Imprinted Polymer Microspheres

Monodisperse, Molecularly Imprinted Polymer Microspheres Prepared by Precipitation Polymerization for Affinity Separation Applications**

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Molecular imprinting is a versatile and facile method for preparing synthetic polymers with predetermined molecular recognition properties[1] (mimics of antibody binding) and hence is presently attracting widespread interest, especially as the technological potential of molecularly imprinted polymers (MIPs) in chromatographic separations,[2] biomimetic sensors,[3] solid-phase extraction (SPE)[4] and catalysis[5] has now been clearly established. To date, one of the simplest methods available for MIP production in the laboratory involves conventional free-radical solution polymerization, wherein a monolith of a highly cross-linked polymer forms upon copolymerization of functional monomer(s) with an excess of cross-linking agent in a porogenic solvent. When discrete, imprinted polymer particles of a particular size range are desired, which is often the case, particularly for HPLC and SPE applications, grinding and sieving of the monolith is necessary. Unfortunately, MIP particles of irregular size and shape are invariably obtained from grinding processes in low to moderate yields (typically less than 50%), and drawbacks associated with handling and application of such heterogeneous products are well documented. To streamline and optimize the production and performance of MIP particles, alternative synthetic strategies for obtaining discrete, imprinted, particulate products that obviate the need for grinding and sieving have been evolved; they include suspension,[6] dispersion[7] and seeded polymerization.[8] Whilst these methods have undoubted value, optimization of reliable experimental protocols can be lengthy, the general applicability is questionable in some cases, and residual emulsifier or stabilizer, which can remain absorbed on the surfaces of the particles, potentially compromises selective rebinding of molecules to the imprinted material.

As an alternative to the above approaches, precipitation polymerization has emerged as an attractive, simple, and seemingly general method for producing high-quality imprinted products as spherical particles. Precipitation poly-

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merization is a surfactant-free method that involves polymerization of monomers in dilute solution ($< 5\%$ w/v) in near-θ solvents.[9] Particle growth predominantly occurs by entropic precipitation of nanogel (seed) particles followed by continuous capture of oligomers from solution. Nearly monodisperse, spherical particles can be routinely prepared in good yields by this method, and it is possible to tune the size and porosity of the particles through control of the polymerization conditions. Application of precipitation polymerization to molecular imprinting has afforded high-quality, imprinted spherical particles with diameters typically less than 1 μm, which have been applied in analytical techniques such as competition assays[10] and capillary electrochromatography (CEC).[11]

To date, however, it has proved difficult to produce larger imprinted monodisperse microspheres by precipitation polymerization that are suitable for direct application in other important affinity-separation applications such as SPE and HPLC. Therefore, the main objective of the present work was to extend significantly the scope of application of imprinted polymers prepared by precipitation polymerization by the evolution of protocols for synthesizing micrometer-scale, spherical particulates. To realize this goal a clear understanding of the growth mechanism of the microspheres was necessary.[12] For example, in the preparation of larger particles, we found that matching the solubility parameter of the developing polymer network to that of the porogenic solvent(s) is particularly important, especially when simultaneous control of polymer morphology is also desirable. These criteria are usually met for copolymerization of divinylbenzene (DVB) in mixtures of acetonitrile and toluene (the solubility parameters for acetonitrile, toluene, and polydivinylbenzene are 24.6, 18.6, and ca. 17–18 $\mathrm{MPa}^{0.5}$, respectively.[13,14]). Thus, when DVB is copolymerized under such conditions, phase separation is delayed and the polymer phase is solvated to a reasonable extent, so that products with well-developed, permanent pore structures and larger average diameters are obtained.[13] In addition, we have since also discovered, perhaps unsurprisingly, that the outcome of a precipitation polymerization is dependent on other factors, such as monomer concentrations and the method of agitation, albeit to a lesser extent. We will report upon these latter findings in full in a subsequent paper.

Under optimized polymerization conditions (see Experimental Section) relatively large microspheres of theophylline-imprinted polymer P1 and non-imprinted polymer P2 were obtained (Figure 1), which were easy to handle and of a size convenient for SPE and HPLC. The particle size distributions, measured by a Coulter Counter, were narrow; P1 and P2 had average particle diameters of 4.27 and 5.36 μm, respectively. Toluene was used as co-solvent in the polymerization of (predominantly) DVB 80; therefore, based on considerations of solubility parameters, P1 and P2 would be expected to be porous and to have high specific surface areas when prepared by precipitation polymerization.[9] This expectation was confirmed by nitrogen sorption porosimetry, which gave adsorption isotherms typical of porous polymer particles with a significant micropore population and BET specific surface areas of 550 and 660 $\mathrm{m^2\,g^{-1}}$ for P1 and P2, respectively. The similar morphologies of P1 and P2 in terms of average pore diameter (2.92 and 2.85 nm), pore volume (0.13 and 0.16 $\mathrm{cm^3\,g^{-1}}$), micropore volume (0.10 and 0.11 $\mathrm{cm^3\,g^{-1}}$), and micropore specific surface area (225 and 285 $\mathrm{m^2\,g^{-1}}$) demonstrated that the presence of template in the precipitation polymerization did not influence significantly the polymer morphology.

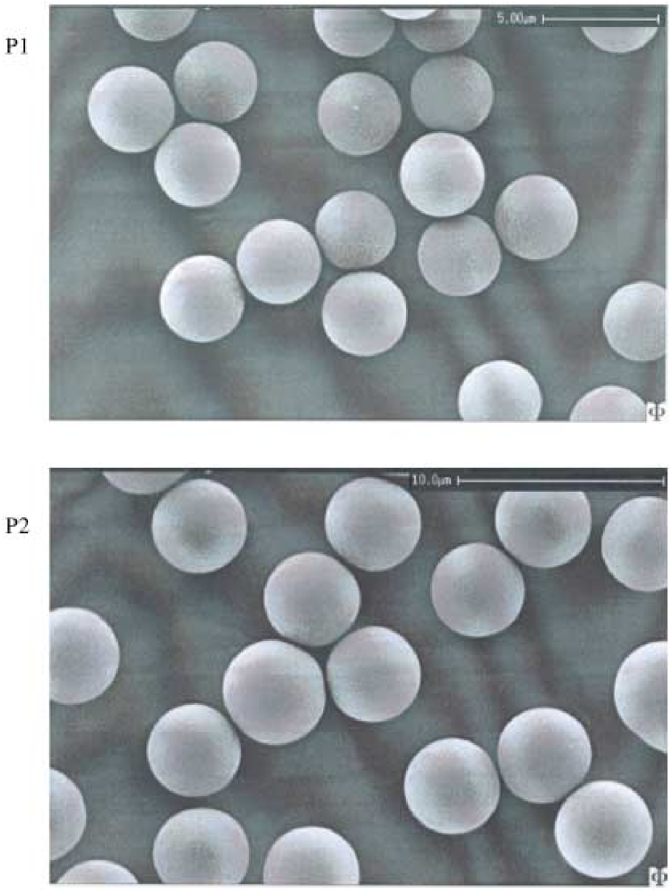

Figure 1. Scanning electron micrographs of theophylline-imprinted (P1, bar = 5 μm) and non-imprinted (P2, bar = 10 μm) microspheres.

The molecular recognition properties of the microspheres were evaluated in HPLC mode after slurry packing of the microspheres, obtained directly after preparation, washing, and drying, into stainless steel HPLC columns (2.1 I.D. × 125 mm) by using an air-driven fluid pump operating at about 200 bar. Acetone was used as the void marker, and the capacity factors calculated according to standard chromatographic theory. The elution profiles under identical chromatographic conditions of theophylline with P1 and P2 as the stationary phase are shown in Figure 2. On the P1 column, theophylline was eluted with a capacity factor of 5.39 and, furthermore, showed the peak-tailing characteristic of an imprinted HPLC stationary phase. In contrast, and as expected, theophylline was relatively poorly retained on the non-imprinted control polymer P2 (capacity factor 2.43) with minimal tailing. The imprinting effect was confirmed by solid-phase extraction experiments under equilibrium rebinding conditions (data not shown), in which the rebinding capacity was found to be about 3 $\mu\mathrm{mol\,g^{-1}}$.

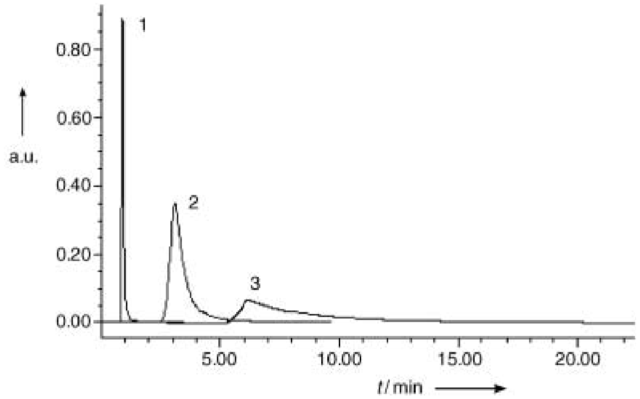

Figure 2. Comparison of retention of theophylline on P1 (peak 3) and P2 (peak 2) and of acetone on P2 (peak 1). Mobile phase: acetonitrile; flow rate: 0.5 mL min^{-1}; detection: 270 nm. 2.5 µg of theophylline in 10 µL chloroform was injected. For acetone, the number of theoretical plates for P1 and P2 were calculated to be 5900 and 5300, respectively.

In summary, theophylline-imprinted and non-imprinted monodisperse, spherical, polymer particles of about 5 µm in diameter have been prepared in one step by precipitation polymerization in good yields and applied to HPLC and SPE separations. The advantages of the precipitation polymerization approach, as outlined herein, include the fact that it can quickly deliver, with minimal optimization, high-quality imprinted products in one step that are surfactant-free, and that it avoids the need for wasteful and time-consuming grinding procedures. In a subsequent paper we will report on how our method can be applied to different analytes, and describe how a variety of particulates can be produced by precipitation polymerization by varying the nature and amounts of functional monomers, cross-linkers, initiators, and porogenic solvents. Apart from the application of the imprinted microspheres to affinity separation described here, these materials can, in principle, be used wherever molecular recognition phenomena are of importance, and they thus have many other potential scientific applications.

Experimental Section

Theophylline-imprinted polymer microspheres (P1) were prepared from divinylbenzene 80 (DVB 80) as cross-linker and methacrylic acid (MAA) as the functional monomer in a near-θ[9] mixture of acetonitrile and toluene. In a typical synthesis, theophylline (1.5 mmol), MAA (6.0 mmol), DVB 80 (28.8 mmol) and free-radical initiator 2,2′-azobisisobutyronitrile (AIBN, 1.9 mmol) were dissolved in a mixture of acetonitrile and toluene (128 mL, 75/25 v/v) in a 250 mL round-bottomed flask. The solution was degassed in an ultrasonic bath for 5 min then sparged with oxygen-free nitrogen for 10 min while cooling on an ice bath. The flask was then attached to the rotor-arm of a Kugelrohr, submerged in a thermostatically controlled oil bath and rotated slowly (ca. 5–10 r.p.m.) to minimize turbulence. The temperature was increased from room temperature to 60 °C over 2 h and then maintained at 60 °C for a further 24 h. At the end of the reaction, the microspheres were separated from the reaction medium by vacuum filtration on a membrane filter, and were then washed successively with methanol/acetic acid (100 mL, 80/20 v/v) and methanol (100 mL) and dried in vacuo overnight at 40 °C. Non-imprinted, control microspheres (P2) were prepared under nominally identical conditions to P1 except that the theophylline template was omitted. By gravimetric analysis, the yields of P1 and P2 were found to be 62 and 55 %, respectively.

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