New Designs of Macroporous Polymers and Supports: From Separation to Biocatalysis

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Reactive polymers play many roles, from supports for solid-phase synthesis or catalysis to media for separations. Although macroporous polymer beads that provide high reactive capacities and excellent solvent tolerance are well established, approaches to monosized beads with optimized pore structures or multiple chemistries segregated within pores of different sizes have expanded their realm of application. Polymer monoliths containing intricate pore networks can be obtained in any desired shape by a simple molding process and provide unique advantages such as fast kinetics, high reactivity, and high throughput. Applications ranging from immobilized enzyme reactors to fast media for the separation of synthetic or biopolymers are presented.

Covalently cross-linked polymers are a large family of materials that are used in a number of applications: vulcanized rubber, cured resins, soft contact lenses, resins for solid-phase synthesis, and ion exchangers are only a few examples. Typically, they do not exhibit any porosity unless swollen in a solvent. Solvation results in the separation of the polymer chains from one another to form "pores," which are solvent-filled voids. This type of porosity is temporary because of the reversible nature of solvation and exists only as long as the solvent remains within the polymer network. Lightly cross-linked beads, often termed swellable or microporous gels, acquire porosity only upon swelling. A well-known example of a cross-linked material with no permanent porosity is the Merrifield resin (*1*), used in the solid-phase synthesis of polypeptides. It consists of slightly cross-linked chloromethylated polystyrene beads that swell in dichloromethane, dimethylformamide, tetrahydrofuran, and toluene. However, the Merrifield-type resins do not swell in protic polar solvents, and reactivity is low because their porosity does not unfold in these solvents. As a result, reactivity of the chloromethyl groups is limited. Grafting long ethylene oxide chains to Merrifield resins leads to more polar matrices that can be used in polar solvents (*2*). All of these swollen materials are usually soft and easily deformed under pressure, which generally prohibits their use in packed beds such as liquid chromatography columns.

In contrast to the polymers that require solvent swelling to become porous, macroporous polymers are characterized by a permanent porous structure formed during their preparation that persists even in the dry state. Their internal structure consists of numerous interconnected cavities (pores) of different sizes, and their structural rigidity is secured through extensive cross-linking. Macroporous polymers emerged in the late 1950s as a result of the search for mechanically resistant ion-exchange resins with enhanced exchange kinetics (*3*). Because the largest application of macroporous polymers involves water treatment in substantial demineralization columns for power plants, it is important to ensure that excellent mass transfer between the liquid and solid phases is achieved. Powdered bulk polymers are totally unsuitable because controlled flow through irregularly shaped or sized particles is difficult. In contrast, columns packed with regular spherical beads generally exhibit good flow characteristics.

Macroporous beads are widely used not only for the preparation of ion-exchange resins but also for use as catalysts, adsorbents, supports, carriers, and chromatographic media (*4*). All of these applications take advantage of the beads' rigid porous structure, which remains unaffected by changes in the environment. Although macroporous materials swell much less than microporous gels in any solvent regardless of its polarity, mass transport in and out of the pores is much faster than that in unswollen homogeneous polymers. Molecules diffuse freely through the pores, which form a labyrinth of tortuous interconnected cavities of different sizes.

After briefly reviewing the state of the art for preparation of such materials, we will focus on new designs of macroporous media such as size monodisperse beads with carefully crafted reactive surfaces and polymeric monoliths that allow convection through their large pores.

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The Shape of Particulate Macroporous Polymers

In the early days of porous materials, the simplest way to obtain small particles for chromatography and enzyme immobilization was to crush bulk polymerized materials. For example, large pieces of porous silica (*5*) or dried dextrane gels (*6*) were crushed in ball mills and then fractionated according to size. Enzymes entrapped within a polyacrylamide matrix were granulated by pushing the gel through a stainless steel mesh (*7*). Although easy to prepare, irregular particles packed poorly in columns and created large voids that robbed the systems of their efficiency, and the many sharp edges of the particles fractured easily, clogging the systems. Spherical beads do not suffer from these acute problems.

In general, five basic techniques are used to prepare the majority of synthetic polymers: bulk, solution, dispersion, emulsion, and suspension polymerization. With few exceptions, the first two do not provide particulate materials. The other three processes do lead to spherical particles, but the size ranges obtained differ because these methods all involve different mechanisms.

Emulsion polymerization starts with a dispersion of monomer in an aqueous phase that contains micelles (aggregates formed from dissolved surfactant molecules). The micelles solubilize the hydrophobic monomer molecules, and the polymerization proceeds within each spherical micelle once a radical enters the micelle from the aqueous phase. Emulsion polymerization leads to a latex, which is an aqueous dispersion of particles, and the particles are nearly always smaller than 1 μm and uniform in size (*8*). Such latices have a broad spectrum of applications: for example, poly(vinyl acetate) latex particles are commonly used in wall paints.

Dispersion polymerization typically involves the polymerization of monomers that are initially soluble in the organic liquid dispersion medium in the presence of a polymeric steric stabilizer. Once a critical chain length is reached during polymerization, the polymer precipitates and forms particulate nuclei that are coated with the polymeric stabilizer, which prevents their coagulation. The process may be optimized to form monodisperse beads in a size range from 1 to 10 μm (*9*). Dispersion polymer-

ization can be used to prepare, for example, the uniformly sized toner particles used in high-resolution laser printing (*10*).

In aqueous suspension polymerization, a water-insoluble monomer containing a dissolved free radical initiator is stirred in a large volume of water to form small droplets of the dispersed organic phase in the continuous aqueous phase. To minimize their interfacial free energy, these droplets adopt a strictly spherical shape, and their average size is roughly controlled by the rate of stirring and the amount and type of suspending agent used (Fig. 1). The polymerization is started by an increase in the temperature of the stirred mixture, resulting in solid polymer beads. This technique, invented in 1912 (*11*), is used for the production of commodity polymers such as polystyrene, poly(styrene-*co*-divinylbenzene), polyacrylates, polymethacrylates, and poly(vinyl chloride) (*12*). Smaller batches of beads are also prepared for special applications such as separation media for high-performance liquid chromatography (HPLC) (*5*) and supports for solid-phase synthesis and combinatorial chemistry (*13*). It is currently the method of choice for the preparation of macroporous beads (*3*).

Formation of the Macroporous Structure

In order to obtain a rigid material that exhibits macroporosity, the dispersed liquid polymerization mixture must contain not only the monomer but also large amounts of a cross-linking divinyl monomer and a porogen. The porogen does not react during the polymerization process but remains within the newly formed beads, where it is surrounded by polymerized material in areas that will ultimately become the pores; it is finally removed during work-up. In most cases, the porogen is a simple organic solvent or even a polymer soluble in the monomer mixture.

To understand pore formation during a typical suspension polymerization, imagine that each droplet is a spherical microreactor, the shape of which is preserved by stirring and interfacial tension. As a result of both cross-linking and solubility changes associated with increased chain length, polymer molecules formed within this microreactor precipitate from the surrounding medium of porogen and remaining monomers. This phase separation occurs at an early stage of the polymerization, leading to the formation of microscopic globular entities that keep growing but do not coalesce because of cross-linking. Eventually, these come into contact with each other and associate to form clusters consisting of both interconnected globules and voids or pores (Fig. 2). In essence, each microreactor or droplet is transformed into a macroporous bead in which the percentage of free volume correlates well with the percentage of porogen used.

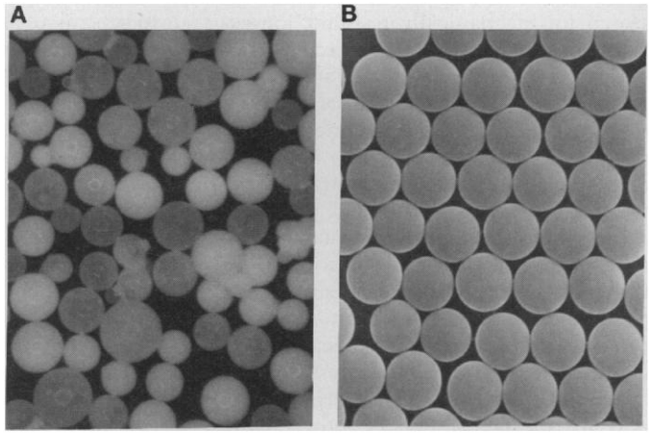

Fig. 1. Scanning electron micrographs of (**A**) 50- to 150-μm polydisperse beads obtained from a "classical" suspension polymerization and (**B**) 10-μm monodisperse beads prepared by a staged templated suspension polymerization process.

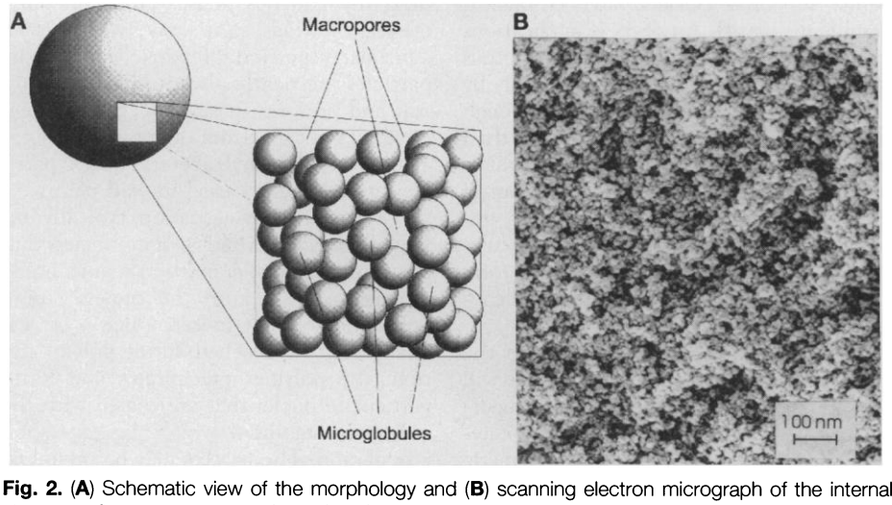

Fig. 2. (**A**) Schematic view of the morphology and (**B**) scanning electron micrograph of the internal structure of a macroporous polymer bead.

Control of pore size distribution. Well-defined pore size distributions are an important consideration in the design of macroporous polymers. For example, small pores and large surface areas are essential for many supported catalysts and gas chromatography packings, whereas the separation of nucleic acids or the immobilization of enzymes require significantly larger pores. Although the number of variables in a suspension polymerization is large, only a few are useful for the control of porous properties. These are the percentage of both the cross-linking monomer and the porogen in the polymerization mixture, the composition of the porogen, and the reaction temperature. Changes in the first two variables also affect both the composition and the mechanical properties of the polymer. The other variables, temperature and type of porogen, are the most useful for the preparation of porous beads with a fixed chemical composition that differ only in their pore size distribution.

Three types of porogens and their mixtures have found use in the creation of porous structures: solvents that solvate the polymer chains, nonsolvents, and linear polymers (*3*). In contrast to solvents, the use of linear polymers as porogens adds more variables to the porogenic system because changes in chemical composition, average molecular weight, and molecular weight distribution all have an effect on porosity.

Toward uniformly sized beads. Although stirring provides some control over the average size of droplets in the initial stages of a "classical" suspension polymerization, the beads that are obtained have a broad distribution of sizes. Because beads are often packed into columns, this size polydispersity leads to a nonideal three-dimensional (3D) array of particles that affects both the flow through the column and its efficiency. As a direct consequence, most commercially available beads for high-end applications

such as HPLC are size-fractionated. The more demanding the application, the tighter the tolerances of the size fractionation process and the smaller the yield of useful beads. In order to optimize the performance of beads and eliminate the considerable waste of material, processes have been developed for the production of uniformly sized spherical particles.

Simple techniques such as emulsion and dispersion polymerization are not suitable for the preparation of macroporous media. Alternative approaches based on the dropwise introduction of a polymerization mixture containing a thermally or photochemically triggered initiator into a heated immiscible liquid in which the polymerization proceeds under conditions that avoid coalescence have met with some success. The droplets are produced by pushing the monomer phase through the thin orifices of syringe needles (*14*), porous glass membranes (*15*), or various capillaries (*16*). Although these simple techniques afford beads with relatively narrow size distributions, they are still polydisperse.

Staged templated suspension polymerization. Because small, nonporous, uniformly sized spherical particles of linear polymers are readily obtained by emulsion polymerization, a new concept for the preparation of large macroporous beads uses these small spheres as "shape templates" in processes that not only increase their size manyfold in a uniform fashion but also introduce pores. A stabilized aqueous dispersion of micrometer-size uniform particles is swollen to the target size of the final macroporous product with a combination of solvents, monomers, and cross-linking agents. The swollen particles are then polymerized. Although the shape and size uniformity of the original templates has been preserved, their size has been increased considerably, and the presence of a solvent during polymerization leads to the desired macroporous structure. In practice, the equilibrium between interfacial energy and swelling forces considerably limits the extent of swelling of the original template beads, but the multistage swelling processes of Wanderhoff (*17*) and Ugelstad (*18*) and their co-workers allow for the preparation of larger monodisperse beads. The size of the final beads is no longer determined by the stirring conditions but by the swelling of the shape-template particles.

Polymer porogens for the preparation of monodisperse beads. Recently we have been able to achieve both size monodispersity and ideal porosity. The choice of porogen has a great influence on the shape and size of pores. Intuitively, it may be expected that the use of a polymer porogen would afford materials with a pore structure particularly well suited for the separation of polymers. As will be shown below, this simple conjecture proved to be true. The preparation of monodisperse macroporous poly(styrene-*co*-divinylbenzene) beads with a linear polymer as porogen is depicted in Fig. 3. Initially, large (4.2 μm), uniformly sized, soluble polymer porogen beads are prepared by polymerization of 1.1-μm shape-template latex particles swollen with a small amount of solvent and styrene. These particles, now serving as both shape templates and porogen, are swollen with a solution of styrene and divinylbenzene containing a free-radical initiator in some toluene. Toluene is only used as a co-porogen to fine tune the porous properties. Once swollen, polymerization of the monosized particles is initiated by an increase in the temperature. Finally, the porogens (solvents and polymers) are extracted from the 7.4-μm beads with toluene, leaving behind pores that account for 50% of the total bead volume (*19*).

The staged polymerization technique with a polymeric porogen is well suited for fine tuning the porous properties of size-exclusion separation media. We have used this technique (*19*) to prepare beads optimized for separations according to size within the very broad molecular weight range from 5×10^2 to 5×10^6. Beads capable of separating small molecules such as alkylbenzenes according to their size in the molecular weight range from 100 to 500 have also been prepared (*20*). Both families of beads are monodisperse and require no size classification, and their size-exclusion chromatography calibration curves are remarkably linear in their respective working areas (Fig. 4).

Although the last swelling and polymerization stage is inevitable, the polymeric porogen beads can be prepared in alternative ways. For example, large shape-template particles can be obtained by dispersion polymerization (*21*) or by the repetitive "seeded" polymerization of a monovinyl monomer described by Wanderhoff and coworkers (*22*).

Control of Surface Chemistry

Macroporous polymers are usually prepared by the copolymerization of a limited number of monomers—such as styrene, vinylpyridine, acrylamide, or glycidyl methacrylate—and cross-linking agents—such as divinylbenzene, ethylene dimethacrylate, or methylenebisacrylamide. As a result, few surface chemistries are available by direct polymerization. This lack of chemistries results mainly from the lack of commercial sources for most other functional monomers. Therefore, access to a broader spectrum of pore surface chemistries is usually achieved through the chemical modification of a few standard copolymers rather than by the use of more exotic functional monomers (*4*, *23*).

The chemical modification of a porous polymer is controlled by kinetic factors, such as concentration of reagents, reaction time and temperature, rate of diffusion, and neighboring-group effects (*23*). Although the external surface of a macroporous bead accounts for only a minute proportion of the overall bead surface area, it may be important to modify it selectively; for example, to prevent access of certain molecules to the inner pores of the bead or their

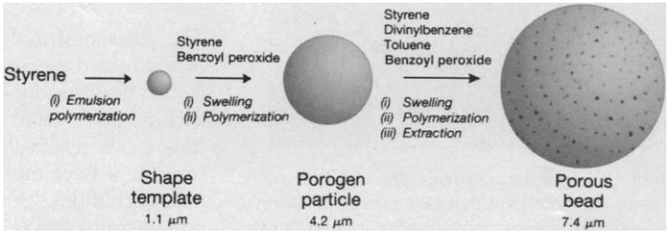

Fig. 3. Preparation of monodisperse macroporous poly(styrene-*co*-divinylbenzene) beads by a staged templated suspension polymerization process.

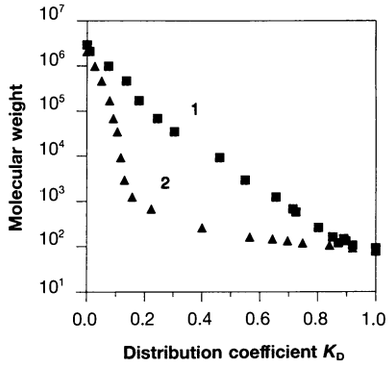

Fig. 4. Size-exclusion calibration curves of (curve 1) poly(vinylphenol-*co*-divinylbenzene) beads and (curve 2) poly(styrene-*co*-divinylbenzene) beads with polystyrene standards and alkylbenzenes in tetrahydrofuran. The distribution coefficient represents the fraction of the total pore volume that is accessible for the respective molecular weight standards. Curve 1 is characteristic of the separation media with broad exclusion limits and lower selectivity, whereas beads with calibration curve 2 are selective for the separation of small molecules with a molecular weight of up to 1000 according to their hydrodynamic size.

adsorption onto the surface. Such "restricted-access media" have been prepared (*24*), but their preparation is elaborate because it is generally not possible to perform modification reactions only at specific sites or selected regions of a porous polymer. Although modification of groups exposed in the most readily accessible parts of the porous polymer may indeed occur first, the modification process has little selectivity and stops only after all available groups are consumed.

To increase the versatility of separation media through tailoring of their surface chemistry, we have developed "site-directed" modification processes that allow either the introduction of functionalities in only the most accessible sites of a resin bead or the incorporation of multiple but segregated chemistries in different areas of each individual bead.

Site-directed modification processes. As an alternative to the tedious method used in the manufacture of the restricted-access media, we have developed a simple approach to achieve outer surface hydrophilization in situ during the suspension polymerization (*25*). In this site-selective modification technique, a modifying monomer (for example, a monomer with a more hydrophilic character) and a water-soluble free-radical initiator are added to the reaction mixture in the late stages of the suspension polymerization. Because of the late timing of this addition, the polymer that is formed covers only the most easily accessed locations of the bead. In practice, this amounts to both the outer surface of the beads and the surface of the largest inner pores. This site-selective modification process not only simplifies the preparation of functionalized beads but also reduces the consumption of the modifying monomer because it is used only to cover selected surface areas of the beads and does not constitute their "mechanical framework." This economy of consumption is particularly important when costly monomers such as single enantiomers must be used. This process has been used to prepare beads with a hydrophilic surface (*25*), thermoresponsive behavior (*26*), and chiral separation media (*27*).

Pore size–specific functionalization. For some applications, even the site-selective modification process is not sufficient, and more selectively targeted modifications to macroporous beads are desirable. Nature itself provides numerous examples of systems that exhibit distinct size specificity. For example, cell membranes allow permeation of small molecules in and out of the cell while large molecules are prevented from crossing the interface. The principle of this size exclusion has been adopted in numerous processes from sieving and advanced membrane technologies to chromatographic separations (*6*, *28*). We have extended this simple concept to the specific modification of selected families of pores within macroporous polymers.

Access to the different pores of a macroporous object, such as a polymer bead, is controlled by the hydrodynamic volume of the dissolved molecules. Thus, the small molecules typically used as reagents in the modification of macroporous beads penetrate virtually all of the existing pores. In contrast, large molecules such as dissolved polymers will penetrate only those pores that are able to accommodate their size, whereas smaller pores remain inaccessible for steric reasons. Therefore, polymeric catalysts and reagents with defined molecular volumes will perform the modification of reactive sites in a polymer bead only in those pores large enough to allow their access. As a result, different surface chemistries, segregated within well-defined areas of a single porous bead or other macroporous object, can be introduced (*29*).

The concept of this pore size–specific functionalization is shown schematically in Fig. 5. The surface of the pores within a poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) bead is completely covered with reactive epoxide groups (labeled A). In the presence of aqueous acid, these epoxide groups are readily hydrolyzed to form more hydrophilic diol groups (labeled B). If the acid used to catalyze the hydrolysis is a polymer such as poly(styrenesulfonic acid), the reaction occurs only in those pores large enough to allow penetration of the large polymeric catalyst, whereas epoxide groups located in smaller, inaccessible pores remain unchanged. After washing the beads to remove the polymeric catalyst, the epoxide groups A that remain in the small pores may now be converted into more hydrophobic groups C by reaction with reagents such as octadecylamine that have a low steric requirement and can penetrate all pores. The resulting beads now contain two different chemistries, hydrophilic and hydrophobic, strictly segregated in pores of different sizes.

The availability of beads with segregated chemistries allows the single-column, 2D HPLC analysis of complex samples such as blood plasma, serum, saliva, and urine without any pretreatment. Any drug or metabolite contained in the biological sample can be separated by reverse-phase chromatography with the hydrophobic surface of the small pores. At the same time, the proteins or other large molecules in the sample can penetrate only the large pores that are lined with hydrophilic groups tuned for their separation. Because proteins bind easily to hydrophobic surfaces and may therefore block an HPLC column, it is important to correlate the size of the polymeric catalyst used in the size-specific modification to that of the smallest protein likely to be present in the sample.

Numerous combinations of chromatographic modes may be introduced in "2D" beads by a proper selection of the modification process and polymeric reagent or catalyst. For example, we have successfully demonstrated combinations such as size exclusion and reverse-phase or ion-exchange chromatography, hydrophobic interaction and reverse-phase chromatography, or ion-exchange and reverse-phase chromatography (Fig. 6). These combinations cannot be achieved by mixing two batches of beads with single chemistries (*30*).

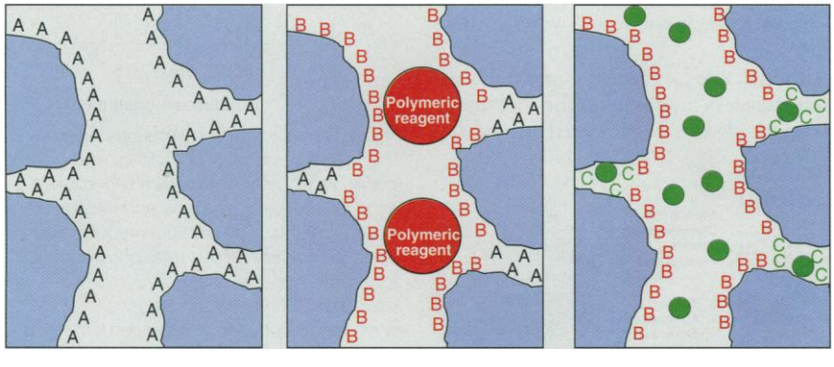

Fig. 5. Concept of pore size–specific functionalization of macroporous polymers. The large polymeric reagent transforms those functional groups A that it comes into contact with (those in large pores only) into B, and the small reagent transforms all remaining functional groups (those in small pores) into C.

The Problem of Mass Transfer in Packed Beds

The passage of molecules within the pores of a standard macroporous material is controlled by diffusion. Typically, small entities such as gases, small organic molecules, and ions move relatively quickly, whereas the transfer of large molecules such as proteins, polysaccharides, or synthetic polymers is considerably slower because their diffusion

coefficients are several orders of magnitude smaller than those of low molecular weight compounds. This effect is detrimental to processes where the speed of the mass transfer limits the overall rate, as is the case in chromatography, catalysis, and adsorption.

Consider what happens when a liquid is forced through a tube packed with standard macroporous particles. The liquid flows readily through the interstitial voids between the particles where resistance against its flow is smallest (Fig. 7). In contrast, the liquid present in the pores does not move and remains stagnant. If a small amount of a substance or a mixture of compounds is injected into the stream of flowing liquid (the mobile phase), these compounds will also be carried through the voids. However, because of the concentration gradient between the solution in the voids and the stagnant liquid within the pores, diffusion occurs, causing transport of these compounds into the pores until their concentration in the stream and the pores is equal. Once the concentration "pulse" has passed by the bead, the amount of compound in the main stream decreases steeply, and the concentration gradient is reversed. The compound then diffuses back from the pores into the surrounding liquid, and eventually only the original stagnant phase remains within the pores (*31*).

Because the diffusion rate for small molecules is quite high and the equilibrium concentration within the pores is reached quickly, the concentration of the molecules in the pores is almost identical to that in the pulse. In the case of macromolecules, the situation is quite different. The slow diffusional mass transfer of macromolecules in macroporous media may be illustrated by the example of biocatalysis in a reactor packed with an immobilized proteolytic enzyme. Once added, the macromolecular substrate (protein) diffuses slowly from the bulk solution into the pores, where it adsorbs onto the active sites of the bound enzyme and reacts to form product. This product then desorbs and diffuses back to the main stream. If diffusion is the rate-determining step, only those active sites that are located in close proximity to the bead surface will be supplied with the substrate. Because of hindered diffusion, no substrate will reach sites located deeper in the bead, and the enzyme capacity will therefore be underutilized. As a result, the efficiency of the immobilized biocatalyst will not reach its full potential, and a larger volume of the supported catalyst will have to be used to achieve the expected throughput (*32*).

A similar diffusion problem encountered with large molecules affects the performance of classical HPLC separations. The slow rate of diffusional mass transfer observed for large molecules within the separation medium results in severe peak broadening. The efficiency of the whole system deteriorates rapidly as the flow rate increases. As a result, longer columns or slower flow rates must be used to achieve the desired separation (*5*).

The use of nonporous particles is the ultimate solution to the problem of diffusion within the pores of a packed column because in the absence of pores, there is no diffusion (*33*). Unfortunately, low porosity translates into very low capacity because a number of applications require direct interaction with surface functionalities. Typical macroporous polymers exhibit surface areas in the range from tens to hundreds of square meters per gram. To achieve such surface areas with nonporous beads, extremely small particles would have to be used, but such small particles are not practical because the high pressure needed to pump the mobile phase through such a column translates into very slow flow rates. Consequently, only relatively short columns can be used. Nonporous 2- to 5-μm beads provide a reasonable compromise between speed and capacity, but these are useful only for analytical purposes.

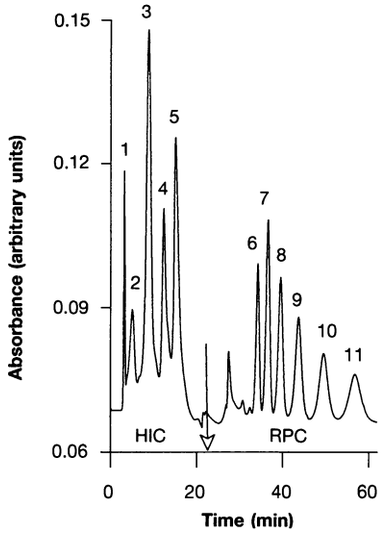

Fig. 6. Two-dimensional separation of a model mixture of proteins and alkylbenzenes in consecutive hydrophobic interaction (HIC) and reverse-phase chromatographic (RPC) modes on beads with bimodal phenyl chemistry (large pores are covered with a few hydrophobic sites interspersed within a hydrophilic surface; small pores are strongly hydrophobic). The proteins are separated without denaturation with a buffered salt solution, and then the mobile phase is changed (at arrow) to a mixture of acetonitrile and water for the reverse-phase separation of alkylbenzenes held within the small pores (*30*). Peaks: cytochrome c (1), ribonuclease A (2), conalbumin (3), lysozyme (4), soya bean trypsin inhibitor (5), benzene (6), toluene (7), ethylbenzene (8), propylbenzene (9), butylbenzene (10), and amylbenzene (11).

Effect of convection on mass transfer. In 1977, Nir and Pismen (*34*) described the positive effect of convection on the efficiency of heterogeneous catalysts and demonstrated a considerable improvement in catalytic activity when a large-pore catalyst was used. In contrast to diffusion, for which the concentration gradient is the driving force, convection uses flow to dramatically accelerate the mass transfer of compounds. However, a major problem is that most pores found in typical macroporous polymers are much too small (<100 nm) to allow convection. According to the Hagen-Poiseulle equation, the pressure needed to force a liquid through a straight tube increases exponentially as the cross section of the tube decreases. In the case of macroporous beads, the pressure needed to achieve convection through typical pores 100 nm or less in diameter is too high to be realistic with today's equipment.

Recently, Regnier and co-workers (*35*) used polymer beads with pores as large as 800 nm for applications in chromatography, diagnostics, and enzyme immobilizations. Although these pores allow some convection, flow around the beads is still preferred and accounts for at least 95% of the total (*36*), thus confirming that the maximum effect of convection can be achieved only if all of the mobile phase is forced through the porous medium.

Unfortunately, beds packed with particulate materials always contain a large void volume between the packed particles. Even an array of ideally packed uniformly sized

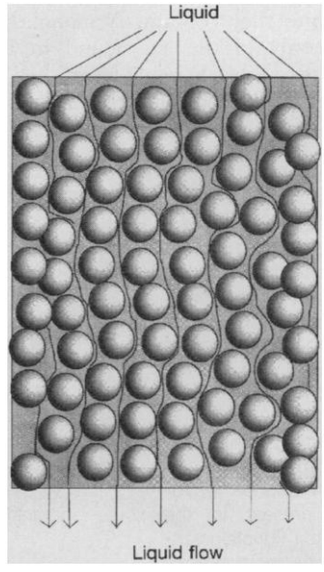

Fig. 7. Schematic view of the flow of a liquid through a bed of uniformly sized beads.

beads still contains about 27% void space. In practice, the void volume is larger, and it is not realistic to envision beads for which flow occurs through tight pores when such a large void interparticle volume is available for essentially unimpaired flow. Therefore, total convection requires material designs that incorporate very little, or preferably none, of the discontinuity that is typical of packed beds.

Design of Continuous Porous Media

Numerous approaches to media with reduced discontinuity have been made. For example, stacked thin membranes based on modified cellulose, nylon, and other polymers (*37*) have been used. Similarly, porous sheets in which macroporous beads are embedded into a web of polymer (*38*) and macroporous sheets (*39*) have been stacked in a cartridge to simulate a column with almost no voids. Rolled cellulose sheets (*40*), woven matrices (*41*), and compressed soft polyacrylamide gels (*42*) placed in the tube of a chromatographic column are other examples of media that exhibit almost no interstitial porosity. However, these media, designed for specific purposes, have found only limited acceptance. In the early 1990s, we developed macroporous monoliths formed by a very simple "molding" process in which a mixture of monomers and solvent is polymerized (Fig. 8) within a closed tube or other container under carefully controlled conditions (*43*).

Hydrodynamic properties and morphology of molded monoliths. Because all of the mobile phase must flow through the monoliths, the first concern is their permeability to liquids, which depends fully on the size of the pores they contain. A monolith with pores only of the size found in typical macroporous beads would be crushed at the extremely high pressures that would be required for flow. Obviously, an ideal monolith should contain both large pores for convection and a connected network of shorter, smaller pores for diffusion. Optimization of the polymerization conditions we use to prepare monoliths—with particular attention paid to temperature (polymerization kinetics) and porogen composition (phase separation process)—leads to highly porous materials with a bimodal pore size distribution. Typically, these monoliths contain not only small diffusive pores but also a large number of flow-through pores with diameters in the range of 700 to 2000 nm (*44*).

Use of monolithic polymers in chromatography and biocatalysis. Because the monoliths allow total convection of the mobile phase through their pores, mass transfer is also dramatically increased. Large-pore materials are almost ideal as supports for processes such as catalysis or for separations in which the transfer of large molecules is required. For example, the immobilization of enzymes onto solid supports is beneficial because it allows for the repetitive use of the biocatalysts and also facilitates work-up and product isolation once an enzyme-mediated reaction has been carried out. However, a recurring problem is that the apparent activity of an immobilized enzyme is generally lower than that of its soluble counterpart. As discussed earlier, this is because the rate-determining step is the slow diffusion of the large substrate molecules to the active sites. With the highly porous monoliths, faster mass transfer should translate into higher activity. Indeed, a comparative study with trypsin immobilized onto both macroporous beads and a fully permeated monolithic support revealed not only the higher catalytic activity of the enzyme bound to the monolith but also the much higher throughput that can be achieved with the latter because efficient mass transfer is achieved even at high flow rates (Fig. 9)(*45*).

The fast mass transfer is also important in HPLC of large molecules. Van Deemter was the first to recognize the detrimental effect of slow mass transfer on the efficiency of a chromatographic column used at higher flow rates (*46*). In contrast to mass transfer through packed columns, mass transfer of the sample within a macroporous monolith occurs by convection, and much faster chromatographic runs can be obtained without sacrificing the separation power of the column (*41*). For example, the separa-

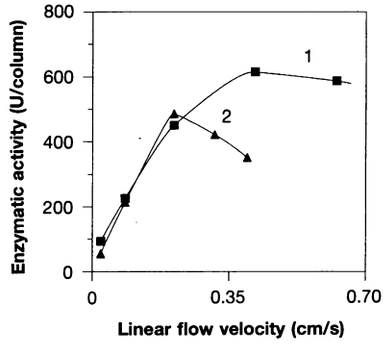

Fig. 9. Effect of linear flow velocity of benzoyl-L-arginine ethyl ester solution on the enzymatic activity of trypsin immobilized on poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) rods (curve 1) and on 10-μm beads (curve 2). With the beads, it is not possible to measure the activity at flow velocities higher than 0.4 cm/s because the back pressure of the packed column bioreactor exceeds limits of the equipment. In contrast, the maximum activity for the rod bioreactor is achieved at high flow velocity and does not decrease very much even at a higher flow rates (*45*).

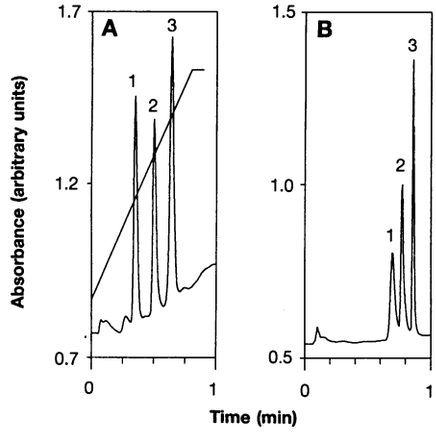

Fig. 10. Fast separation of (**A**) proteins by reverse-phase chromatography in a gradient of acetonitrile in water and of (**B**) polystyrene standards by precipitation-dissolution chromatography in a gradient of tetrahydrofuran in methanol on a 50-mm-long molded poly(styrene-*co*-divinylbenzene) column with an 8-mm inside diameter. Proteins: cytochrome c (1), myoglobin (2), and ovalbumin (3); polystyrenes: molecular weight 9200 (1), 34,000 (2), and 980,000 (3).

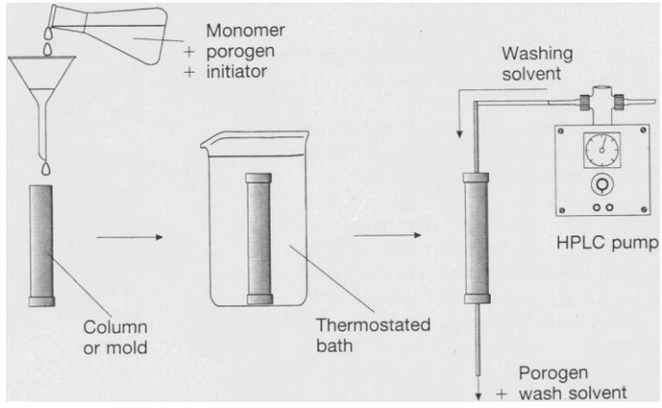

Fig. 8. Formation of macroporous monolith by a "molding" process. The mold is filled with the polymerization mixture, sealed, and transferred to a heated bath. After the polymerization is completed, the column is attached to the HPLC system. A solvent is pumped through the column to remove all soluble compounds (porogens) present in the monolith. Functionalization of the porous polymer or immobilization of a catalyst may be achieved by pumping the appropriate reagent through the column. Finally, the column is used for the desired application.

tion of protein mixtures (*47*) or polystyrene standards (*48*) can be achieved in a very short time (Fig. 10). The high speed of these separations indicates that these monoliths can be used for real-time process control and the design of smaller units with the very high throughput required for industrial separations, fast diagnostics, sensors, and many other applications.

Although much remains to be done in studies of advanced macroporous polymers, recent achievements in the preparation of both polymer beads and monoliths should open new avenues for the preparation of supports and separation media with exactly tailored properties.

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RESEARCH ARTICLE

Transcription Processivity: Protein-DNA Interactions Holding Together the Elongation Complex

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The elongation of RNA chains during transcription occurs in a ternary complex containing RNA polymerase (RNAP), DNA template, and nascent RNA. It is shown here that elongating RNAP from *Escherichia coli* can switch DNA templates by means of end-to-end transposition without loss of the transcript. After the switch, transcription continues on the new template. With the use of defined short DNA fragments as switching templates, RNAP-DNA interactions were dissected into two spatially distinct components, each contributing to the stability of the elongating complex. The front (F) interaction occurs ahead of the growing end of RNA. This interaction is non-ionic and requires 7 to 9 base pairs of intact DNA duplex. The rear (R) interaction is ionic and requires approximately six nucleotides of the template DNA strand behind the active site and one nucleotide ahead of it. The nontemplate strand is not involved. With the use of protein-DNA crosslinking, the F interaction was mapped to the conserved zinc finger motif in the NH_2-terminus of the β′ subunit and the R interaction, to the COOH-terminal catalytic domain of the β subunit. Mutational disruption of the zinc finger selectively destroyed the F interaction and produced a salt-sensitive ternary complex with diminished processivity. A model of the ternary complex is proposed here that suggests that trilateral contacts in the active center maintain the nonprocessive complex, whereas a front-end domain including the zinc finger ensures processivity.

In the advancing elongation complex, RNAP combines two contradictory biochemical features: (i) exceptional stability for dissociation and (ii) the ability to easily translocate along DNA. Thus, elongating RNAP simultaneously behaves as a strong