Synthesis and Characterization of Molecularly Imprinted Microspheres

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ABSTRACT: A novel method for the preparation of molecularly imprinted microspheres has been developed. Theophylline, caffeine, and 17β-estradiol are used as template molecules to demonstrate the generally applicable methodology. In the presence of a template molecule, cross-linking polymerization starting from a dilute solution of methacrylic acid and trimethylolpropane trimethacrylate yields polymer microspheres bearing imprinted binding sites. Radioligand binding analysis confirms that very high binding specificity is retained when the molar ratio of functional monomer:cross-linker is controlled between 1:1 and 2.3:1. Nonspecific binding of molecularly imprinted microspheres decreases when an increasing amount of template molecule is used for polymer preparation. Compared with previously reported suspension polymerization and seed polymerization, the present method is highly efficient, is generally applicable to various template molecules, and gives uniform microspheres in good yield.

Introduction

Defined structures in both organic and inorganic materials have been synthesized using template polymerization; e.g., polystyrene latex spheres have been used as a template for the synthesis of highly ordered macroporous inorganic oxides.[1] Similarly, colloidal silica spheres have been used as a template to give a cross-linked polymer with ordered mesopores.[2] These templated materials have organized pore networks, which give them great potential in the areas of catalysis, separation technology, and biomaterials science. In addition to latex and silica spheres, other templates that have been successfully employed include surfactant,[3] inorganic crystal,[4] and bacteria.[5] An interesting approach has been demonstrated using a surfactant bilayer of vesicles as the template, which leads to a hollow, spherical polymer shell.[6] Furthermore, molecular templates have been realized in the creation of polymeric receptors that possess specific binding sites toward the template.[7−9] In molecular imprinting the complex formed between the template molecule and the functional monomer is fixed via polymerization in the presence of an excess amount of cross-linking monomer. After removal of the template, the molecularly imprinted polymer (MIP) can rebind the original template very specifically. Given their high binding affinity and specificity, MIPs have been successfully used for chiral separations of pharmaceutical compounds,[10] for sample preparation using solid-phase extraction,[11] for drug determination using competitive ligand binding assay,[12] and as recognition elements in the construction of biomimetic sensors.[13] Analogous to the production of catalytic antibodies, imprinted polymers have been synthesized against transition-state analogues (TSAs). In many cases, enzyme-like catalysis has been obtained with these polymeric catalysts.[14,15] In addition, MIPs have been used for screening chemical libraries,[16] for in-situ product removal in the biotransformation process,[17] and for downstream product purification following chemical synthesis.[18]

In their most common format, MIPs are prepared in the form of a macroporous monolith that is then ground and sieved to the desired particle size. The grinding and sieving process is time-consuming and yields only moderate amounts of "useful" imprinted polymers. The obtained polymer particles are also irregularly shaped, which is not ideal for chromatographic purposes, and the grinding process may also be detrimental to some of the binding sites. Suspension polymerization, emulsion polymerization, and seed polymerization have been introduced to address some of these issues. However, these methods are neither straightforward nor generally applicable in that they require use of special dispersing phases,[19] functional surfactants,[20] and multistep swelling operations in interfering media.[21] A more general method that can be applied in various imprinting systems (noncovalent, covalent, and semicovalent) is highly desired. If we consider noncovalent imprinting as an example, a molecular imprinting process involving only the indispensable elements such as the template molecule, functional monomer, cross-linking monomer, and initiator ideally will not be affected by addition of substances (e.g., surfactants) that are used in dispersion and suspension polymerization.

The formation of a macroporous structure of imprinted polymers results from phase separation during cross-linking polymerization. Various pores are formed when microgels are fused to give the interlinked polymer phase with the pores occupied by the solvent volume. High-magnification scanning electron microscopy (SEM) showed that, in many cases, the globular microgels are densely fused with each other in the conventionally imprinted polymers that display high mechanical strength. Discrete microgels can be produced starting from dilute monomer solutions with the formation of narrow-disperse microspheres.[22,23]

In this paper we study the preparation of affinity microspheres using molecular imprinting in cross-linking polymerization starting from a dilute monomer solution[24]—a novel, efficient, and generally applicable methodology. The effect of functional monomer:cross-linker ratio and template feed are investigated. The

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Table 1. Preparation of Molecularly Imprinted Microspheres

polymer[a]	template	MAA (mmol)	EDMA (mmol)	TRIM (mmol)	condition
MIP 2	17β-estradiol, 0.734 mmol	0.734	3.713		UV 366 nm[b]
CP 2		0.734	3.713		UV 366 nm[b]
MIP 3	17β-estradiol, 0.734 mmol	3.454		1.485	UV 350 nm
CP 3		3.454		1.485	UV 350 nm
MIP 4	theophylline, 0.255 mmol	0.911	3.640		60 °C
CP 4		0.911	3.640		60 °C
MIP 5	theophylline, 0.255 mmol	2.813	2.813		60 °C
CP 5		2.813	2.813		60 °C
MIP 6	theophylline, 0.255 mmol	1.884		1.884	UV 350 nm
CP 6		1.884		1.884	UV 350 nm
MIP 7	theophylline, 0.255 mmol	2.567		1.711	UV 350 nm
CP 7		2.567		1.711	UV 350 nm
MIP 8	theophylline, 0.255 mmol	3.134		1.567	UV 350 nm
CP 8		3.134		1.567	UV 350 nm
MIP 9	theophylline, 0.511 mmol	3.454		1.485	60 °C
CP 9		3.454		1.485	60 °C
MIP 10	theophylline, 0.0511 mmol	3.454		1.485	60 °C
CP 10		3.454		1.485	60 °C
MIP 11	caffeine, 0.511 mmol	3.454		1.485	60 °C
CP 11		3.454		1.485	60 °C
MIP 12	caffeine, 3.708 mmol	3.454		1.485	UV 350 nm
CP 12		3.454		1.485	UV 350 nm

[a] Various microspheric polymers (polymers 2−12) were synthesized. MIP = molecularly imprinted polymer; CP = control polymer. [b] For the preparation of polymer MIP 2 and CP 2, 0.122 mmol of AIBN was used. A laboratory UV lamp at 366 nm (CAMAG, Bubendorf, CH) was employed.

imprinting effect of the microspheres is compared to that of the traditionally prepared imprinted polymers. The narrow-disperse, molecularly imprinted microspheres are highly desirable in many applications such as competitive ligand binding assays, solid-phase microextraction (SPME), sensor development using deposited microspheres, and capillary electrochromatography. Furthermore, imprinting on microgels provides a chance to investigate the distribution of specific binding sites that are created by molecular imprinting. In other words, we want to know whether intact "cavities" are still present on discrete polymer particles down to the submicrometer size. We conclude that specific binding sites are distributed on the microgel particles in the conventional, imprinted polymer monolith and that these binding sites are kept intact when the fused microgels are dissected by large excess solvent.

Experimental Section

Materials. Theophylline, caffeine, theobromine, xanthine, 17α-estradiol, 17β-estradiol, 17α-ethynylestradiol, and estrone were from Sigma (St. Louis, MO) (purity >98%). The radiochemical, [8-^{3}H]theophylline (specific activity 18.5 Ci mmol^{-1}), was purchased from Sigma (St. Louis, MO). [8-^{14}C]Caffeine (specific activity 53.3 mCi mmol^{-1}) and [2,4,6,7-^{3}H(N)]estradiol (specific activity 72.0 Ci mmol^{-1}) were from NEN Life Science Products, Inc. (Boston, MA). The scintillation liquid, Ecoscint O, was from National Diagnostics (Manville, NJ). Methacrylic acid (MAA, >99%), ethylene glycol dimethacrylate (EDMA, >98%), trimethylolpropane trimethacrylate (TRIM, >90%), and azobis(isobutyronitrile) (AIBN, >98%) were from Merck (Darmstadt, Germany) and used as supplied. Anhydrous solvents used for polymer syntheses were from Lab-Scan (Stillorgan, Co. Dublin, Ireland). Other solvents were from commercial sources and of HPLC grade.

Preparation of Molecularly Imprinted, Ground Particles against 17β-Estradiol. To prepare the traditional, molecularly imprinted polymer particles with irregular shapes (MIP 1), we used 17β-estradiol as the template following the previously described approach.[16] Briefly, the template molecule, 17β-estradiol (2 mmol), was dissolved in anhydrous acetonitrile (18 mL). MAA (12 mmol), EDMA (60 mmol), and AIBN (0.761 mmol) were successively added. The solution was saturated with dry nitrogen for 5 min and polymerization thermally induced at 60 °C for 16 h. The polymer block was ground with a mechanical mortar (Retsch, Haan, Germany) and suspended in water/ethanol, and the slurry was forced through a 25 μm test sieve (Retsch). Fine particles were removed by repeated sedimentation in acetone. Polymer particles ranging from 10 to 25 μm were collected and dried under vacuum (yield: approximately 40%). The template was extracted by extensive washing with methanol:acetic acid (9:1, v/v), followed at 280 nm with a spectrophotometer until no more template molecule could be detected. A control polymer (CP 1) was prepared in the same way except that the template molecule was omitted during the whole process.

Preparation of Molecularly Imprinted Microspheres. Molecularly imprinted microspheres were prepared using precipitation polymerization under the conditions described in Table 1. The template molecule was dissolved in 40 mL of anhydrous acetonitrile in a borosilicate glass tube equipped with a screw cap. The functional monomer (MAA), the crosslinker (EDMA or TRIM), and 0.0852 mmol of the initiator (AIBN) were then added. The solution was saturated with dry nitrogen for 5 min and the tube sealed under nitrogen. Polymerization was induced either by placing the tube in a 60 °C water bath or by UV irradiation (350 nm) at 20 °C using an RMA-400 Raynet photochemical reactor from Southern New England Ultraviolet Co. (Bradford, CT). In both cases the polymerization was continued for 16 h. The microspheres obtained were collected by centrifugation at 10 000 rpm for 10 min using an RC5C superspeed refrigerated centrifuge from Beckman (Palo Alto, CA). The template molecule was extracted by washing repeatedly with 15 mL of methanol containing 10% acetic acid (v/v) for 3 × 1 h, followed by a final wash in the same volume of acetone. The microspheres were finally dried in vacuo (yield: 85%). As a control, the nonimprinted microspheres were prepared and treated in exactly the same way, except that the template molecule was omitted from the polymerization stage.

Chromatographic Analysis. The irregularly shaped polymer particles (MIP 1 and CP 1) were suspended in chloroform and packed into a 200 × 4.6 mm stainless steel HPLC column with an air-driven fluid pump (Haskel Engineering Supply Co., Burbank, CA) at 300 bar using acetone as the packing solvent. Chromatographic analyses were performed using a Pharmacia LKB type 2249 solvent delivery system and a variable wavelength monitor model 2141 (Pharmacia LKB Biotechnology), together with a software package EZChrom (Scientific Software, CA). Acetonitrile containing 1% acetic acid (v/v) was used

as mobile phase at a flow rate of 0.5 mL/min, and the analytes were monitored at 280 nm. An acetone solution in the mobile phase (1%, v/v) was used as the void marker to calculate the capacitor factor (k') and the separation factor (α) using basic chromatography theory.

Radioligand Binding Analysis. The binding capacity of the imprinted polymers was estimated from saturation studies. Varying amounts of imprinted and control polymers were incubated in polypropylene microcentrifuge tubes overnight and at room temperature with 1.62 pmol (1110 Bq) of [8-^{3}H]-theophylline, 417 fmol (1110 Bq) of [2,4,6,7-^{3}H(N)]estradiol, or 375.2 pmol (750.4 Bq) of [8-^{14}C]Caffeine in 1 mL of acetonitrile. A rocking table ensured gentle mixing. The polymer particles were then separated by centrifugation at 14 000 rpm for 5 min, 500 μL of supernatant was mixed with 10 mL of scintillation liquid, and the radioactivity was then measured using a model 2119 Rackbeta β-radiation counter from LKB Wallac (Sollentuna, Sweden).

For competitive analysis, fixed amounts of polymer particles were suspended in acetonitrile by sonication to form the polymer stock suspensions, 200 μL of which was transferred into each microcentrifuge tube. Varying amounts of non-radiolabeled ligand and the same amount of radioligand as used in the saturation studies were added, and the final volume was adjusted to 1 mL with acetonitrile. The competition for binding was allowed to proceed overnight by incubation at ambient temperature, using a rocking table for gentle mixing. The amount of bound radioligand was estimated by measuring the radioactivity from 500 μL of supernatant following centrifugation at 14 000 rpm for 5 min.

Results and Discussion

Molecular Recognition by Imprinted Ground Porous Particles and Microspheres. In the conventional imprinted polymer particles, pores are formed after removal of the porogenic solvent that previously occupied the pore volume during the cross-linking polymerization and phase separation processes. Typically, the volume concentration of monomer is approximately 40−60%. The obtained polymer block is composed of densely fused, interlinked microgels that display high mechanical strength and therefore able to withstand high pressure and chemical treatment without loss of binding specificity.

To verify the imprinting effect, imprinted polymer MIP 1, in which 17β-estardiol was used as the template and a control polymer, CP 1, were used as stationary phases in chromatographic analyses. Compounds that had similar structures to the template molecule were loaded onto the column, and the separations of these different compounds were compared. Figure 1 shows the separation of three compounds, estrone, 17α-estradiol, and 17β-estradiol, using both the imprinted and the control polymer. On the control polymer (CP 1), estrone was less retained and readily separated from the other two compounds; however, there was no separation between 17α- and 17β-estradiol. When the imprinted polymer (MIP 1) was used, complete separation between 17α- and 17β-estradiol was realized, since the imprinted polymer displayed the strongest binding affinity to the original template molecule, 17β-estradiol. In addition to the present demonstration, we have shown that the elution profile in a chromatographic separation of steroids can be tailored to ensure the longest retention for a target compound. This was achieved by simply using the specific compound to be retained as the template for the preparation of the imprinted stationary phase.[25]

Besides chromatographic analysis, we used radiolabeled ligand to characterize the binding efficiency of the

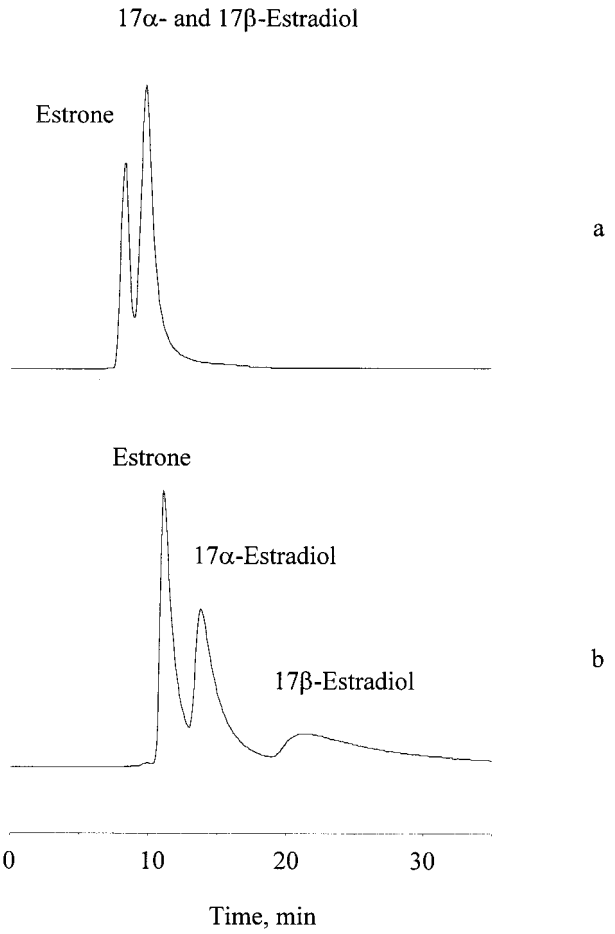

Figure 1. Chromatographic separation of estrone, 17α-estradiol, and 17β-estradiol (20 nmol for each) using (a) a control polymer, CP 1, and (b) an imprinted polymer, MIP 1, as the stationary phase. Acetonitrile containing 1% acetic acid was used as the mobile phase at a flow rate of 0.5 mL/min. Analytes were monitored at 280 nm.

template, 17β-estradiol, on the imprinted polymer (MIP 1) and the control (CP 1). Saturation studies showed that in acetonitrile the imprinted polymer bound up to approximately 30% of the added 17β-estradiol, whereas the control polymer bound less than 13% (Figure 2). Since the template molecule is highly hydrophobic, binding affinity of the polymers in organic solvent should be low. However, the difference in binding efficiency between the imprinted and the control polymers was quite obvious. In a homologous, competitive binding experiment, radiolabeled ligand was displaced from MIP 1 by unlabeled 17β-estradiol. In contrast, when the control polymer was used as the binder, there was no effective competition between the labeled and the unlabeled 17β-estradiol. These results suggest that competition for binding can take place in the specific binding sites.

The imprinted polymer obtained by grinding the polymer monolith was irregular in shape and size (Figure 3a). It was comprised of densely fused microgels that were approximately globular in shape (Figure 3b). This feature can be found in various macroporous, cross-linked polymers, prepared either from homogeneous monomer solution or by suspension polymerization in the presence of a porogenic solvent. When a cross-linking polymerization is started from a dilute, homogeneous monomer solution, spherical microgels with narrow size distribution can readily be formed. In this

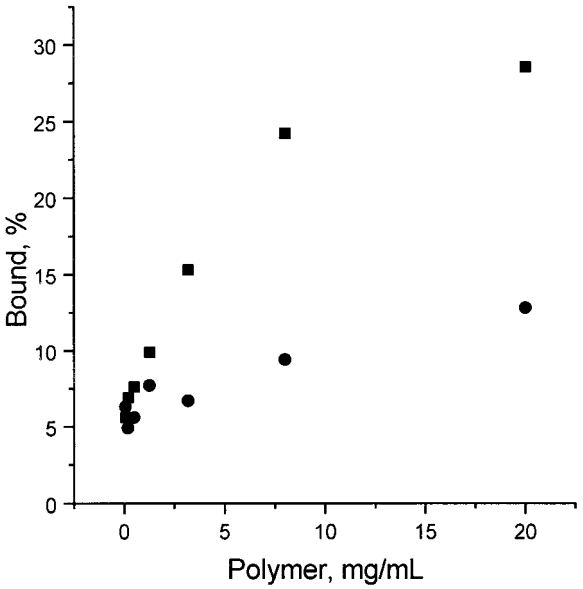

Figure 2. Binding isotherm for radiolabeled 17β-estradiol on MIP 1 (■) and CP 1 (●).

paper we investigated whether we would obtain imprinted binding sites on these micro-/nanospheres and the influence of reaction parameters such as the cross-linking density and template molecule feed.

Since we were convinced that we had a positive imprinting against 17β-estradiol via the conventional method, we initiated preparation of microspheres imprinted against the same template in a similar polymer system. In a preliminary experiment, common cross-linking monomers such as EDMA, TRIM, and divinylbenzene (DVB) could be combined with various functional monomers (MAA and vinylpyridine (Vpy)) to give satisfactory microspheres, when the polymerization was performed in commonly used imprinting solvents (acetonitrile, toluene, and dichloromethane). For comparison to the ground particles, we have focused on imprinting in acetonitrile. MIP 2 (microspheres) was prepared using the same molar ratio between MAA and EDMA as that used for the preparation of MIP 1 (monolith), except that the polymerization started with a much more dilute monomer solution (approximately 2 vol % total monomer loading relative to the reaction solvent). In acetonitrile, while 20 mg of the imprinted microspheres (MIP 2) bound 14% of the radiolabeled 17β-estradiol, an equivalent amount of the control (CP 2) bound less than 3%. Because of the presence of excess solvent that interferes with molecular interactions between the functional monomer and the template, imprinting is less efficient and ligand binding for the imprinted microspheres lower than that of the conventional monolith. However, the difference between the imprinted and the control microspheres is quite obvious. Both MIP 1 and MIP 2 bind specifically 17β-estradiol via hydrogen-bond interactions that are formed between the carboxyl groups of the polymer and the hydroxyl groups of the template. An attractive feature of the microspheres is that even the most concentrated stock polymer suspension is easy to prepare and dispense using a pipet. This is due to the microspheres with such a small size (<1 μm) being relatively free from sedimentation during the process of sample preparation. A fast separation of the microspheres can, however, be readily obtained by centrifugation.

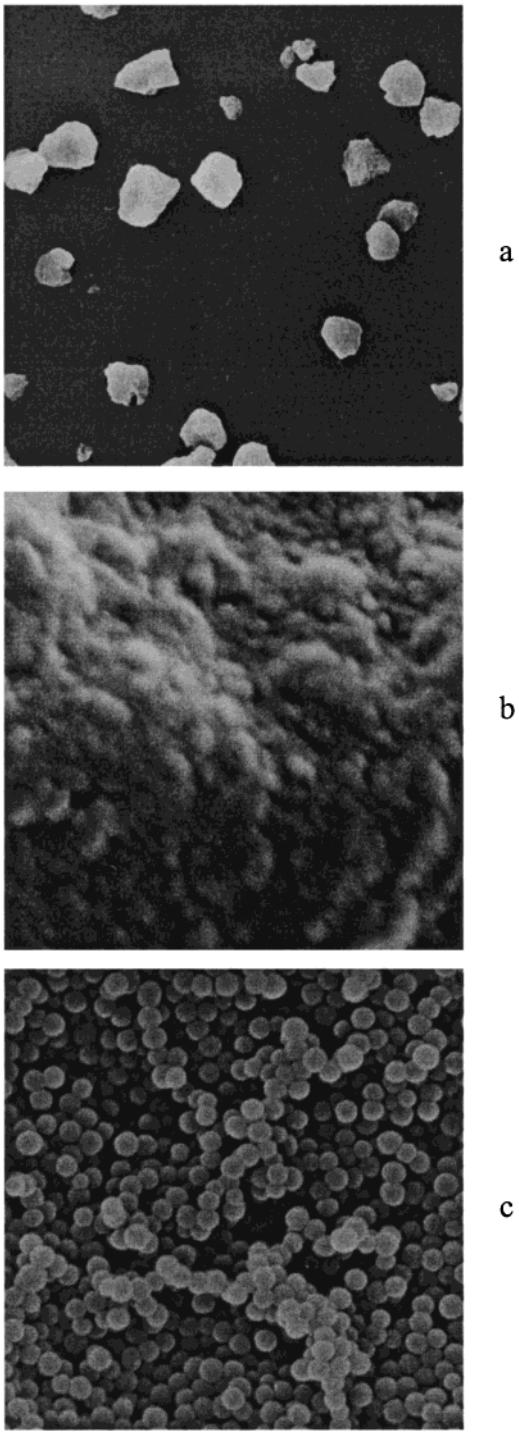

Figure 3. SEM of (a) irregularly shaped MIP 1 with 525× magnification, (b) MIP 1 with 30 000× magnification, and (c) MIP 3 with 15 000× magnification.

Since the cross-linking monomer, EDMA, was the main component for MIP 2, which hardly contributed binding affinity for the template, we tried to use as little of the inert cross-linker as possible—just enough to ensure cross-linking density for the specific binding on the imprinted microspheres. TRIM, with intrinsically higher cross-linking efficiency and similar chemical structure, was therefore tested. When a molar ratio between MAA and TRIM of 1:1 was used, highly specific imprinted ground particles were reportedly obtained.[26] We used a molar ratio between MAA and TRIM of 2.3:1 in our study, and narrow disperse, spherical polymer beads in the submicrometer range were obtained (Fig-

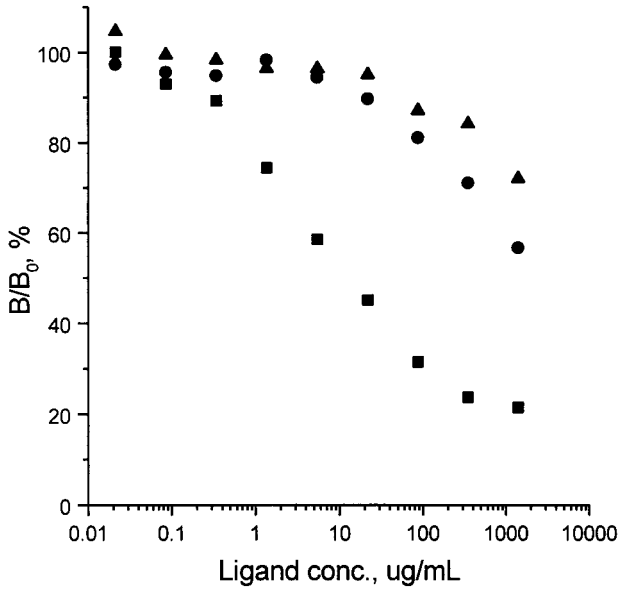

Figure 4. Inhibition of binding radioactive 17β-estradiol on 30 mg of MIP 3 by 17β-estradiol (■), 17α-estradiol (●), and 17α-ethynylestradiol (▲). B/B_0 is the ratio of the amounts of radioligand bound in the presence and absence of displacing ligand.

ure 3c). Binding of 17β-estradiol by the imprinted poly(MAA-*co*-TRIM) microspheres (MIP 3) in acetonitrile was much higher than by the poly(MAA-*co*-EDMA) microspheres (MIP 2). For the poly(MAA-*co*-TRIM) microspheres, while 30 mg of the imprinted (MIP 3) bound approximately half of the labeled 17β-estradiol, binding of the same ligand by the control (CP 3) was 3 times less than that of MIP 3.

Binding specificity of the molecularly imprinted microspheres was studied by competitive binding assay. Various template analogues were used as the competing ligand to inhibit radioligand binding by the imprinted MIP 3. Figure 4 shows that all the analogues had difficulty inhibiting the binding of 17β-estradiol. Cross-reactivity of 17α-estradiol and 17α-ethynylestradiol was lower than 1%, which confirmed the presence of highly specific binding sites on the imprinted microspheres.

To investigate the general applicability of making imprinted microspheres against different template molecules, we chose theophylline as an alternative template. This was based on the following considerations: (1) theophylline has quite different hydrophobicity from that of estradiol; (2) theophylline-imprinted monolithic poly(MAA-*co*-EDMA) has been successfully prepared previously.[27] In addition to the poly(MAA-*co*-EDMA) microspheres (MIP 4 and CP 4), for which a molar ratio of MAA/EDMA = 1/4 was used, poly(MAA-*co*-TRIM)-based microspheres (MIP 9 and CP 9) with a molar ratio of MAA/TRIM = 2.3/1 were also prepared. Figure 5 shows the results from the saturation study and competitive radioligand binding experiments. Obviously, highly specific binding sites for theophylline were obtained in both cases, although the imprinted poly(MAA-*co*-TRIM) microspheres (MIP 9) displayed much higher binding capacity due to its higher content of carboxyl groups.

Effect of Cross-Linking Density on Binding Specificity by Imprinted Microspheres. While the functional monomer ultimately becomes the binding group in the imprinted polymer, in many cases binding specificity is augmented by the rigid three-dimensional

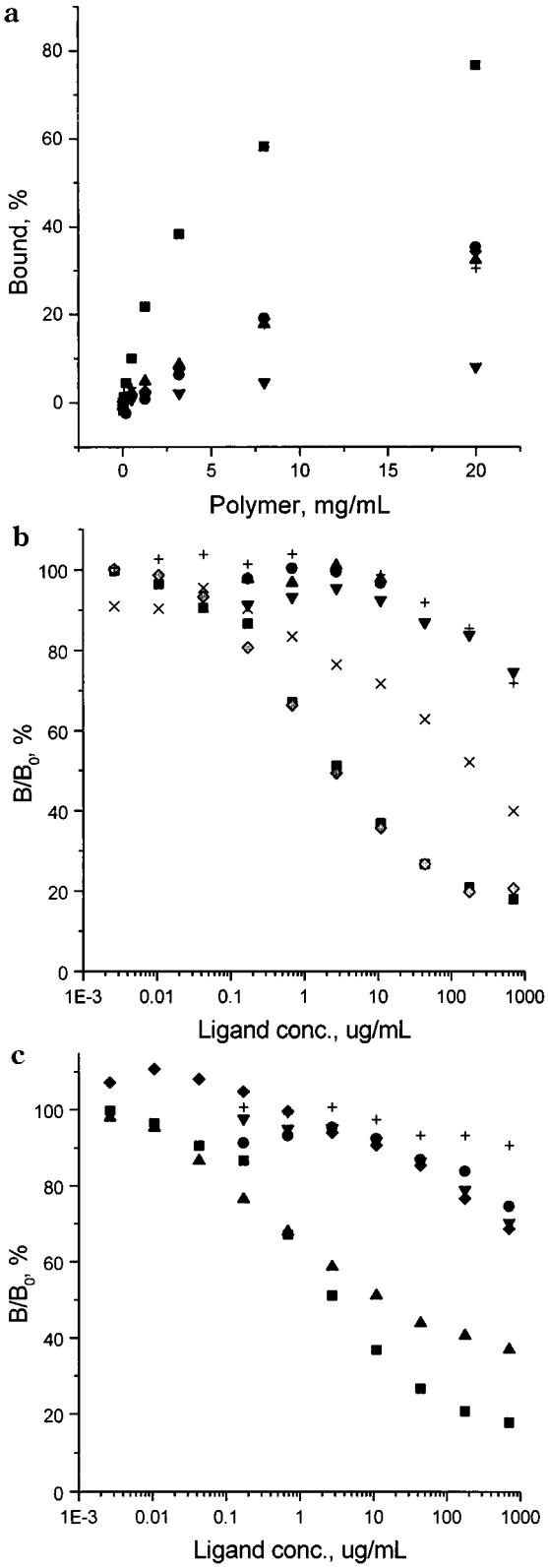

Figure 5. (a) Binding isotherm for radiolabeled theophylline on MIP 4 (▲), CP 4 (▼), MIP 5 (◆), CP 5 (+), MIP 9 (■), and CP 9 (●). (b) Inhibition of binding radioactive theophylline on 20 mg of MIP 4 by theophylline (◆) and caffeine (+), on 20 mg of MIP 5 by theophylline (×), and on 5 mg of MIP 9 by theophylline (■), caffeine (▼), theobromine (●), and xanthine (▲). B/B_0 is the ratio of the amount of radioligand bound in the presence and absence of displacing ligand. (c) Inhibition of binding radioactive theophylline on 5 mg of MIP 9 by theophylline (■) and caffeine (●), on 10 mg of MIP 10 by theophylline (▲) and caffeine (▼), and on 30 mg of CP 9 by theophylline (◆) and caffeine (+).

structure of the polymer. Appropriate levels of cross-linking are important in maintaining binding specificity of an imprinted polymer. When the functional groups are not sufficiently fixed by cross-linking, i.e., the levels of cross-linking are too low, the binding specificity by the imprinted polymer decreases. At the other extreme, when the levels are too high, the loading capacity of the polymers is reduced, and the diffusion of substrates into the imprinted cavities during rebinding may also be hindered.

In general, molecularly imprinted poly(MAA-*co*-TRIM) has a higher load capacity than poly(MAA-*co*-EDMA), since TRIM has one more vinyl group than EDMA and therefore less is required to ensure the same backbone rigidity that contributes to binding specificity. The influence of cross-linking density on the performance of noncovalent, molecularly imprinted ground particles has been reported previously.[28] In the present study we investigate the effect of cross-linking on the binding characteristics of imprinted microspheres. For the poly(MAA-*co*-EDMA) microspheres, when the molar ratio of MAA:EDMA was increased to 1:1 (MIP 5 and CP 5), the polymer chain became too flexible to maintain binding specificity, and the increased nonspecific binding could not be efficiently blocked by the addition of unlabeled theophylline (Figure 5a,b). In contrary, a change in the MAA:TRIM molar ratio from 1:1 (MIP 6), 1.5:1 (MIP 7), to 2:1 (MIP 8) for the imprinted poly(MAA-*co*-TRIM) microspheres only slightly altered the binding efficiency; i.e., 5 mg of respective microspheres could bind approximately 40−60% of radioactive theophylline in acetonitrile. In addition, binding of radioligand by these microspheres was always effectively inhibited in the presence of unlabeled theophylline. As seen previously, when the same molar ratio was increased to 2.3:1, favorable microspheres were still obtained with the desired binding performance.

Effect of Template Molecule Feed on Specific Binding by Imprinted Microspheres. Figure 5c shows the influence of the amount of theophylline used during polymer preparation on binding performance of the obtained microspheres. When the amount of theophylline used for microsphere preparation is reduced, the difference between the inhibition of binding the radiolabeled theophylline by theophylline and caffeine decreases. The B/B_0 value at the bottom plateau of the sigmoid displacement curve reflects an approximation of the extent of nonspecific binding. The figure shows that nonspecific binding increases when a decreasing amount of template molecule is involved in polymer preparation. For the nontemplated microspheres, inhibition of binding radiolabeled theophylline by both theophylline and caffeine was much less pronounced.

Although many applications of theophylline-imprinted polymers have been reported,[29,30] verification of the imprinting effect has not been carefully studied. As we observed, theophylline generally has a higher affinity than caffeine for polyacrylate polymer carrying a free carboxyl group. When caffeine is used as a template molecule, and the same imprinting protocol is followed, we should be able to produce a caffeine-specific polymer. For this purpose we prepared a caffeine-imprinted microspheric polymer (MIP 11) following the same recipe as that used for preparing the theophylline-imprinted polymer (MIP 9). Analyzed in a similar radioligand binding experiment, the uptake of caffeine by the caffeine-imprinted microspheres in acetonitrile was much lower than the binding of theophylline by the theophylline-imprinted microspheres. Simply increasing the template amount and using UV, instead of thermal polymerization, only gave a slight improvement in the binding of caffeine (data not shown). However, despite the low binding efficacy of the caffeine-imprinted microspheres, specific binding on the microspheres was confirmed by comparing the inhibition of binding radioactive caffeine by adding caffeine and theophylline as competitors. Figure 6 shows that on the caffeine-imprinted microspheres (MIP 11) binding of labeled caffeine is inhibited to the highest level by caffeine, whereas on the theophylline-imprinted microspheres (MIP 9), theophylline becomes the most powerful competitor to labeled theophylline. In other words, the most effective inhibition for binding the radiolabeled template by imprinted microspheres can be realized only when the respective template molecule is introduced as the competitor.

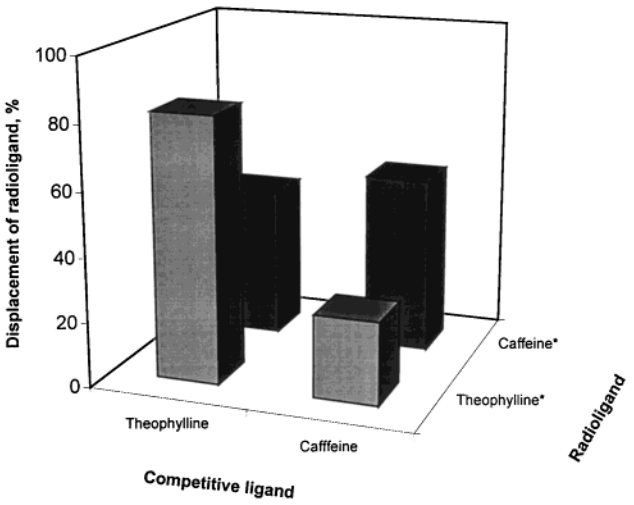

Figure 6. Maximum displacement of radiolabeled theophylline from 5 mg of anti-theophylline MIP 9 and displacement of radiolabeled caffeine from 20 mg of anti-caffeine MIP 11 using theophylline and caffeine (700 μg/mL for each) as the competitor.

Conclusion

The specific binding sites of imprinted materials are present in microgel particles that form during the cross-linking polymerization in the presence of various template molecules. Imprinted microspheres are readily obtained via precipitation polymerization starting from dilute monomer solution in the presence of appropriate amount of template molecules. While binding groups from functional monomer generally enhance total binding, specificity of binding may be sacrificed if effective cross-linking is insufficient. For molecular imprinting on microspheres, using TRIM as a cross-linker at a molar ratio of between 1:1 and 1:2.3 with respect to a functional monomer, generally gives polymer beads with superior binding performances as far as load capacity and binding specificity are concerned.

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