Preparation of Molecularly Imprinted Polymers in Supercritical Carbon Dioxide

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ABSTRACT: Molecularly imprinted polymer nanoparticles were prepared in supercritical carbon dioxide using a noncovalent imprinting approach. In the present work, propranolol was used as a model template, methacrylic acid as a functional monomer, and divinylbenzene as a crosslinker. Under a high dilution condition, the heterogeneous polymerization resulted in discrete crosslinked polymer nanoparticles. Compared with the nonimprinted polymers, the imprinted nanoparticles displayed much higher propranolol uptake in a low polarity organic solvent. The use of a single enantiomer (*S*)-propranolol as the template clearly demonstrated that the imprinted binding sites are chiral-selective, with a cross-reactivity towards (*R*)-propranolol of less than 5%. The overall binding performance of the imprinted nanoparticles was comparable to imprinted polymers prepared in conventional organic solvents. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 2863–2867, 2006

Key words: molecular imprinting; supercritical carbon dioxide; propranolol

INTRODUCTION

Molecular imprinting is one of the most efficient methods to prepare synthetic materials with predesigned recognition properties.[1–4] Binding affinity and specificity of molecularly imprinted polymers (MIPs), in many cases, are challenging biological receptors (e.g., antibodies and enzymes), which makes MIPs ideal recognition components to develop binding assays and biomimetic sensors. Traditionally, MIPs are prepared in organic solvents by free radical polymerization of functional monomers in the presence of a target molecule. The target molecule is used as a template to generate binding sites in the resulting crosslinked polymer matrix, which can readily bind the target and its structural analogues. The most common MIPs are prepared in a monolithic form, which are subsequently ground and fractionated into appropriate particle sizes for different applications. In a typical imprinting preparation, polymerization is carried out in a porogenic solvent, which brings in appropriate porous structure in the final polymer matrix. The use of some unconventional solvents such as perfluorocarbon liquid and mineral oil in suspension polymerization systems to prepare imprinted polymer beads has been described.[5,6] In these cases the solvents were used purely as a continuous phase to provide a quasistable dispersion of monomer droplets, often in the presence of additional surface active reagents. Molecularly imprinted nanoparticles have been prepared by emulsion and miniemulsion polymerization methods using water as the continuous phase.[7,8] For many polar template compounds, polymerization in an inverse emulsion system has proven viable to give desirable imprinting effect.[9] Compared with bulk materials, imprinted nanoparticles contain much more accessible binding sites. They are also easy to handle with common liquid dispensing tools, allowing fast sample preparation for analytical applications.

In previous studies we, and others, have demonstrated that under optimized reaction conditions, imprinted polymer nanoparticles and microspheres can be easily synthesized using precipitation polymerization.[10–13] This method does not require special surfactant to be added in the reaction mixture, which makes MIP purification straightforward. A typical imprinting precipitation polymerization starts from a dilute solution of template and monomer in a near theta solvent. For MIP beads prepared using acrylate or styrene-type crosslinking monomers, acetonitrile has proven a valuable reaction solvent.

The drawback of inverse emulsion and precipitation polymerization is that they generate a large amount of organic solvent waste. To minimize the consumption of organic solvent, we attempted to use supercritical carbon dioxide ($scCO_2$) as reaction medium to prepare imprinted polymers in a heterogeneous reaction system. In a previous study, Cooper and coworkers synthesized dye-impregnated poly(divinylbenzene-*co*-methacrylic acid) nanoparticles in $scCO_2$, using a CO_2-soluble

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diblock copolymer as stabilizer.[14] Although it was suggested that $scCO_2$ may be used to prepare imprinted polymer microspheres, no successful demonstration has been reported until now. In the present study, we selected to use propranolol as a model template to prepare imprinted polymer nanoparticles directly in $scCO_2$. The imprinted nanoparticles were characterized by scanning electron microscopy, and by radioligand binding analysis to confirm the presence of successfully imprinted binding sites. Except for being nontoxic and having tunable solvent property, $scCO_2$ represents a particular type of ''inert'' reaction medium that is unlikely to interrupt noncovalent template-functional monomer interactions. For these reasons $scCO_2$ may be considered generally applicable for the preparation of MIPs against a broad range of template structures.

EXPERIMENTAL

Chemicals and methods

Methacrylic acid (MAA) and azobis-isobutyronitrile (AIBN) were purchased from Merck (Darmstadt, Germany). AIBN was purified by re-crystallization from methanol before use. Divinylbenzene (DVB, technical, mixture of isomers, 80%) from Aldrich was passed through an aluminum oxide column to remove the stabilizer, 4-*tert*-butylcatechol prior to use. (*R,S*)-Propranolol hydrochloride, (*R*)-propranolol hydrochloride and (*S*)-propranolol hydrochloride were purchased from Fluka and converted into free base form before use. (*S*)-[4-^{3}H]-Propranolol (specific activity 15.0 Ci $mmol^{-1}$) was purchased from NEN (Boston, MA). Solvents and other reagents were of analytical grade unless otherwise stated. Scanning electron microscopy (SEM) images were obtained with a JEOL JSM-840A microscope at the Department of Materials Chemistry, Chemical Center, Lund University. Supercritical CO_2 was generated by pumping liquid CO_2 into a 50-mL cylindrical stainless steel reactor, using a refrigerated liquid delivery system SFE-100 from Thar Technologies, Inc. (Pittsburg, PA).

Polymer preparation

Imprinting polymerization was carried out in supercritical CO_2, with and without addition of small amount of acetonitrile as modifier, using MAA and DVB as functional monomer and crosslinker, respectively, (Table I). Briefly, the template compound was dissolved in a mixture of MAA (132 mg, 1.53 mmol) and DVB (795 mg, 6.10 mmol). After addition of AIBN (20 mg, 0.122 mmol), the solution was purged with argon for 5 min and transferred into the batch reactor that was prewarmed to an elevated temperature. The reactor was then pressurized with liquid CO_2 using the SFE-100 delivery system to a preset pressure. When the desired condition (pressure and temperature) was reached, the reactor was disconnected from the pumping system. Polymerization was continued under magnetic stirring at the fixed temperature for 18 h.

After polymerization, CO_2 was slowly released. Methanol was added into the batch reactor to assist collection of the polymer particles. The particle suspension was briefly sonicated and centrifuged to remove the solvent. The template was removed by repetitively washing the polymer in methanol: acetic acid (90:10, v/v). Centrifugation was performed between two washing steps to assist solvent change. Polymer particles were finally washed in methanol and dried in vacuum. As a control, a nonimprinted polymer was prepared in the same way except for the omission of the template in the polymerization step.

Equilibrium binding analysis

Different amount of polymers were incubated with radioisotope labeled (*S*)-propranolol (2 pmol) in 1 mL of solvent for 16 h. In competitive binding experiments, different unlabeled analyte was added in the same solution. A rocking table was used to provide gentle mixing. After the incubation, samples were centrifuged to separate the labeled analyte bound on the solid particles. Supernatant (200 μL) was taken and mixed with scintillation liquid Ecosint A (10 mL), and counted for 1 min using a Rackbeta 2119 liquid scintillation counter

TABLE I
Preparation of Molecularly Imprinted Polymers and Target Binding in Organic Solvent

	Template (mmol)				(*S*)-propranolol bound (%)[a]		
Polymer	(*R, S*)-propranolol	(*S*)-propranolol	Pressure (bar)	Temperature (°C)	Imprinted polymer	Nonimprinted polymer	Specific binding[b]
MIP1	0.192	–	250	80	29.3	0	29.3
MIP2[c]	0.384	–	125	60	69.2	44.6	24.6
MIP3	0.384	–	125	60	64.2	4.1	60.1
MIP3S	–	0.384	125	60	85.0	4.1	80.9

[a] The amount of labeled (*S*)-propranolol bound to 1.8 mg of polymers in toluene containing 0.5% acetic acid.
[b] Defined as the difference of (*S*)-propranolol uptake by the imprinted and the non-imprinted polymers.
[c] The prepolymerization mixture contained 1 mL of acetonitrile.

(LKB Wallac, Sollentuna, SE). The amount of labeled (*S*)-propranolol bound to polymer nanoparticles was calculated by subtraction of the free fraction from the total amount added.

RESULTS AND DISCUSSION

In previous studies, $scCO_2$ has been used to afford template extraction from irregular MIP particles,[15] and as a mobile phase to perform supercritical liquid chromatography separation on MIP-packed columns.[16] Although $scCO_2$ has been used as a reaction medium for the synthesis of crosslinked poly(divinylbenzene) microspheres,[14] direct preparation of imprinted polymers in $scCO_2$ has not been realized. One difficulty of using $scCO_2$ for MIP synthesis is that $scCO_2$ has a poor miscibility with the most commonly used acrylate crosslinkers, e.g., ethylene glycol dimethacrylate (EDMA) and trimethanolpropane trimethacrylate (TRIM). For molecular imprinting in $scCO_2$, it appeared to us that a less polar crosslinker should be employed. In a previous work we used divinylbenzene (DVB) as crosslinker and methacrylic acid (MAA) as functional monomer to prepare polymer microspheres in acetonitrile. The propranolol-imprinted polymer microspheres displayed binding performance superior to MIPs obtained with an acrylate crosslinker.[11] DVB was thus chosen as the crosslinker to prepare MIP particles in the present study. Although most functional monomers used in noncovalent imprinting have high polarity, they only account for a small volume fraction in the monomer mixture, and should be possible to disperse in $scCO_2$, especially after forming stable complexes with template molecules.

The polymers prepared according to Table I were all composed of similar particle aggregates. The particle aggregates could be easily separated by ultrasonic treatment after being suspended in toluene or methanol. Addition of organic modifier (acetonitrile) in $scCO_2$ (for preparation of MIP2) did not bring in obvious change in particle morphology. Figure 1 shows representative SEM images for the propranolol-imprinted polymers. The high magnification SEM images [Figs. 1(c)–1(d)] indicate the average size of the imprinted polymer particles of around 100 nm. Compared to MIP microspheres (up to 2 μm in diameter) synthesized in acetonitrile from the same monomer mixture, the present propranolol MIPs obtained in $scCO_2$ have a much smaller particle size. Obviously, the new reaction medium had a profound effect on polymer nucleation and particle growth, presumably due to the special supercritical property of $scCO_2$.[17,18]

To evaluate the molecular imprinting effect, equilibrium binding experiment was initially carried out in toluene containing 0.5% (v/v) acetic acid. The use of very low concentration of radioligand allowed us to study the difference of propranolol affinity of the best imprinted sites among the different polymers (Table I). For the MIPs prepared using racemic propranolol template (MIP1–MIP3), the imprinted polymer (MIP3) pre-

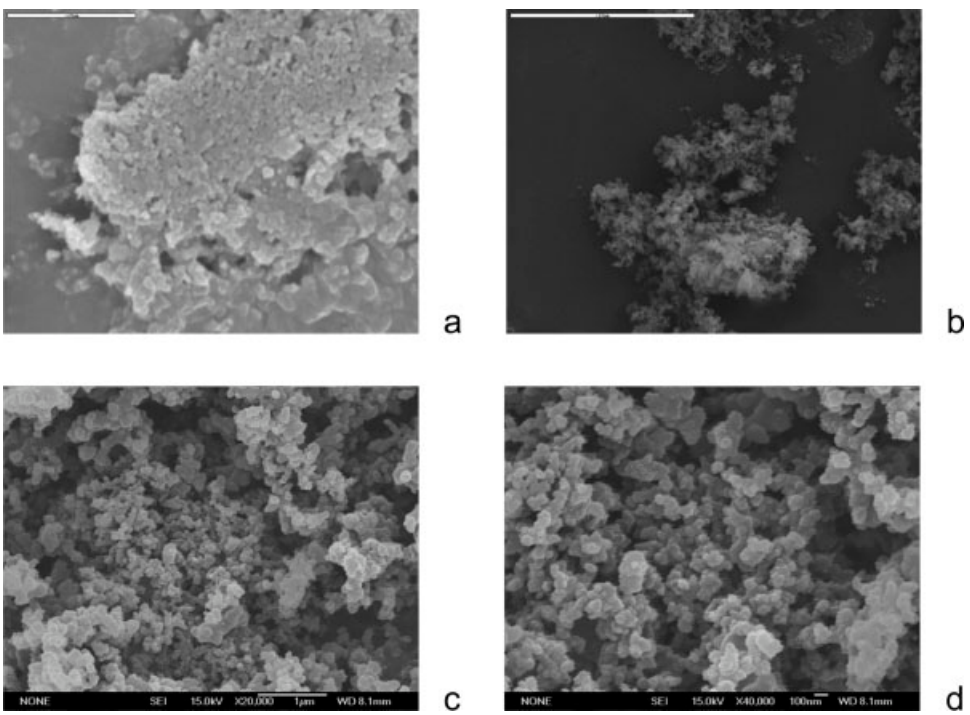

Figure 1 SEM micrographs of MIP3 (a) and MIP3S (b, c, d). The scale bar corresponds to 10 μm (a, b), 1 μm (c), and 100 nm (d).

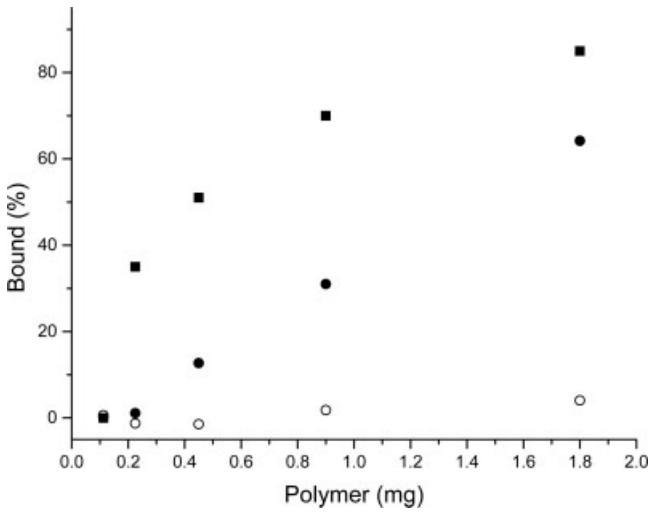

Figure 2 Equilibrium binding of (*S*)-propranolol to polymers MIP3 (●), NIP3 (○), and MIP3S (■) in toluene containing 0.5% acetic acid (v/v).

pared using high template loading and low polymerization temperature and pressure gave the highest specific binding. The nonimprinted control polymer prepared under the same condition showed negligible propranolol binding, suggesting that in the absence of the template, most MAA existed as hydrogen-bounded dimers during the crosslinking reaction, therefore became inaccessible in the polymer matrix.

In conventional molecular imprinting systems, a single propranolol enantiomer has been used as template to generate chiral selective binding sites.[11,13,19] To confirm that the same chiral cavities could be obtained in $scCO_2$, a new imprinted polymer (MIP3S) was prepared under the same reaction condition as used for MIP3, except that (*S*)-propranolol was used to replace the racemate (*R*,*S*)-propranolol. Compared to MIP3, the new polymer (MIP3S) should have two-folds of imprinted chiral sites to bind (*S*)-propranolol. The equilibrium binding results in Figure 2 confirmed that MIP3S indeed had largely increased propranolol uptake. The amount of polymers needed to obtain 50% of radioligand binding was approximately 1.2 mg for MIP3 and 0.45 mg for MIP3S.

The (*S*)-propranolol-imprinted nanoparticles also showed favorable template binding in aqueous buffer. To demonstrate chiral selectivity, MIP3S was incubated with radioactive (*S*)-propranolol in the presence of different amount of unlabeled (*R*)- and (*S*)-propranolol until equilibrium. The unlabeled compounds competed for the limited binding sites, thus reducing the amount of bound radioligand. The potency of the displacement from each compound represents their relative affinity to the imprinted sites. Figure 3(a) shows the displacement curves obtained with both (*R*)- and (*S*)-propranolol in aqueous solution. As seen, the cross-reactivity of MIP3S towards (*R*)-propranolol was less than 5%. The IC_{50} value of the present competitive assay for (*S*)-propranolol was approximately 600 ng mL^{-1}, similar to that obtained with the (*S*)-propranolol-imprinted microspheres prepared previously in a conventional organic solvent.[11]

The homologous competition binding results from (*S*)-propranolol can be converted into a binding iso-

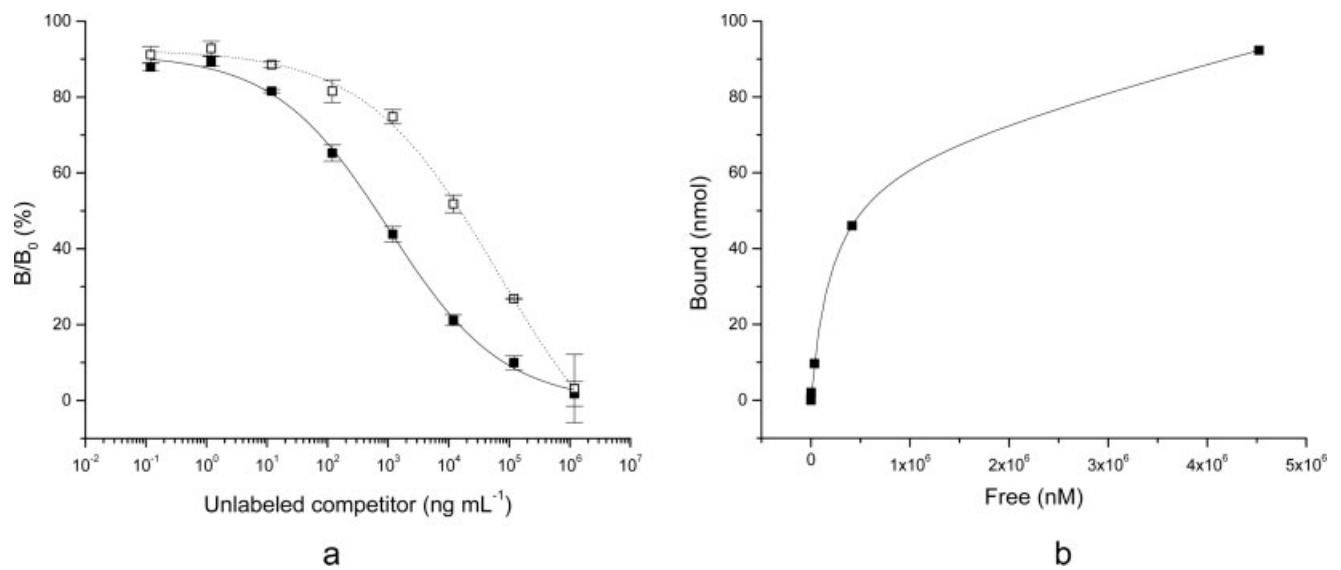

Figure 3 (a) Displacement curves of (*S*)-propranolol (■) and (*R*)-propranolol (□) obtained with 0.2 mg of polymer MIP3S. The displacement experiment was carried out in 25 m*M* citrate buffer (pH 6.0) containing 50% acetonitrile (v/v). *B* and B_0 are the amount of bound labeled (*S*)-propranolol in the presence and absence of the competing unlabeled compounds. Data are mean values of triplicate determinations. (b) The binding isotherm of (*S*)-propranolol with MIP3S, as calculated from the homologous competition data for (*S*)-propranolol.

therm plot [Fig. 3(b)].[20] With a simplified one-site binding model, the total (*S*)-propranolol uptake can be divided into an apparent specific portion and a nonspecific portion using the following equation:

$$\text{Bound} = B_{\max}\text{F}/(K_{\text{D}} + \text{F}) + N\text{F}$$

K_{D} is the apparent mean dissociation constant and $B_{\max}$ the apparent capacity of the imprinted sites. From Figure 3(b), the K_{D} and $B_{\max}$ of the (*S*)-propranolol-imprinted nanoparticles are estimated to be (2.13 $\pm$ 0.26) $\times$ 10^{-4} *M* and (3.28 $\pm$ 0.17) $\times$ 10^{-4} mol g^{-1}, respectively. In the present aqueous solvent, the apparent template affinity and binding capacity of MIP3S are comparable to most noncovalently imprinted polymers reported in the literature.[21]

CONCLUSIONS

The present study demonstrated that noncovalent molecular imprinting can be readily carried out in supercritical carbon dioxide. By controlling appropriate reaction conditions, molecularly imprinted nanoparticles can be prepared directly under a high dilution condition in $scCO_2$. The imprinted nanoparticles obtained in the present work had binding performance comparable to the traditional MIPs synthesized in conventional organic solvents. Because of its nontoxic character, use of $scCO_2$ as reaction medium can greatly reduce the consumption of volatile organic compound (VOC) to minimize environmental pollution. We believe that further research in this direction will create new opportunities for industrial applications of MIPs that are produced on a large scale.

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References

1. Zimmerman, S. C.; Lemcoff, N. G. Chem Commun 2004, 5.
2. Ansell, R. J. J Chromatogr B 2004, 804, 151.
3. Ye, L.; Haupt, K. Anal Bioanal Chem 2004, 378, 1887.
4. Mayes, A. G.; Whitcombe, M. J Adv Drug Deliv Rev 2005, 57, 1742.
5. Mayes, A. G.; Mosbach, K. Anal Chem 1996, 68, 3769.
6. Kempe, M.; Kempe, H. Macromol Rapid Commun 2004, 25, 315.
7. Pérez, N.; Whitcombe, M. J.; Vulfson, E. N. J Appl Polym Sci 2000, 77, 1851.
8. Vaihinger, D.; Landfester, K.; Iris, K.; Herwig, B.; Tovar, G. E. M. Macromol Chem Phys 1965, 2002, 203.
9. Markowitz, M. A.; Kust, P. R.; Klaehn, J.; Deng, G.; Gaber, B. P. Anal Chim Acta 2001, 435, 177.
10. Ye, L.; Cormack, P. A. G.; Mosbach, K. Anal Commun 1999, 36, 35.
11. Ye, L.; Surugiu, I.; Haupt, K. Anal Chem 2002, 74, 959.
12. Wang, J.; Cormack, P. A. G.; Sherrington, D. C.; Khoshdel, E. Angew Chem Int Ed 2003, 42, 5336.
13. Spégel, P.; Schweitz, L.; Nilsson, S. Anal Chem 2003, 75, 6608.
14. Cooper, A. I.; Hems, W. P.; Holmes, A. B. Macromolecules 1999, 32, 2156.
15. Ellwanger, A.; Berggren, C.; Bayoudh, S.; Crecenzi, C.; Karlsson, L.; Owens, P. K.; Ensing, K.; Cormack, P. A. G.; Sherrington, D.; Sellergren, B. Analyst 2001, 126, 784.
16. Ellwanger, A.; Owens, P. K.; Karlsson, L.; Bayoudh, S.; Cormack, P. A. G.; Sherrington, D. C.; Sellergren, B. J Chromatogr A 2000, 897, 317.
17. Wells, S. L.; DeSimone, J. Angew Chem Int Ed 2001, 40, 519.
18. Cooper, A. I. J Mater Chem 2000, 10, 207.
19. Andersson, L. I. Anal Chem 1996, 68, 111.
20. Boonpanggrak, S.; Prachayasittikul, V.; Bülow, L.; Ye, L. J Appl Polym Sci 2006, 99, 1390.
21. Shimizu, K. D. In Molecularly Imprinted Materials: Science and Technology; Yan, M.; Ramström, O., Eds.; Marcel Dekker: New York, 2005; pp 419–434.