Synthesis of Molecularly Imprinted Microspheres for Recognition of *Trans*-Aconitic Acid

Yong Jiang, Ai-Jun Tong

Department of Chemistry, Tsinghua University, Beijing 100084, China

Received 10 September 2003; accepted 8 April 2004
DOI 10.1002/app.20954
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In the presence of a template molecule, *trans*-aconitic acid and, using acetonitrile as solvent and dispersing medium, monodispersed microspheres with a diameter of 600–700 nm bearing molecularly imprinted binding sites were prepared by precipitation polymerization. It was found that the concentrations of template, monomer, and crosslinking agent as well as the chemical structure of the template greatly affect the polymer configuration. Microspheres are produced only when the concentration of the template molecule and the functional monomer are finely tuned. Comparison with the performance of a conventional imprinted polymer monolith showed that the imprinted microsphere had obvious advantages in specific binding to template molecule. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 542–547, 2004

Key words: molecularly imprinted microsphere; precipitation polymerization; aconitic acid; molecular recognition; hydrogen-bonded complex

INTRODUCTION

Molecular imprinting is a technique for constructing tailor-made receptor binding sites in a three-dimensional, crosslinked polymer matrix that is called a molecularly imprinted polymer (MIP).[1,2] Since Vlatakis *et al.*[3] first reported the synthesis of MIPs through a noncovalent approach, interest in the molecular imprinting technique has blossomed.[4–8] We have reported selective recognition of histamine with a fluorescent molecularly imprinted polymer by using a commercially available reagent zinc(II)–protoporphyrin (ZnPP) as a novel fluorescent functional monomer.[9] Synthetic conditions for preparation of a β-estradiol imprinted monolithic polymer were also reported.[10] We found that the porogen solvent and the other polymerization conditions greatly affected the binding ability of a MIP to a certain molecule. However, most of the reported MIPs are prepared in the form of a macroporous monolith that is then ground and sieved to an appropriate particle size to obtain a surface large enough for the template molecules to diffuse in when being rebound.[4,7] The grinding and sieving process is labor intensive and polymer wasteful. It yields only moderate amounts of "useful" product and decreases specific binding degree because of destruction of imprinted binding sites. The resulted MIP particles are also irregularly shaped and not ideal for chromatographic purposes. Thus the imprinting method yielding uniform MIP spheres is highly desired.

Traditional suspension polymerization methods[11] for producing spheres require an aqueous or a highly polar organic suspension medium (e.g., an alcohol), which weakens the hydrogen bonding interaction between the functional monomer and the template molecule in the noncovalent MIP synthesis. In contrast to the highly polar suspension medium, Mayes and Mosbach[12] developed a new suspension polymerization method for molecular imprinting by using perfluoro(methylcyclohexane) (PMC), a dipersion medium largely immiscible with most organic solvents. Although it was applicable to a range of conditions typically used for yielding an imprinted microsphere, the reagent is so expensive that it restricted the broad acceptance of this method in molecular imprinting. An attempt has also been made to use the aqueous multistep swelling method[13] to make monodispersed microspheres[14,15] for imprinted polymer preparation. With uniformly sized seed polymer beads as the template, microspheres were produced in very high yields compared with the suspension polymerization method. Unfortunately, this method also suffers from the need for an aqueous phase during the swelling procedure, so it is applicable only to a narrow range of template molecules.

A more economical and labor-saving imprinting method for synthesizing uniform microspheres bearing molecularly imprinted binding sites, called precipitation polymerization,[16] was reported.[17,18] With acetonitrile as the porogen and dispersing medium, mo-

Correspondence to: A. J. Tong (tongaj@chem.tsinghua.edu.cn).

lecularly imprinted microspheres were produced without any other postimprinting treatment. Although Ye *et al.*[17,18] discussed the effect of dilution on the configuration of the polymer, other detailed experimental conditions for getting imprinted microspheres with this precipitation polymerization method is still not clear.

In this study, the experimental conditions for preparation of spherical MIP with the precipitation polymerization have been discussed. We also compared the binding ability of the imprinted microsphere with the conventional MIPs prepared as a bulky monolith. Evidence of much higher specific binding by the microspheric imprinted polymers than the monolithic MIPs was found.

EXPERIMENTAL

Materials

Trans-aconitic acid (99%), *cis*-aconitic acid (98%), and diethylstilbestrol (99.0%, HPLC grade) were purchased from Fluka and used as received. Methacrylic acid (MAA, 99.5%) was obtained from Acros Organics, Belgium. Trimethylol-propane trimethacrylate (TRIM) was from Tonghua Chemical Plant, Beijing, China. Azobisisobutyronitrile (AIBN, chemical pure) was from Shengshi Fine Chemical Co. Ltd, Wuhan, China. Acetonitrile (99.9%, HPLC grade) was from Siyou Biomedicine Co. Ltd, Tianjin, China.

Preparation of imprinted microspheres by precipitation polymerization

Synthesis of the *trans*-aconitic acid imprinted microspheres was carried out with 1 mmol TRIM, 0.25 mmol *trans*-aconitic acid, 0.8 mmol MAA, and 0.15 mmol AIBN in 0.5 mol acetonitrile in a 50- ml round-bottom flask. The solution was treated with ultrasound for 5 min and then was purged with nitrogen flow for 10 min. Polymerization was carried out in a water bath at 60°C for 24 hs. The microspheres obtained were collected by centrifugation at 3,500 rpm for 30 min. The template was removed by washing five times with 10 ml methanol containing 10% (vol/vol) acetic acid. The microspheres were finally rinsed with acetone and then dried in a vacuum desiccator for 48 h before use. As a control, the nonimprinted microspheres were prepared and treated in exactly the same way except that the template was absent in the polymerization procedure.

Evaluation of the binding ability

Four milliliters of 6×10^{-5} mol/L *trans*-aconitic acid acetonitrile solution was mixed with 40 mg of the microspheric polymer in a 5-ml glass tube with screw cap. The mixture then was dispersed by ultrasound and incubated with frequent shaking on a rocking table at room temperature for 24 hs. After centrifugation and careful separation of the polymer microspheres, the solution was filtered through a membrane filter. The concentration of the *trans*-aconitic acid after adsorption by the polymer was determined by measuring the solution absorbance at 215 nm. The average binding percentage was calculated with nine repeated binding processes. UV spectra were taken on a Shimadzu UV-2100s type absorption spectrometer.

RESULTS AND DISCUSSION

Although precipitation polymerization in acetonitrile provides a method yielding spherical molecularly imprinted polymers,[17] detailed synthesis conditions have not been reported. With acetonitrile as solvent and dispersion medium, *trans*-aconitic acid as template molecule, MAA as functional monomer, and TRIM as crosslinking agent, monodispersed molecularly imprinted microspheres are synthesized at certain experimental conditions. The effects of template molecule, functional monomer, and crosslinking agent on shape and size of polymer are summarized in Table I.

From Table I it can be seen that, with different synthesis conditions in polymerization, polymers can have various physical configurations. The increase in concentration of crosslinking agent at constant amounts of porogen drastically resulted in coagulation of the microparticles. In Figure 1, both imprinted microspheres (MIP-A) and nonimprinted amorphous particles (NMIP-A) coagulated to bulky monoliths MIP-C and NMIP-C with the increase of TRIM concentration from 40.8 to 410 mmol/L. In fact, the conventional imprinted and nonimprinted polymer monoliths were produced with a total crosslinker concentration of 25% (vol/vol) with respect to porogen. When the polymerization occurs, solid polymer appears and absorbs porogen. The lack of porogen makes the growing polymers occupy the entire volume of the vessel with little porogen left after initiation, which results in coagulation.

From Table I it can also be seen that, although the increase of crosslinker concentration makes the microparticles coagulate, it is not the unique control factor that affects the formation of spherical polymer. In the absence of the template molecule *trans*-aconitic acid, more functional monomer than that needed for synthesis of imprinted microsphere or synthesis of amorphous imprinted particles should be added to obtain spherical nonimprinted polymer NMIP-B. However, excess of template molecule or functional monomer will also coagulate microspheres to one another (Fig. 2). Microspheres are produced only when the concentration of template molecule and functional monomer is finely tuned while low concentration leads to amor-

TABLE I
Polymer Configuration and Size under Different Synthetic Conditions[a]

Entry	Template molecule (mmol/L)	Functional monomer MAA (mmol/L)	Crosslinker TRIM (mmol/L)	Configuration and size (nm)
MIP-A	*Trans*-aconitic acid 10.3	32.8	40.8	Microsphere 600–800[b]
MIP-B	*Trans*-aconitic acid 10.3	187.4	40.8	Coagulum >1,000
MIP-C	*Trans*-aconitic acid 10.3	32.8	410	Monolith >10,000[c]
MIP-D	*Trans*-aconitic acid 10.3	32.8	122	Coagulum >1,000
MIP-E	Diethylstilbestrol 10.3	32.8	40.8	Amorphous <100
MIP-F	*Cis*-aconitic acid 10.3	32.8	40.8	Coagulum >1,000
MIP-G	*Trans*-aconitic acid 10.3	70.3	40.8	Microsphere 800–900
MIP-H	*Trans*-aconitic acid 10.3	70.3	81.6	Microsphere 1,500–2,000
MIP-I	*Trans*-aconitic acid 10.3	32.8	40.8	Microsphere 400–500[b]
NMIP-A	None	32.8	40.8	Amorphous <100
NMIP-B	None	468	40.8	Microsphere 600–700
NMIP-C	None	32.8	410	Monolith >10,000

[a] The porogen solvent is acetonitrile. The concentration of AIBN is 6.09 mmol/L. MIP and NMIP refer to the imprinted or non imprinted polymer, respectively.
[b] The total reaction volume of MIP-A is 25 ml while that of MIP-I is 250 ml.
[c] Ground carefully to collect the particles with sizes below 900 nm in binding experiment.

phous particles and high concentration leads to coagulum. Higher concentrations of the template and the monomer led to higher "concentration" of the hydrogen-bonded template–monomer complex and the aggregation of monomer themselves, which resulted in coagulum of the polymer particles. The results indicate that the "concentration" of hydrogen-bonded complex is one of the most important factors controlling the microsphere formation.

Because template molecules with different steric structures have different affinities with the functional monomer, even template molecules of steric isomers will lead to different "concentrations" of hydrogen-bonded template–monomer complex with exactly the same amounts of added template molecule and functional monomer. In fact, different configurations of molecularly imprinted polymer result with *cis*- and *trans*-aconitic acid as the template (Fig. 3). The coagulum of imprinted polymer is produced when *cis*-aconitic acid is used as the template molecule but imprinted microsphere is obtained at the same concentration with *trans*-aconitic acid as the template. Another template molecule, diethylstilbestrol, results in amorphous particles for its lowest affinity to MAA of the three templates used.

The total reaction volume affected the size of microspheres even if the concentrations of template and monomer didn't change. As shown in Figure 4, 600 to 800-nm microspheres were produced when the total reaction volume was 25 ml while the size became less

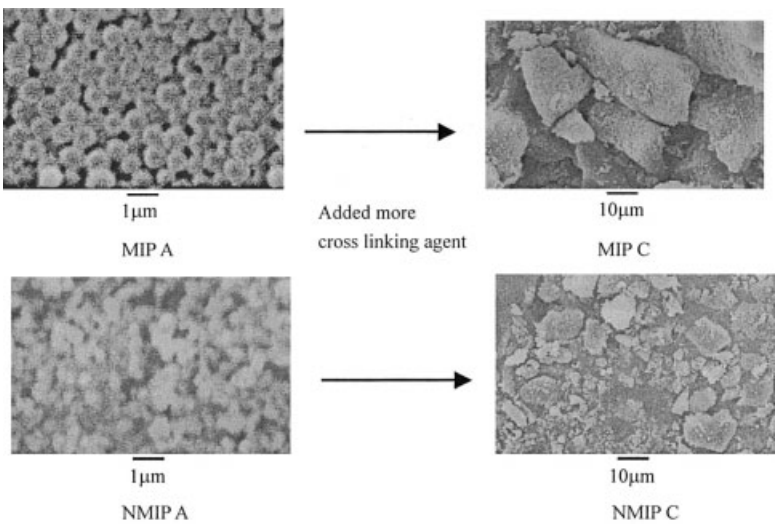

Figure 1 Effect of crosslinker concentration on the configuration of the polymer. Polymer synthetic conditions are the same as shown in Table I.

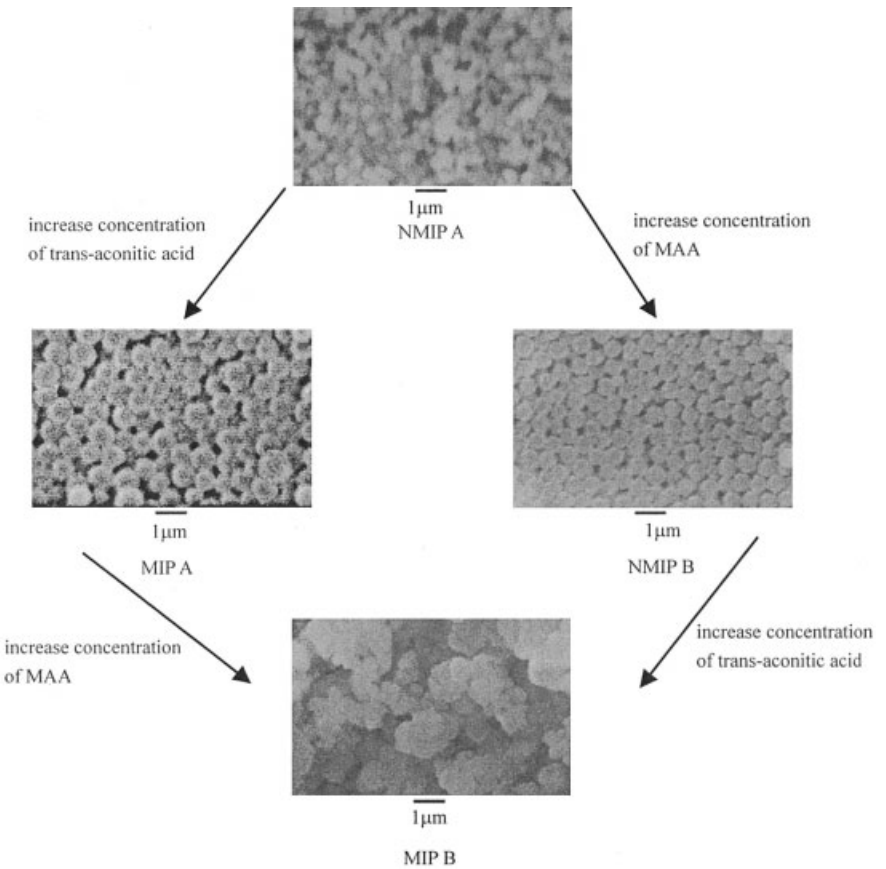

Figure 2 Concentration effect on polymer configuration and size. Increase in the concentration of the template and the monomer means an increase in the concentration of hydrogen-bonded template–monomer complex and the concentration of monomer aggregate. Polymer synthetic conditions are the same as shown in Table I.

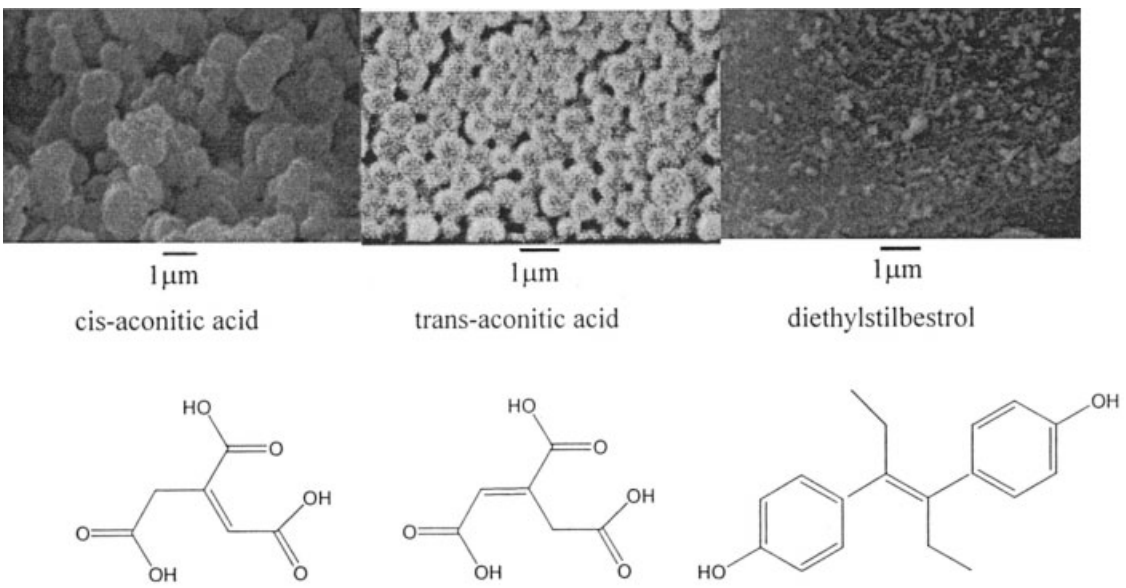

Figure 3 Configurations of polymers synthesized with different template molecules. The chemical structures of the corresponding template molecules are shown below. Polymer synthetic conditions are the same as shown in Table I.

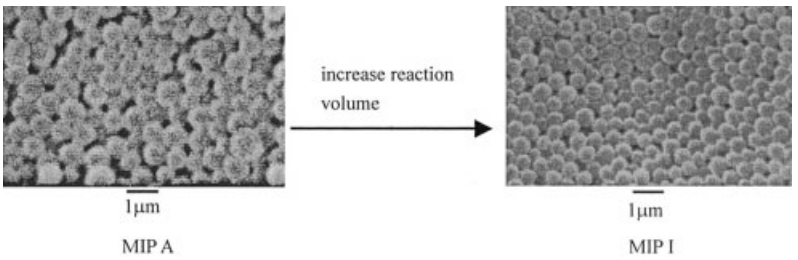

Figure 4 Effect of the total reaction volume. Polymer synthetic conditions are the same as shown in Table I.

than 500 nm when the reaction volume turned to 250 ml with preparation of the *trans*-aconitic acid imprinted polymer.

Binding performance of molecularly imprinted polymers with different configurations and particle sizes for *trans*- and *cis*-aconitic acid was studied by using a batch adsorption method. Adsorption percentages were calculated by measuring *trans*- or *cis*-aconitic acid concentration in the supernatant with UV detection. Direct comparison was difficult due to the experimental restrictions, i.e., under the same template, monomer, and crosslinker concentration, the polymer configuration was different while both the polymer composition and configuration affect their binding performance. From the synthetic practice, we found that monolithic MIP could not be obtained at the same dilute concentration as that in preparation of MIP-A. Monolithic MIP-C was produced with a large excess of crosslinking agent. To eliminate the particle-size effect, the MIP-C was ground as finely as possible and then the particle size below 900 nm was collected with the aid of a scanning electron microscope. For nonimprinted polymers, NMIP-A was of the same polymer composition as MIP-A but the particle size and configuration were different; NMIP-B was monodispersed sphere and has a similar particle size as MIP-A except its polymer composition was different.

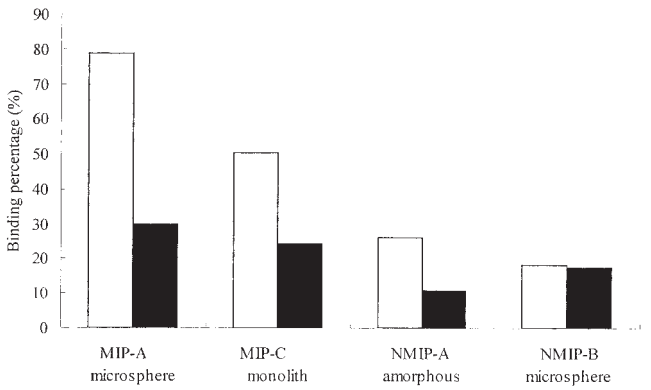

Figure 5 Binding of *trans*-aconitic acid (open column) and *cis*-aconitic acid (solid column) on different types of polymers. Polymer synthetic conditions are the same as shown in Table I.

Figure 5 compares the binding of *trans*- and *cis*-aconitic acid with these different kinds of polymers. *Trans*-aconitic acid imprinted polymers MIP-A and -C bind more efficiently to the template molecule than the nonimprinted polymers. They also show more affinity to the template *trans*-aconitic acid than its isomer *cis*-aconitic acid. The imprinted microsphere MIP-A and the imprinted monolith MIP-C were of a similar size in the binding experiment. The percentage of binding on imprinted microsphere with *trans*-aconitic acid was 78.87% while it was 50.32% on the imprinted monolith. The imprinted microsphere shows an obvious advantage over the conventional monolith in specific binding. The spherical polymer also shows an advantage in nonspecific binding. Results of the binding analysis indicated that the nonspecific binding of spherical NMIP-B was invariable with both template molecule (18.15%) and its isomer (17.72%), while amorphous NMIP-A bound more template molecules (26.26%) than its isomer (10.68%); this is because of the nonuniformity of the surface of the amorphous nonimprinted polymer where the nonspecific binding takes place.

CONCLUSION

In summary, our results show that the concentrations of template, monomer, and crosslinking agent as well as the chemical structure of the template greatly affect the polymer configuration. The concentration effect of the hydrogen-bonded template–monomer complex is one of the most important factors controlling the microsphere formation by the precipitation polymerization method. The microspheric imprinted polymers had obvious advantages in specific binding to the template molecule than the monolithic MIPs.

References

1. Al-Kindy, S.; Badía, R.; Suárez-Rodríguez, J. L.; Díaz-García, M. E. Crit Rev Anal Chem 2000, 30, 291.
2. Kriz, D.; Ramström, O.; Mosbach, K. Anal Chem 1997, 69, A345.
3. Vlatakis, G.; Andersson, L. I.; Muller, R.; Mosbach, K. Nature 1993, 361, 645.
4. Venn, R. F.; Goody, R. J. Chromatographia 1999, 50, 407.
5. Titirici, M. M.; Hall, A. J.; Sellergren, B. Chem Mater 2002, 14, 21.

6. Masci, G.; Aulenta, F.; Crescenzi, V. J Appl Polym Sci 2002, 83, 2660.
7. Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. Anal Chem 1998, 70, 2025.
8. Dickert, F. L.; Tortschanoff, M. Anal Chem 1999, 71, 4559.
9. Tong, A. J.; Dong, H.; Li, L. D. Anal Chim Acta 2002, 466, 31.
10. Dong, H.; Tong, A. J.; Li, L. D. Spec Chim Acta A 2003, 59, 279.
11. Svec, F.; Fréchet, J. M. Anal Chem 1992, 64, 820.
12. Mayes, A. G.; Mosbach, K. Anal Chem 1996, 68, 3769.
13. Hosoya, K.; Jean, M.; Fréchet, J. J Polym Sci A Polym Chem 1993, 31, 2129.
14. Hosoya, K.; Yoshizako, K.; Shirasu, Y.; Kimata, K.; Araki, T.; Tanaka, N.; Haginaka, J. J Chromatogr A 1996, 728, 139.
15. Fu, Q.; Sanbe, H.; Kagawa, C.; Kunimoto, K.; Haginaka, J. Anal Chem 2003, 75, 191.
16. Naka, Y.; Kaetsu, I.; Yamamoto, Y.; Hayashi, K. J Polym Sci A Polym Chem 1991, 29, 1197.
17. Ye, L.; Cormack, P. A. G.; Mosbach, K. Anal Commun 1999, 36, 35.
18. Ye, L.; Cormack, P. A. G.; Mosbach, K. Anal Chim Acta 2001, 435, 187.