A New Method for the Synthesis of Gigaporous Polymer Beads

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Introduction

Liquid chromatography has been a center-stage technology for the purification of biomolecules in modern-day biotechnology. Various stationary phase media were developed in the last century for liquid chromatography. They include rigid inorganic particles (such as silica beads), soft polymeric gel particles (such as agarose beads), and rigid polymeric particles. Rigid polymer particles are much stronger mechanically than soft gels, and they provide a wider applicable pH range (between 1-12) than inorganic supports such as silica beads that are unstable at alkaline pH. Cross-linked polystyrene resins are the most popular rigid polymeric particles (Afeyan et al., 1990; 1991; Gustavsson and Larsson, 1996; Gracia et al., 2000).

The common preparation method of macroporous polymer microspheres is suspension polymerization in the presence of an appropriate porogen at an appropriate level in the comonomer phase that causes phase separation of crosslinked polymer. In the polymerization process, each polymer microsphere is composed of a crosslinked polymer phase and a continuous porogen phase, the latter acting as a template for the permanent porous structure of the resin. Removal of the porogen yields rigid macroporous polymer. The point when the phase separation occurs depends on the nature of the porogen, its compatibility with the incipient polymer matrix and the feed amount. These are the key factors that control the pore size distribution of the macroporous polymer microspheres. Nearly all porous polymer particles are prepared by the method described above. Pore diameters in these polymer particles are typically greater than 50 Å, and most polymers contain pores in 100 -300 Å (Sherrington, 1998).

In recent years, biomolecular products from biotechnology industry present new challenges for liquid chromatography. Because some of the biomolecules have very large and complex structures and are fragile, it is desirable that purification process is conducted as quickly as possible. Due to

their large molecular sizes, some biomolecules require the polymeric particles to have very large macropores. The conventional polymer particles have the problem of a longer separation time for the biomolecules due to their slow diffusion rate through the interior of the stationary phase particles (Sherrington, 1998). Some polymer particles are not spherical resulting in a less desirable column packing structure. In order to facilitate mass transfer and to make binding sites more accessible to large biomolecules, spherical polymer particles with twp sets pf pores are desired. The conventional diffusive pores provide the large surface area while the large through pores allow convective flow and better access to the binding sites on the diffusive pore surface.

Gigaporous polymer particles have a pore to particle diameter ratio of $d_{pore}/d_{particle} > 0.01$ (Frey et al., 1993; Tallarek et al., 1999). The gigaporous through pores must transect individual particles and are sufficiently large to allow a significant fraction of mobile phase to pass through the through pores inside the particles in addition to the flow in the interstitial space between the particles. Near the end of 80's, Afeyan et al. (1990) reported a new chromatographic technique named "perfusion chromatography." Their perfusion chromatography used gigaporous particles that have two sets of pores: through pores (6000~8000Å) and diffusive pores (800~1500Å). This bimodal pore structure significantly enhanced mass transfer of molecules in a column. Afeyan et al. (1990) claimed that separations using the gigaporous media were 10~100 times faster than in conventional chromatography, while resolution and column loading capacity were maintained. The synthesis method for perfusive particles is not in the open literature. It was said that Each perfusive particle consisted of a plurality of interadhered small polymer spheres. The interstitial spaces between the small particles form through pores and diffusive pores (Afeyan et al., 1991; 1998; Fulton et al., 1991; McCoy, 1996; Whitney et al., 1998). This apparently points to a two-step synthesis. In the first step, small subparticles were synthesized. They were subsequently linked together in the second step.

In 1996, Li and Benson (1996) patented a technique for the synthesis of HIPE (high internal phase emulsion) spherical polymer particles. HIPE structures are formed when an aqueous phase, a monomer phase and a suitable emulsifier are mixed with agitation. The aqueous phase is dispersed in the monomer phase. As aqueous phase concentration increases, the viscosity

of the mixture becomes high. The resulting substance is called HIPE. It is then suspended in a large amount of water in the presence of an appropriate surfactant to form HIPE droplets. After polymerization, the mcirospheres have a macroporous structure with interconnected cavities. The diameter of the cavities has the range of one micron to several decade microns (Li and Benson, 1996; 1997; Li et al., 1999; 2000). HIPE microspheres offers a very low pressure drop in a packed column. However, its surface area is relatively small due to its extremely high porosity. The extremely high porosity also makes the microspheres weak in physical strength and this can be a problem in packed bed operations.

Inorganic particles can also act as the template in the preparation of macroporous polymer particles. Sun et al. used organic solution and inorganic particles as double porogen. They were used to prepare small pores and large pores respectively (Zhang and Sun, 2001). This method is difficult to produce continuous macropores if the content of inorganic particles is below 70%. While, the polymer beads tend to break as the content of inorganic particles is high, and how to get the inorganic particles out is not easy.

This work presented a new method to prepare gigaporous polymer microspheres produced using suspension polymerization. The new particles have integral structure. They are suitable for liquid chromatography applications when mass transfer improvement or better access to binding sites desired. The particles can also find applications in fermentation and cell culture as microcarriers as well as in drug delivery and in gene therapy.

Preparation of Microspheres

An oil phase was prepared by mixing certain amounts of styrene (monomer), divinylbenzene (crosslinking agent), initiator, diluent and surfactant A (a key component that played a key role in the formation of large pores in a reaction vessel). A water phase was prepared by dissolving a suspension agent (polyvinyl alcohol acetate), a surfactant and an inhibitor in distilled water. Then, an emulsion was prepared by dispersing the oil mixture into the water phase at a stirring rate of 150 rpm. After sparging the reaction mixture with nitrogen gas, its temperature was raised. The polymerization was carried out under a nitrogen atmosphere for 20 h at a stirring rate of 160 rpm. The polymer beads were filtrated and washed with water and then with ethyl

alcohol for several times. The beads were vacuum dried for one day. SEM was used to characterize the polymer beads. The beads produced using an optimized recipe were found to have a pore surface area of 204 m^2/g, porosity of 83.6%, and minimal through pore size around 5000 angstroms. The particle diameter was in the range of 50 - 100 micron. Smaller particles sizes and other porosities can also be obtained by varying the recipe.

Effect of the amount of surfactant A on the morphology of microspheres

Observed from experiments, macroporous structure appeared as the amount of surfactant A reached a critical level. This was different from conventional preparation method of macroporous microspheres. The amount of surfactant A had remarkable influence on the morphology of microspheres, as shown in Fig. 1.

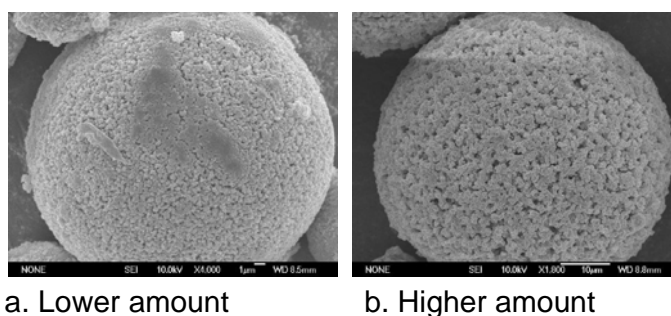a. Lower amount   b. Higher amount

Fig. 1. SEM images showing the effect of the amount of surfactant A on the morphology of microspheres with a crosslinking degree of 13.8%.

The structures of the two samples in Fig. 1 were characterized using Hg porosimetry. As seen in Table 1, the total intrusion volume and the porosity increased by 1 mL/g and 18%, respectively. In the pore size distribution analysis shown in Fig. 2, the first peak (from left) of each curve corresponds to the pore size distribution in the microspheres. The peak value increased from 50 nm to 500 nm as the amount of surfactant A increased. The second peak corresponds to the interstice between the microspheres and any large cavities in side the microspheres.

Table 1. Porosimetry measurement results of microspheres prepared with different amounts of surfactant A.

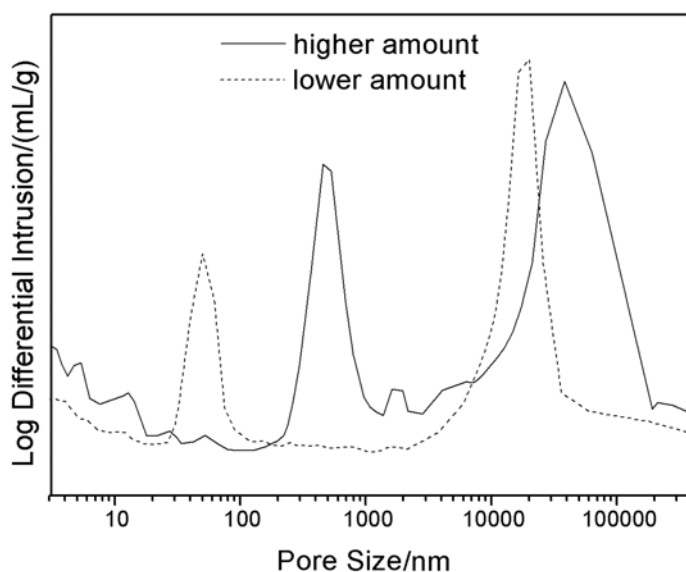| Amount of surfactant A	Total intrusion volume /(mL/g)	Total pore area/(m^2/g)	Average size /nm	Porosity /%
higher	2.65	203.8	52.1	83.6
lower	1.62	126.9	51.0	65.5

Fig. 2. Hg porosimetry results of microspheres prepared with different amounts of surfactant A.

It is well known that the strength of polymer skeleton gets higher at higher degree of crosslinking. To investigate this the crosslinking degree was increased from 13.8% to 27.5%. Fig. 3 shows the effect of the amount of surfactant A when the crosslinking degree became 27.5%.
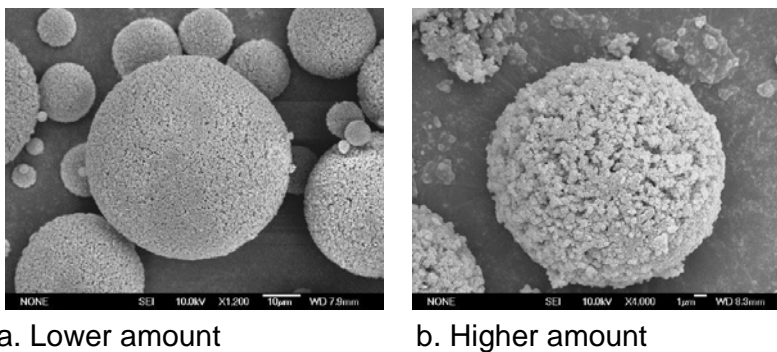

a. Lower amount   b. Higher amount

Fig. 3. SEM images showing the effect of surfactant A on the morphology of microspheres with a crosslinking degree of 27.5%.

Effects of different surfactants

In addition to surfactant A, the effects of surfactants B and C were also investigated. The effect of surfactant B was similar to that of surfactant A as shown in Fig. 4. However, higher amount of surfactant B was needed in order to get similar pore size. As the case of surfactant C, the pore sizes also increased with the increased amount of surfactant C. When the pore size reached beyond 1 μm, the microspheres were no longer intact as shown in Fig. 5.
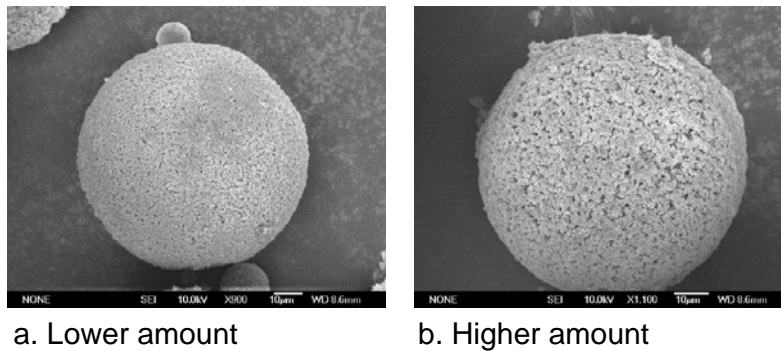

a. Lower amount   b. Higher amount

Fig. 4. Microspheres obtained with different amount of surfactant B.

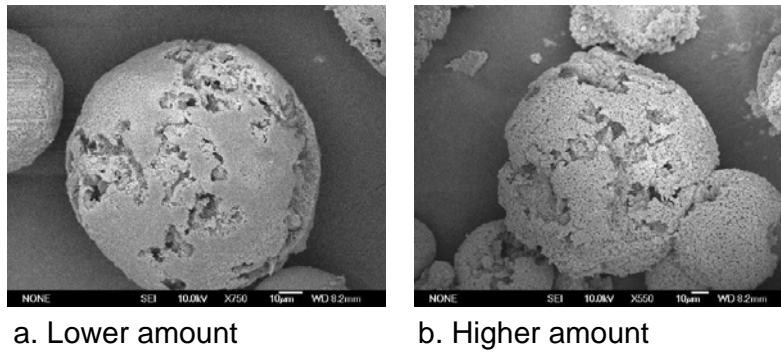a. Lower amount   b. Higher amount

Fig. 5. Microspheres obtained with different amount of surfactant C.

Effects of different types of diluent

Three kinds of diluent were tested. When the amount of diluent A was increased, large pores appeared. Too much diluent A caused microspheres to break apart (Fig. 6). Fig. 7 shows that using the same amount of diluent, diluent A provides largest pores.
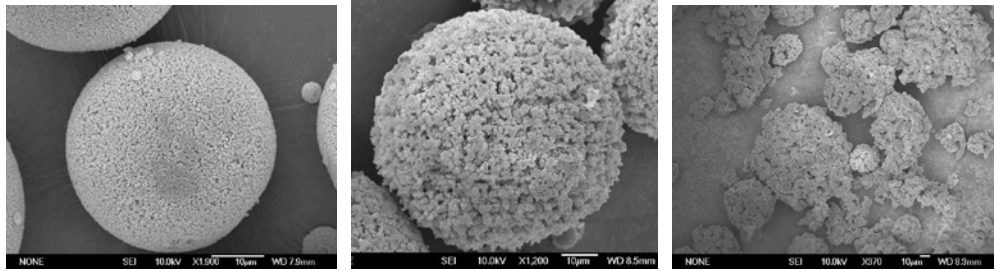
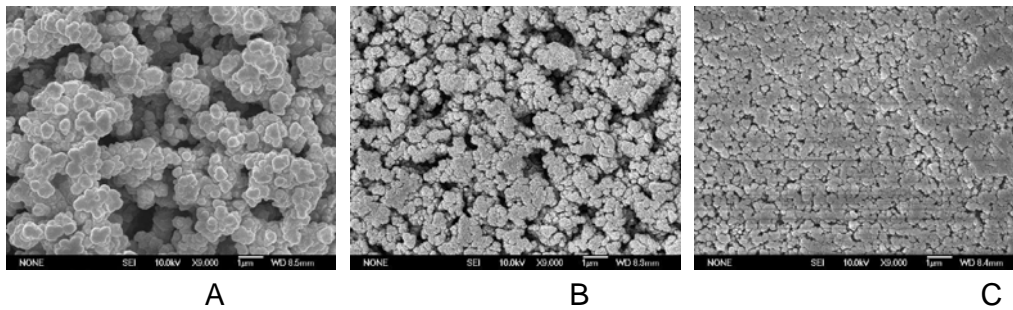Fig. 6. Microspheres prepared with increasing amount of diluent A.

| A | B | C |

Fig. 7. Microspheres prepared with the same amount of diluent A, B and C

Conclusions

A new method was developed for the synthesis of integral gigaporous particles. Effects of surfactants used in the synthesis were discussed. The particles have potential applications in bioseparations as well as other areas of biotechnology when highly porous microspheres are desired.

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