

RIGID GIGAPOROUS CHROMATOGRAPHIC MEDIA AND THEIR POTENTIAL IMPACT ON DOWNSTREAM PROCESSING

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Abstract More and more biomolecules are being produced by the biotechnology industry for applications ranging from medicine and food to engineering materials. Liquid chromatography plays a center-stage role in a typical downstream process producing biomolecules such as recombinant proteins. Rigid gigaporous media are porous particles possessing large transecting through-pores with a pore-to-particle diameter ratio of $d_{\text{pore}}/d_{\text{particle}} > 0.01$. They allow convective flow in the large through-pores, while the smaller diffusion-pores (typically several hundred angstroms in size) supply the needed surface areas. Because of the transecting gigapores, a portion of the mobile phase flows through the pores in addition to fluid flow in the interstitial spaces between the particles in a packed-bed column. This considerably lowers the operating column pressure drop. This lower pressure drop makes axial-direction scale-up of chromatographic columns possible to avoid pancake columns that invariably degrade separation resolution. The large gigapores also make the binding sites on the diffusion pore surfaces more accessible, thus increasing the loading capacity of large protein molecules that can be hindered sterically if only diffusion pores are present. This work discusses the development of rigid gigaporous media and their potential impact on the design of multi-stage downstream process from the angle of multi-scale analysis.

Keywords Gigapore, chromatography, bioseparation, downstream process, multi-scale, diffusion

1. Introduction

Biotechnology underwent tremendous growth in the second half of the twentieth century. Its continued advance in the twenty-first century involves the production of more and more valuable biomolecules for various applications to benefit mankind in many ways. In a typical production process, the feed from upstream fermentation or cell culture is a dilute solution of the product biomolecule. Various unit operations such as microfiltration, centrifugation, homogenization, extraction, precipitation, ultrafiltration, lyophilization and different forms of liquid chromatography are used in cascade to obtain a purified product (Ladisch, 2001; Ahuja, 2000; Flickinger & Drew, 1999). Downstream processing consumes up to 70% or more of the total processing cost in the production of a biomolecular product (Ladisch, 2001). An overall yield of 50–80% is typical for a downstream process with eight to ten unit operations (Walter, 1998). A lower yield is expected for a downstream process consisting of more unit operations for purification. Liquid chromatography plays a center-stage role because of its versatility and high resolution. High pressure liquid chromatography (HPLC) is often used to increase resolution. Many products such as biopharmaceuticals are required to be “HPLC pure” for product safety reasons. In a downstream process, the steps before liquid chromatography serve the purposes of reducing the feed volume and removing the majority of impurities. These steps help retain the resolution and prolong the life expectancy of the relatively expensive chromatography columns. The steps after liquid chromatography are polishing steps aimed at solvent or water removal.

The multiscale approach has gained considerable popularity in recent years (Li & Kwauk, 2003, 2004; Li et al.,

2004) for complex systems such as chemical reactors (Braatz et al., 2004). Based on the multiscale classification of biochemical engineering by Charpentier and McKenna (2004), the interactions of biomolecules with the binding sites on liquid chromatography media can be considered nanoscale events. The pore structures in the media are at the microscale, while the mesoscale corresponds to the unit operations in a downstream process. The macroscale and megascale are used to describe the production plant and the interactions with the biosphere. The different scales are interrelated. The microscale pore structure in a liquid chromatography medium has great impact on the separation performance of the column. This in turn can change the mesoscale arrangement of the unit operations in the entire downstream process through the possible elimination of one or more unit operations.

Mass transfer in a chromatography column plays a critical role in the scale-up of chromatography columns (Farnan et al, 1997). Traditional rigid chromatography media (with the exception of size-exclusion chromatography that relies on large pores for separations based on molecular size and shape) are porous particles with pore sizes in the range of 100 to 300 angstroms. Because these pores are relatively small, mass transfer inside the pores relies exclusively on molecular diffusion (i.e., the Brownian movement of molecules). Due to the lack of interconnecting channels, a considerable portion of these pores is not easily accessible. This decreases the overall availability of binding sites thus resulting in lower loading capacity. On the other hand, a large portion of these diffusive pores requires very long diffusional path. This causes band spreading that is detrimental to the separation resolution of a chromatography column. Fast chromatography is desired for real time or near real time process monitoring. It is also

beneficial when the biomolecular product is fragile for which short separation time helps retain product bioactivity (Afeyan et al., 1990; Fulton et al., 1991).

Gigaporous media for liquid chromatography provide answers to the drawbacks of the traditional media. Gigaporous polymer particles have very large macropores ($d_{\text{pore}}/d_{\text{particle}} > 0.01$) (Frey et al., 1993; Tallarek et al., 1999). For particle sizes of 10 and 50 microns, the pores sizes are larger than 1000 and 5000 angstroms, respectively. Superporous agarose was reported by Gustavsson and Larsson (1996) for chromatographic applications. However such a gel matrix is too weak to endure high pressure drop. Columns using this kind of media are usually scaled up in the radial direction causing flow distribution problems that lead to low resolution. POROS[®] perfusion media were the first rigid gigaporous commercial products introduced in the late 1980s. These first generation gigaporous media were synthesized in two steps. The small nanosized subparticles were synthesized first and then assembled into large particles with transecting gigapores ranging from 5000 to 8000 angstroms in size. With a particle size between 10 and 50 microns, these gigapores permit convective flow inside the particles in addition to flow in the interstitial spaces between the particles. This reduces column pressure drop considerably and thus allows much faster flow. This kind of chromatography using perfusive media is known as perfusion chromatography (Afeyan et al., 1991; McCoy et al., 1996; Whitney et al., 1998; Garcia et al., 2000).

To void fines and fragmentation of the particles in column operation, stronger integral gigaporous particles are desired. Developments in suspension polymerization technology in recent years have made a single-step synthesis possible. By using a porogen to control the gigapores sizes, it is practical to produce designer particles with controlled pore sizes. This work discusses the synthesis of such second generation gigaporous media and their potential impact on the downstream processing of biomolecular products.

2. Experimental Methods

There are several methods currently used to produce gigaporous media. While the details on the two-step synthesis method used to make POROS[®] still remain proprietary, Li and Benson (1996) patented a method to synthesize polyHIPE (high internal phase emulsion) spherical polymer particles. The HIPE structures (Cameron & Sherrington, 1996) are produced during polymerization when an aqueous phase, a monomer phase and an emulsifier are mixed. The aqueous phase is a dispersed phase in the monomer phase (oil phase). During agitation, the mixture becomes increasingly viscous when the aqueous phase concentration increases, resulting in a HIPE. PolyHIPE droplets are subsequently formed by suspending the mixture in a water-surfactant solution. The produced microspheres contain transecting macropores with pore sizes

ranging from one micron to several decade microns (Li & Benson, 1996, 1997; Li et al., 1999, 2000; Sherrington, 1998). PolyHIPE microspheres can be made with extremely high porosities ranging from 60% to 99%. However, when the porosity is very high, their weak physical strength becomes unsuitable for column packing. PolyHIPE microspheres have also found applications in drug delivery and gene therapy (Landgraf et al., 2003, 2005).

A new method (Zhou et al., 2005) was recently developed at the Institute of Process Engineering (IPE) of the Chinese Academy of Sciences in Beijing, China to produce gigaporous poly(styrene + divinylbenzene) and poly(glycidyl methacrylate) (polyGMA) particles. This is also a suspension polymerization method involving one oil phase and two aqueous phases. To produce integral polystyrene microspheres, an oil phase is prepared by mixing suitable amounts of styrene (monomer), divinylbenzene (crosslinking agent), an initiator, a diluent and a special surfactant that plays a critical role in the formation of gigapores. A water phase is prepared by mixing deionized water with a suspension agent, a surfactant and an inhibitor. An emulsion is created by dispersing the oil phase mixture in the water phase. At the end, integral microspheres with gigapores are obtained. The specific surface area of such microspheres can be as high as 200 m²·g⁻¹ or higher. Microspheres with porosity ranging from 68 to 85% and particle size ranging from 50 to 80 microns are typical. Smaller particle sizes are also possible by varying the polymerization recipe. The density of such microspheres is slightly higher than that of water, unlike typical polyHIPE particles that are much lighter than water.

3. Results and Discussion

POROS[®] perfusion chromatography media are available commercially in various forms such as POROS[®] HQ and PI anion exchangers and POROS[®] R1 and R2 reversed phase media. Fig. 1 is an SEM image of a commercially available POROS[®] R1 particle. The nanosized subparticles and the transecting gigaporous structure are clearly visible in the SEM image of the interior of the particle, as shown in Fig. 2. The mercury intrusion porosimetry (MIP) analysis of a POROS[®] R1 sample shows its pore size distribution in Fig. 3.

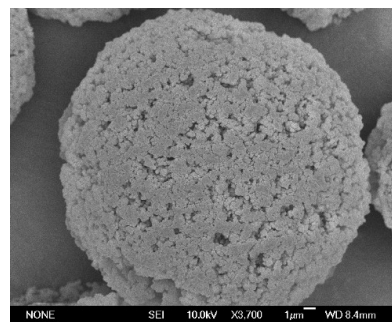


Fig. 1 SEM image of a POROS[®] R1 particle.

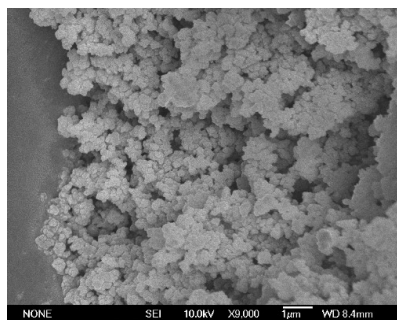


Fig. 2 SEM image showing the interior structure of a POROS® R1 particle.

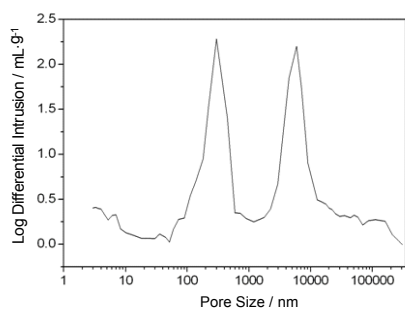


Fig. 3 Pore size distribution of POROS® R1 particles.

In comparison, an integral gigaporous polystyrene particle synthesized using our new method is shown in Fig. 4. Fig. 5 is a schematic illustrating convective flow inside the through-pores of our gigaporous particle.

Figure 6 is an SEM image showing the gigaporous through-pores in our polystyrene particle. The pore size distribution of our particles presented in Fig. 7 indicates that their pore structure characteristics are somewhat analogous to those displayed in Fig. 3 for a POROS® R1 sample.

The polystyrene and polyGMA microspheres can be functionalized using existing technologies to produce various chromatographic media such as for hydrophobic interaction, affinity, ion-exchange, etc. Functionalization of chromatography media can be carried out in three different ways. One is to use monomers already possessing functional ligands (binding sites) for polymer synthesis. Another approach is to perform functionalization polymerization by derivatizing the media surface molecules to create functional groups for attaching ligands. The most popular way is to utilize monomers that already have functional groups for ligand attachment reactions. Several of these popular functional monomers are commercially available.

Due to the lower pressure drop for gigaporous particles, scale-up of a chromatography column will no longer be limited to the augmentation of column diameter. Column volume can be increased by increasing the column length in addition to increasing column diameter. The loss of resolution due to column diameter increase can be offset by the increased column length, thus maintaining the resolution obtained in a small column. This will make scale-up more flexible.

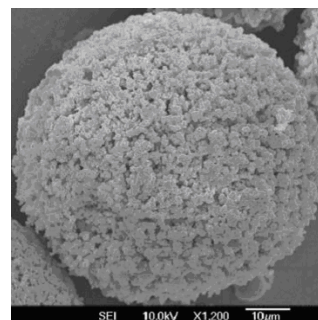


Fig. 4 SEM image of our gigaporous poly(styrene + divinylbenzene) particle produced at IPE.

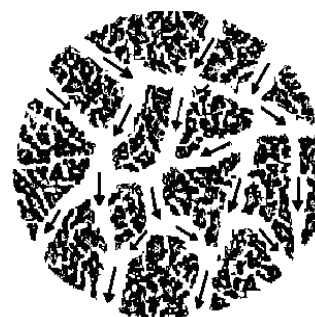


Fig. 5 Schematic of convective flow through our gigaporous particle with transecting gigapores.

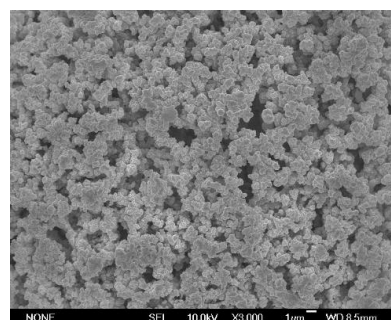


Fig. 6 Close-up SEM image showing through-pores in our gigaporous particle.

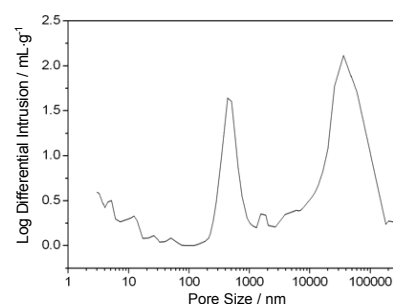


Fig. 7 Pore size distribution of our new integral gigaporous sample.

A more efficient chromatography step can require fewer service steps needed to concentrate and pre-purify the feedstock. In terms of spatio-temporal multi-scale structure

as outlined by Li et al. (2004), the intelligent design of the gigaporous particles at the product engineering scale (micro scale) has a significant impact on the column operation at the process engineering scale (macro scale) and the overall arrangement of unit operations in a downstream process for a production plant at the process system engineering scale (mega scale).

It is worthwhile to note that instead of using the gigaporous particles to pack chromatography columns, a monolithic column with gigapores can be used (McCalley, 2002; Xie et al., 2002). A monolithic medium is prepared in a molding process in which polymerization occurs in a confined mold. Currently, monolithic columns are restricted to small disks or small analytical columns (Josic et al., 2001). A breakthrough in column fabrication is necessary to produce large monolithic columns for preparative- and large-scale applications. This is certainly a promising research field to be explored.

4. Conclusions

The intelligent design of pore structures at the micro-scale can greatly impact the selection and arrangement of unit operations in a downstream process at the mesoscale to show how gigaporous chromatography media can significantly improve productivity. Rigid gigaporous media have two important advantages that have major implications on the performance and scale-up of liquid chromatography columns. Convective flow in the gigaporous through-pores greatly increases the volumetric flow rate of the mobile phase, thus making axial-direction scale-up feasible. Apart from this, the large through-pores make the smaller diffusive pores more accessible, thus increasing the loading capacity of a column. The gigaporous media approach is clearly multi-scale because the pores at the microscopic level directly impact the design of large-scale chromatography columns. With improved resolution and loading capacity, the large-scale chromatography columns will in turn change the landscape of a downstream process because the unit operations before chromatography do not have to concentrate or pre-purify the feed to the same extent as before. This means that the pre-treatment step before the chromatography step may be simplified resulting in a better process and higher overall products yield.

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