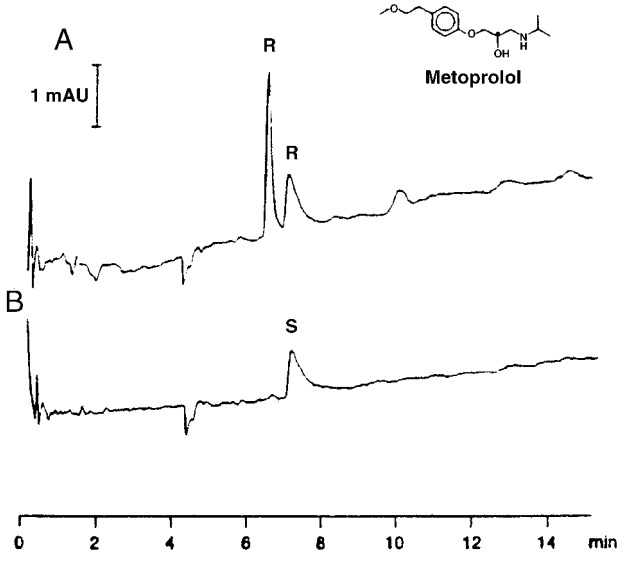

Fig. 11. Electrochromatographic separation of 100 μM *rac*-metoprolol (a) and 50 μM (*S*)-metoprolol (b) using a monolithic capillary containing imprints of (*S*)-metoprolol. (Reprinted with permission from [116], copyright 1997 American Chemical Society).

ing size exclusion electrochromatography[118] and chiral separations. For example, very efficient chiral monolithic media have been prepared by the direct copolymerization of a chiral selector,[120] while numerous reports describe molecular imprinting methods.[121] To illustrate the performance of this type of material, Figure 11 shows the CEC chiral separation of racemic metoprolol on a monolithic column imprinted with the (*S*)-enantiomer.[116] Clearly, the stationary phase contains sites that preferentially bind the imprinted enantiomer. However, the fidelity of these imprints in response to changes in temperature and the solvating power of the mobile phase employed remains to be explored.

3.9. On-Chip Separations

Although the performance of monolithic materials in CEC is encouraging, the true potential of this technology may lie in its possible use in further miniaturized separation systems. Numerous groups have already reported on-chip electrophoretic separations involving the migration of charged species through open channels under the influence of an applied electric field.[121–125] However, only a limited number of truly chromatographic on-chip separations of neutral molecules have been reported.[126–127] In each case, the "stationary" phases are the surface modified channel walls or micromachined features rather than real packings. The easy introduction of a solution and subsequent in situ formation of a porous monolithic structure provides an attractive alternative option. Further refinements including photoinitiation coupled with the use of appropriate masks and polymerization mixtures may allow the simple preparation of more complex chip-based systems possessing multiple monolithic structures with easily controllable position, size, and chemistry for use in a variety of functions.

4. Conclusion

Rigid macroporous polymer monoliths possess a number of unique properties compared to their more traditional macroporous counterparts. Although these materials are

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unlikely to completely replace particulate supports, they can serve as an effective complementary option, since their unique properties can often be used to specific advantage in a variety of applications. In addition to the number of their documented uses, these materials also show promise as potential flow-through heterogeneous catalyst supports, polymeric scavengers and reagents for combinatorial chemistry, and novel stationary phases in a variety of less common formats such as membranes, capillaries, and media for lab-on-chip devices.

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