Synthesis and Characteristics of Imprinted 17-β-estradiol Microparticle and Nanoparticle with TFMAA as Functional Monomer

Qiujin Zhu,[1,2] Jian Tang,[1,3] Jun Dai,[3] Xiaohong Gu,[3] Shangwei Chen[3]

[1]*Key Laboratory of Food Science and Safety, Ministry of Education, Southern Yangtze University, Wuxi, Jiangsu, 214036 China*
[2]*School of Life Science, Guizhou University, Guiyang, Guizhou, 550025 China*
[3]*Testing & Analysis Center, Southern Yangtze University, Wuxi, Jiangsu, 214036 China*

Received 2 May 2006; accepted 26 September 2006
DOI 10.1002/app.25564
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Submicron-scaled molecularly imprinted polymer (MIP) particles for 17-β-estradiol were prepared by precipitation polymerization method using 2-(trifluoromethyl) acrylic acid as functional monomer and trimethylolpropane trimethacrylate as cross-linker in acetonitrile. The obtained submicron-scaled MIP particles were subsequently packed into stainless steel columns and the chromatographic characteristics of the MIP stationary phase were investigated. Interestingly, it was found that tandem MIP columns of 17-β-estradiol-based MIP with two different ratios of the template could achieve almost complete separation of isomers (17-α-estradiol and 17-β-estradiol) with a good separation factor (2.45). Morphological characteristics and adsorption kinetics of the submicron-scaled MIP particles, effect of flow-rate, injection amount, and column temperature on separation were also investigated in detail. Results indicated that the submicron-scaled MIP particles had both good selectivity and high affinity to the template molecule 17-β-estradiol. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1551–1558, 2007

Key words: β-estradiol; molecularly imprinted polymers; precipitation polymerization; molecular recognition; hormone; food safety

INTRODUCTION

Steroid hormones have an influence on meat quality, such as the collagen level and solubility.[1,2] Thus, exogenous steroid sex hormones could be illegally applied in promoting animal weight gain. The occurrence of a wide range of steroid hormones has therefore to be monitored. It is a pressing need to monitor both the occupational and environmental exposures sure to develop a rapid, sensitive, and economical analytical method, although there are many methods for the analysis of steroid hormones in pharmaceuticals, biological fluids, and foods. The most commonly used methods include fluorescence spectroscopy,[3,4] mass spectrometry,[5] liquid chromatography,[6] gas chromatography,[7] thin-layer chromatography,[8] and immunoassay,[9] and a combination of these techniques, except for immune technology, involves costly investment, fussy procedure, and use of large amounts of organic solvents. Although immunoassay method has allowed considerable progress in endocrine research, it has considerable limits with respect to specificity and sensitivity, and it is dependent on the availability of antisera.[10]

A molecularly imprinted polymer (MIP) has the potential power of playing the above role according to reports from recent decade researches in this field. Molecular imprinting is a simple method for making polymeric artificial receptors that can be applied to most small molecules.[11] It can be comprehended to be a molding process in which a print molecule is used as a template for the creation of a substrate-selective macromolecular matrix.[12] Synthesis of MIP involves covalent, semicovalent, and noncovalent distinct approaches of molecular imprinting. Application of noncovalent approach is the broadest among the three, because it is much more flexible and facile than the covalent or semicovalent approach.[13] MIPs have been employed in a wide area, such as liquid chromatography, solid-phase extraction, membranes, sensors, artificial antibodies, catalysis, biotransformation process, and diagnostic tools for drug assays,[14,15] and the sensors with MIPs as sensitive material have been capable of developing a rapid, sensitive, and economical analytical instrument.[16–18] Separation of estradiol isomers by molecular imprinting technique might be an interesting topic, and there have been some reports about MIPs of steroid hormones in different studies as a tool to detect biological hormone or in investigation of the

Correspondence to: J. Tang (guxh@sytu.edu.cn).
Contract grant sponsor: Guizhou Province Natural Science Foundation; contract grant number: 20052013.

TABLE I
Composition and Condition of the Synthesized Molecularly Imprinted Polymers for 17-β-estradiol

Polymers	17-β-estradiol (mmol)	TFMAA (mmol)	MAA (mmol)	MMA (mmol)	TRIM (mmol)	Porogen (15 mL)	Condition
MIP1	0.125	1			1	MeCN	60°C, 190 rpm, 24 h
CP1		1			1	MeCN	60°C, 190 rpm, 24 h
MIP2	0.125	1			1	MeCN	UV 365 nm, 3 h, 60°C, 190 rpm, 24 h
CP2		1			1	MeCN	UV 365 nm, 3 h, 60°C, 190 rpm, 24 h
MIP3	0.125	1			1	MeCN	UV 365 nm, 48 h
CP3		1			1	MeCN	UV 365 nm, 48 h
MIP4	0.25	1			1	MeCN	UV 365 nm, 48 h
MIP5	0.125		1		1	$CHCl_3$	UV 365 nm, 2 h, 60°C, 24 h
CP5			1		1	$CHCl_3$	UV 365 nm, 2 h, 60°C, 24 h
MIP6	0.125			1	1	MeCN	UV 365 nm, 48 h
CP6				1	1	MeCN	UV 365 nm, 48 h

roles of these functional groups in the recognition process.[19] The polymerization methods published on 17-β-estradiol MIPs involve bulk polymerization,[20] two-step swelling polymerization[21] methods, etc. Precipitation polymerization has been applied for the preparation of MIPs in a variety of different analytes and applications.[20,22,23] Since no interfering components as surfactants or stabilizers are involved in the precipitation method, the obtained MIPs are easy to handle.

It may be a key problem for MIP application on how to prepare a high selectivity polymer containing recognition site. This can be achieved by carrying out polymerization in the presence of a desired target molecule (template), crosslinker, and functional monomer(s), which are capable of forming noncovalent complexes or covalent bonds with the template.[24] A functional monomer with double functional groups undertakes an important role for forming a recognition site, and the structure ensures formation of hydrogen bonding, electrostatic or hydrophobic interaction with the template. On the other hand, the functional monomer could be incorporated into the polymer matrix in a specific orientation.

In this work, precipitation polymerization was employed to prepare molecularly imprinted microparticles and nanoparticles as the HPLC stationary phase for separation of the 17-β-estradiol and other position isomers or/and homologues utilizing noncovalent interaction. In a special way, the structural differences between the isomers 17-α-estradiol and 17-β-estradiol which are only slightly in position of —OH and —H at carbon atom number 17(C-17) were reviewed.[25] The combination of 2-(trifluoromethyl) acrylic acid (TFMAA) and trimethylolpropane trimethacrylate (TRIM) were selected as functional monomer and crosslinker, respectively. The ratio of 17-β-estradiol versus functional monomer and induct type were studied, and the recognition properties of MIP were investigated under different flow rate, injection amount, temperature, and acetonitrile eluent containing different water. In addition, the adsorption kinetic parameters were determined by static adsorption test.

METHODS

Materials

17-α-estradiol, 17-β-estradiol, 4-androstene-3, 17-dione, and progesterone were purchased from Sigma (St. Louis, MO) (purity > 98%) 2-(trifluoromethyl)acrylic acid (TFMAA) from Aldrich (purity > 98%), TRIM (>90%) from (Sigma-Aldrich, Germany), and azobis-(isobutyronitrile) (>98%) were from Sihe (Shanghai, China), and were used as supplied. α-Methacrylic acid and anhydrous solvents used for polymer syntheses were from Guoyao Group (Reagent, Shanghai, China). Methacrylic acid methyl ester (MMA, chem. grade) was made in Wulian factory (Shanghai, China). HPLC grade acetonitrile and methanol were from Hanbang Group (Huaian, Jiangsu, China). All other chemicals used were of analytical grade.

Preparation of MIPs

MIPs were prepared using precipitation polymerization under the conditions described in Table I. The template molecule was dissolved in 15 mL of anhydrous acetonitrile in a 100-mL borosilicate glass bottle equipped with a rubber cap. The functional monomer TFMAA 1 mmol, the crosslinker TRIM 1 mmol, and 0.0852 mmol the initiator azobis- (isobutyronitrile) were then added. The solution was saturated with dry nitrogen for 5 min and the bottle was sealed under nitrogen. Polymerization was induced either by placing the bottle in a 60°C water bath at 190 rpm or by UV irradiation (365 nm) at 0–8°C using a WD-9403E Ultraviolet analysis equipment from Beijing Liuyi Equipment (Beijing, China). In both cases, the polymerization was conducted over 24 h. The template molecule was extracted by wash-

ing 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 submicron and nanoparticles were collected by removing fine particles after deposition in acetone. The submicron-scaled MIP particles were finally dried in a vacuum. As a control polymer (CP), i.e., the nonimprinted submicron-scaled MIP particle was prepared and treated in exactly the same way, except that the template molecule was omitted at the polymerization stage. The preparation strategies of MIPs and CPs are listed in Table I.

Chromatographic analysis

The dried polymers were packed into a $4.6_{i.d}$ × 100 mm stainless steel HPLC column by manual method repeatedly, and acetone as packing solvent was pumped into the packed column by a pump (Waters 510 model) at flow rate 1 mL/min. Chromatographic analysis was performed on an HPLC system equipped with a Waters model 510 pump and a Waters 490E programmable multiwavelenghth detector, together with a software package ANASTAR® chromatography data system. 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 281 nm for the isomer or 235 nm for other sterol. An acetone solution in the mobile phase (1%, v/v) was used as the void marker to calculate the rentention factor (κ′) and the separation factor (α) using basic chromatography theory.

Characteristics of surface morphology for TFMAA-*co*-TRIM imprinted polymers

The surface morphology analyses were characterized with scanning electron microscopy (SEM, Quanta-200, FEI Company, Holand) at an acceleration voltage of 10 kV, and atomic force microscope (AFM) images were obtained with a Benyuan CSPM4000 scanning probe microscope (Benyuan, China).

Adsorption kinetics studies

Adsorption kinetics was investigated by a saturated adsorption method. Ten milligram MIPs weighted was loaded for each in 6 × 5 mL polypropylene microcentrifuge tubes, and 1 mL acetonitrile solution respectively, containing 1 m*M* β-estradiol added to each tube. The samples were kept at room temperature and stirred intermittently. The mixture was filtered by a micromembrane (Ø0.3 μm) filter linked with a 5-mL syringe, and a triplicate injection was applied on the HPLC system. HPLC conditions were as follows: column, Agilent Hypersil ODS ($4.0_{i.d}$ × 125 mm); mobile phase, acetonitrile (HPLC grade); detection, 281 nm; flow-rate, 1 mL/min; inject amount, 10 μL; column temperature, 25°C.

RESULTS AND DISCUSSION

Stationary phase chromatography of TFMAA-*co*-TRIM imprinted polymers

In most cases, submicron-scale and nanoscale particles would lead to a low throughput and high back pressure. Thus, 17-β-estradiol molecularly imprinted nanoparticles prepared by precipitation polymerization were seldom characterized by HPLC system.[19–21,26–28] In this work, it was difficult to overcome low column throughput and high pressure while polymers were synthesized with MAA and MMA as functional monomer instead of TFMAA. Also, the swollen degree of the MIPs with MAA as monomer was significantly changed when the powder was transited into wettish phase, and SEM image of MAA-MIPs was gel and nonparticle morphology polymer.

TFMAA was a more appropriate monomer for the preparation of MIP for basic nicotine,[29] *d*-chlorpheniramine,[30] and isoproturon[31] than MAA, owing to the electron-withdrawing effect of TFMAA, which was able to interact more strongly through hydrogen bonding, and cause the effect of TFMAA-*co*-DVB (divinylbenzene) polymers being able to trap and retain quantitatively in a wider concentration range all the tested analytes.[31] The results in Table II confirmed that the more acidic TFMAA as functional monomer had an advantage over MAA/MMA imprinting neutral 17-β-estradiol molecules. Moreover, TFMAA-*co*-TRIM imprinted polymers had a more steady size than MAA-*co*-TRIM polymers as stationary phase for the lower dilatability. Thus, the mobile phase could flow through the column loaded with TFMAA-*co*-TRIM imprinted polymers at higher flow rates and at a lower pressure than the column loaded with MAA-*co*-TRIM polymers. On comparing with MAA-*co*-TRIM polymers, TFMAA-*co*-TRIM imprinted polymers had higher capacities, similar to the study by Tamayo.[31] But Lai had different conclusion that the affinity was in the order 4-VP-*co*-DVB > 4-VP-*co*-EDMA > MAA-*co*-DVB >TFMAA-*co*-DVB according to the rebinding test for benzo[*a*]pyrene.[32] Therefore, with the present case, it can easily be explained that the TFMAA-*co*-TRIM is more important than the other monomer in the overall affinity of polymers to the template molecule, and the cooperation displays stronger affinity for 17-β-estradiol than those prepared with other combinations of monomer and crosslinker. Therefore, it is necessary to explain the influence and do further research, but surface morphology and inner cavity and the ratio of submicron-particles versus nanoparticles in the

TABLE II
Chromatographic Data Obtained from HPLC Analysis of Imprinted Nanoparticles Polymers (Column: $4.6_{i.d} \times 100$ mm)[a] in Comparison with Imprinted Microspheres Prepared by One-Step Precipitation Polymerization Routes Described in Literature

Data source	Analytes	k_{cp}[b]	k_{mip}[b]	α[c]	IF[d]	S[e]
Imprinted microparticles and nanoparticles MIP4/CP3	17-β-estradiol (template)	0.707	2.127	1.000	3.009	1.000
TFMAA-co-TRIM polymers	17-α-estradiol	0.707	0.933	2.279	1.320	2.280
	4-androstene-3,17-dione	0.249	0.310	6.857	1.245	2.416
	Progesterone	0.422	0.410	5.186	0.973	3.094
Imprinted microgels MIP5/CP5	17β-estradiol (template)	1.432	1.983	1.000	1.385	1.000
MAA-*co*-TRIM polymers	4-androstene-3,17-dione	0.682	0.801	2.476	1.175	1.179
Imprinted microparticles MIP6/CP6	17β-estradiol (template)	1.279	1.801	1.000	1.408	1.000
MMA-*co*-TRIM polymers	17α-estradiol	–	1.571	1.146	–	–
	4-androstene-3,17-dione	0.437	0.563	3.198	1.288	1.093
	Progesterone	–	0.736	2.445	–	–
Wei et al.[23 f]	17-β-estradiol (template)	2.37	3.01	1.00	1.27	1.00
MAA-*co*-DVB polymers	17-α-estradiol	2.14	2.08	1.45	0.97	1.31

[a] The ratio of template vs functional monomer was 1 : 4, acetonitrile containing 1% acetic acid was used as the mobile phase, and flow rate is 0.5 mL/min. Analytes were monitored at 281 nm/235 nm and room temperature.
[b] The retention factor κ′ was calculated as $(t - t_0)/t_0$, where t is the retention time of the estrogens, and t_0 the retention time corresponding to the column void volume.
[c] The separation factor $\alpha = \kappa'$ (17-β-estradiol)/κ' (compound).
[d] The imprinting factor is defined by $I = \kappa'\text{imp}/\kappa'\text{cp}$ where $\kappa'\text{imp} = \kappa'\text{cp}$ on the imprinted and nonimprinted polymers.
[e] The selectivity factor is the ratio of the imprinting factors S = I(17-β-estradiol)/I(compound).
[f] HPLC conditions: acetonitrile as mobile phase; flow rate: 1 mL/min; detection at 280 nm; column: $4.6_{i.d} \times 150$ mm.

column should be extremely correlative with maintaining desired chromatographic performance.

Molecular recognition of TFMAA-*co*-TRIM imprinted polymers

The chromatographic characterization of molecularly imprinted nanoparticles prepared by using precipitation polymerization resulted in a good separation between 17-α-estradiol and 17-β-estradiol. Figure 1 shows the chromatogram (a) and (b) for separation of the isomers, which were detected using $4.6_{i.d} \times 100$ mm stainless columns loaded with MIP3 and MIP4, respectively. The chromatogram (c) was obtained from tandem linking of column (a) and (b). The baseline in chromatogram (a) is fluctuant owing to the low S/N ratio of waters 490E programmable multiwavelenghth detector. Ten microliter mixture sample was injected, which was acetonitrile containing 0.5 m*M* 17-α-estradiol and 17-β-estradiol in (a)

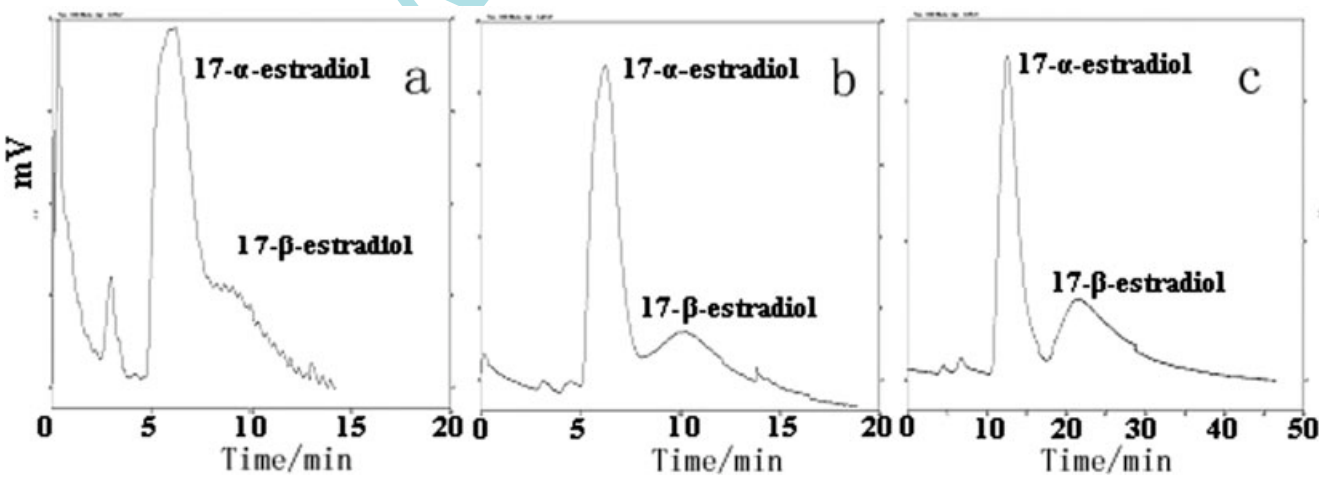

Figure 1 Chromatograms of the isomers (17-α-estradiol and 17-β-estradiol) separation using $4.6_{i.d} \times 100$ mm stainless column loaded with (a) MIP3, (b) MIP4, (c) tandem column with (a) and (b). HPLC conditions: mobile phase, acetonitrile containing 1% HAc; flow rate, 0.5 mL/min; detector, 281 nm; operation temperature, 12–14°C (room temperature); injection amount, each 10 μL acetonitrile solution containing 0.5 m*M* isomers in (a) and (b), (c) 20 μL acetonitrile solution of 0.25 m*M* 17-α-estradiol and 0.5 m*M* 17-β-estradiol.

and (b), and the injected amount of C was 20 μL acetonitrile solution of 0.25 m*M* 17-α-estradiol and 0.5 mmol/L17-β-estradiol. Acetonitrile containing 1% acetic acid was used as the mobile phase. Flow rate was kept at 0.5 mL/min. Analytes were monitored at 281 nm. (a) low column pressure (300PSI) was kept during the stage of separation. The retention times for 17-α-estradiol in chromatogram (a), (b), and (c) were 6.01, 6.28, and 12.57 min, respectively, and that for 17-β-estradiol were, respectively, 8.32, 10.16, and 21.71 min, and the void time detected with acetone was 3.25, 3.25, and 6.28 min, respectively. Thus, the separation factors (α) for (a) (MIP3) and (b) (MIP4) were 1.84 and 2.28, respectively, and that for tandem column (c) was 2.45. Obviously, the tandem column (c) perfected the separation of the isomers. The result implicated that the separation effect of 17-α-estradiol and 17-β-estradiol could be improved on adopting the higher ratio (1:4) of TFMAA-*co*-TRIM for formation of high quality specificity binding sites. In addition, there are two reasons for explaining the high separation factors in the columns loaded with MAA-*co*-TRIM polymers but incomplete baseline separation. On the one hand, peak width of 17-α-estradiol broaden in Figure 1 because of the slight discrimination between 17-α-estradiol and 17-β-estradiol, which cause the isomers binding sites of the TFMAA-*co*-TRIM imprinted polymers were highly similarity in cavity, a certain extent, the MIP3 and MIP4 have nonspecific recognition for 17-α-estradiol because of the similarity tridimensional cavities; on the other hand, steroid hormone separation properties were affected by polarity and compositions of mobile phase, low dissolution for 17-α-estradiol caused the peak stretching.

The data on HPLC characterization are shown in Table II to illuminate the effect of specific recognition of MIP4. The imprinted factor was calculated according to the formula in Table II. At the same time, the relative values were also presented. For MIP4 and CP3 on a $4.6_{i.d} \times 100$ mm column, the lipophilic isomer had a fairly high IF value (3.01) compared with studies carried out,[19,20,25,27] and MIP4 had more outstanding specific recognition for 17-β-estradiol than MIP3 and other polymers. Many steroids as like estradiol belong to lipophilic molecule with few functional groups. It remains a challenge for many steroids MIPs on how to promote capability of specific recognition increase. Hydrogen bonding between 17-β-estradiol and TFMAA in low polar and nonproton acetonitrile solvents is a means of ensuring the strength and fidelity of the binding. More so, the polymerization temperature controlled below 8°C and UV irradiation (365 nm) are predominant for improvement of specific recognition through further tests.

The binding characteristics of MIP4 were evaluated in Figure 2 by determining the retention time

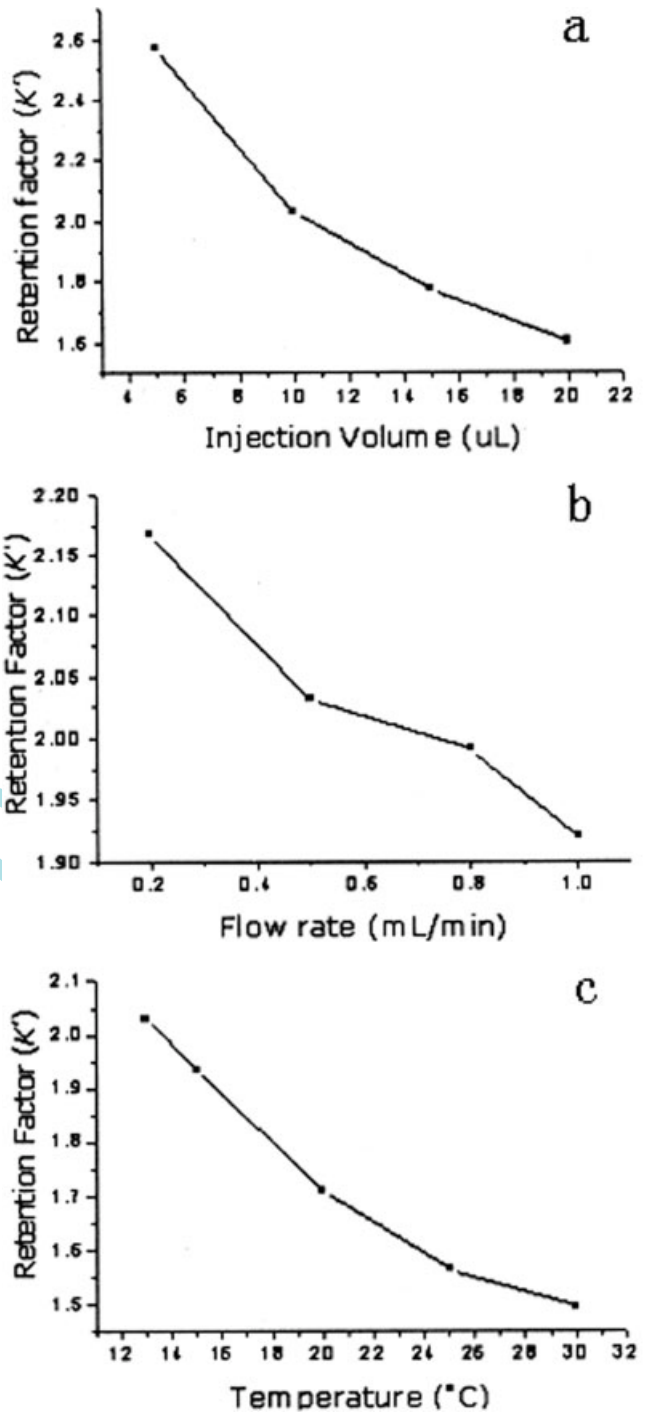

Figure 2 Effects of sample injection volume (a), flow rate of mobile phase (b), and column temperature (c) on the retention factors of 17-β-estradiol on the column loaded with MIP4. HPLC conditions: column size, $4.6_{i.d} \times 100$ mm; mobile phase: acetonitrile containing 1% HAc (v/v); flow rate, 0.5 mL/min; monitor at 281 nm.

as the change of sample injection amount (a), flow rate of mobile phase (b), and column temperature (c). The capacity factor ran down along with *x*-axis as it shifted forward to the right. The chromatographic characteristic inferred that lower flow rate,

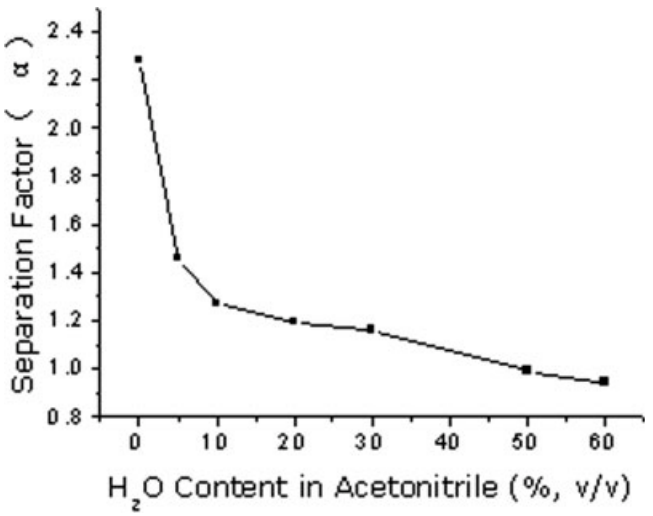

Figure 3 Effects of mobile phase on the separation factors of 17-β-estradiol on the column loaded with MIP4. HPLC conditions as Figure 2 except that the eluent was a mixture containing 1% HAc (v/v) of H_2O and acetonitrile.

lower injection amount, and lower temperature benefited the separation on MIPs.

To investigate the influence of water on separation factor, a mixed solution containing 1% acetic acid (HAc) was used as mobile phase, and was the routine eluent as characterizing MIPs by HPLC.[20,23] The purpose of adding slight HAc (0.5–1%) in acetonitile is in favor of restraining the ionization of 17-β-estradiol hydroxyl group and improving the retention value of 17-β-estradiol in the column, and this resulted in increase of MIPs capacity and optimization of the recognition for 17-β-estradiol. Chromatographic separation of 17-α-estradiol and 17-β-estradiol showed a better separation as shown in Figure 1, with acetonitrile containing 1% acetic acid was as eluent at a flow rate of 0.5 mL/min than the eluent without acetic acid. But as the increment in polarity of solvent takes place, hydrophobic interaction between functional monomer and template may be weakened. Thus, 1% acetic acid was maintained in the mixture eluent to compare with prior data, only the water content and acetonitrile in mixture was varied. The separation factors of MIP4 for the isomers were inspected by HPLC. Figure 3 showed the separation factor loss about 50% as mobile phase only contains 5% distilled water, and had 60% loss using acetonitile containing 10% distilled water as eluent, which was because of the interference of polar H_2O molecules in the hydrophobic interaction of the template with functional monomer.

Adsorption kinetic of MIP3 and MIP4 in Figure 4 showed that the latter had better adsorbing capacity than the former. This implied that TFMAA-*co*-TRIM imprinted polymers can be applied to determination of 17-β-estradiol as core sensitive material. The adsorption balance for MIP4 was approached at 50 min, and balance response time might be shortened once the technique of MIP nanofilm is developed.

Characteristics of surface morphology for TFMAA-*co*-TRIM imprinted polymers

SEM and AFM images showed that TFMAA-*co*-TRIM imprinted polymers were predominantly defined submicron and nanoparticles. All the samples including CP3 (a), MIP1 (b), MIP2 (c), and MIP4 (d) were not especially milled and dispersed, those from TFMAA-*co*-TRIM imprinted polymers which were dried after removing fine particles in acetone.

Particle size of MIP2 from 1 μm to 2 μm was bigger than other polymers between 300 nm and 1.5 μm. Figure 6 shows AFM images of CP3, and (a) and (b) were, respectively, 3-dimensional and 2-dimensional images of the sample. No monodisperse microspheres and nanospheres were found in these pictures. The dosage of porogen (15 mL) in preparation formula should be the reason for formation of congeries of particles the size being lower than 5 μm. A good morphologic property for MIPs more likely originated from the polymerization process, and polymerization temperature, composition of prepolymerized solution (e.g., the type and amount of crosslinker), and porogenic solvent were at least three factors.[33] To synthesize uniform, crosslinked polymer microspheres in various polymer system, crosslinking polymerization under high dilution conditions were needed.[34] Wei et al. synthesized the microspheres and nanospheres selective for 17-β-estradiol yielding imprinted, and the performance of the imprinted microspheres prepared by the one-step synthetic route developed was superior or equal to the performance of microspheres prepared by multistep swelling/polymerization tech-

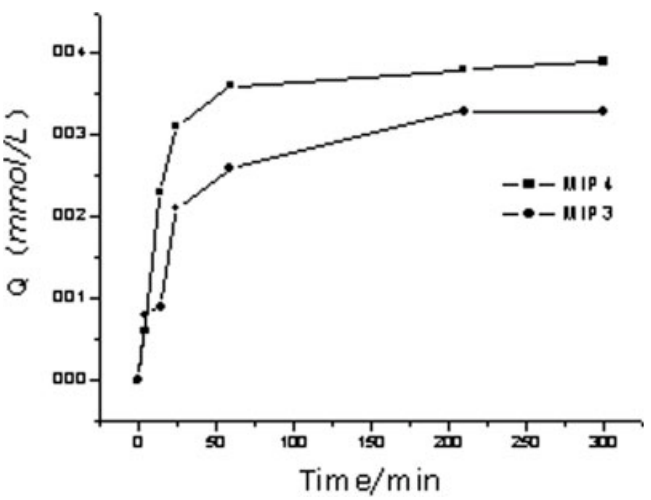

Figure 4 Adsorption kinetic curves of MIP3 and MIP4 (m = 10 mg; c_0 = 1 mM; V = 1 mL; t = 25°C).

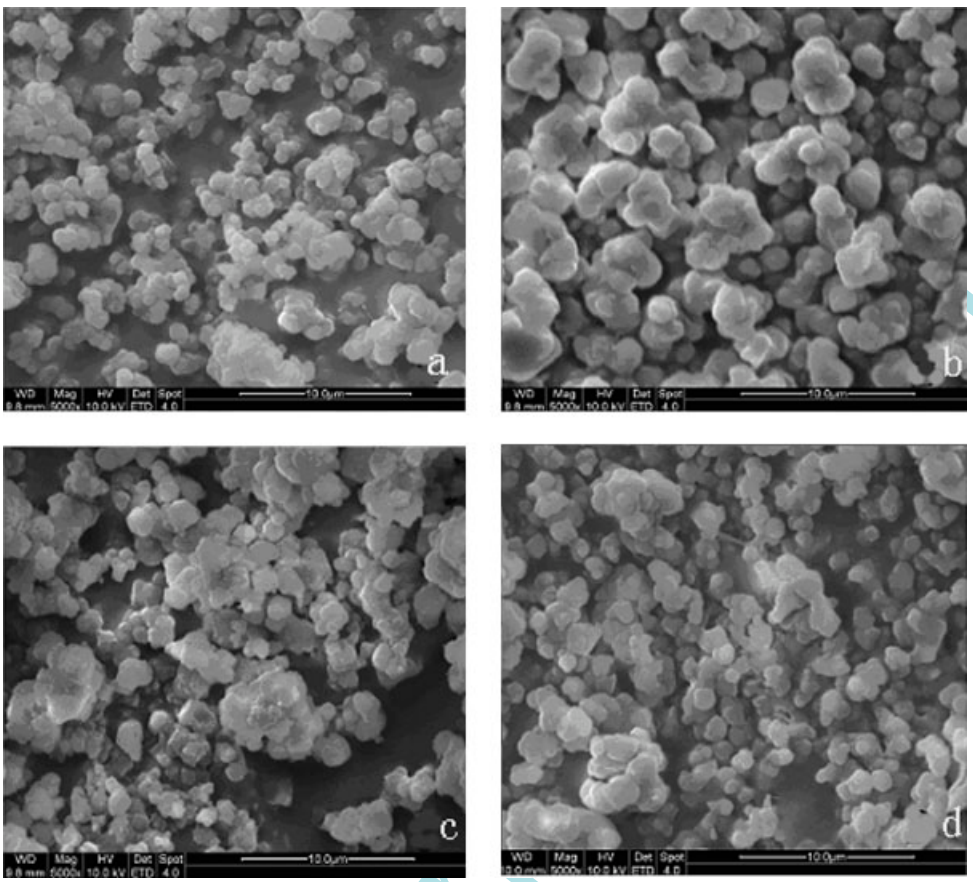

Figure 5 SEM of various polymers (a), is CP3; (b), is MIP1; (c), is MIP2; (d), is MIP4.

niques.[23] TFMAA-*co*-TRIM imprinted polymers for 17-β-estra-diol yielding imprinted were better perfectly imprinting and separating performance for the isomers than MAA-*co*-DVB polymers[23] in Table II, although the latter obtained good morphologic properties. Therefore, the recognition property is more important than the morphologic property for MIPs. Only on the basis of the stable recognition properties, it is necessary for various applications to further improve the morphologic properties of MIPs.

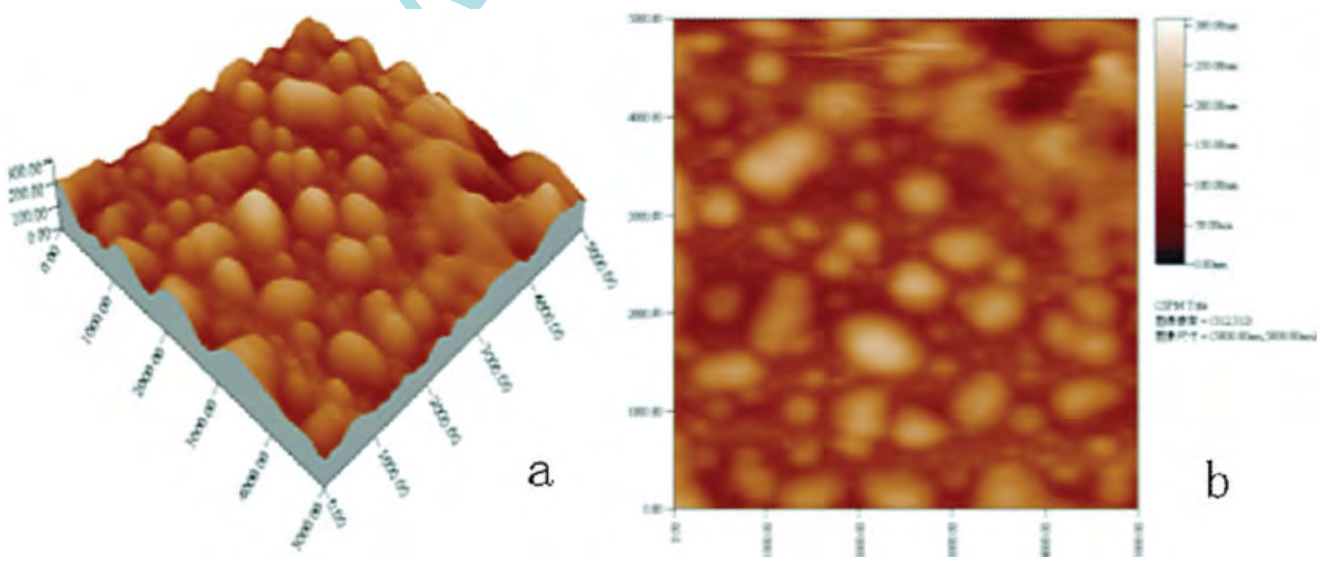

Figure 6 AFM images of CP3; (a) and (b) are, respectively, 3-dimensional and 2-dimensional images of the sample. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

Submicron-scaled MIP particles for 17-β-estradiol have been prepared by a precipitation polymerization method using TFMAA as functional monomer and TRIM as crosslinker in acetonitrile. The size of TFMAA-*co*-TRIM imprinted polymers obtained via precipitation polymerization is approximately between 300 nm and 1.5 μm, according the images of SEM and AFM. The TFMAA-*co*-TRIM imprinted polymers showed good throughput as stationary phase. The imprinted TFMAA-*co*-TRIM submicron and nanoparticles had a much higher binding capacity to 17-β-estradiol than the nonimprinted TFMAA-*co*-TRIM imprinted polymers. For MIP4 and CP3 on $4.6_{i.d} \times 100$ mm columns, the lipophilic isomer had a fairly high IF value (3.01) and separation factor (2.28). The tandem columns could lead to better separation with a good separation factor (2.45). The results of HPLC confirmed that the TFMAA-*co*-TRIM submicron and nanoparticles had specific discrimination ability to the isomers of 17-α-estradiol and 17-β-estradiol. The chromatographic properties of MIP4 inferred that lower flow rate, lower injection amount, and lower temperature benefited for separation of the isomers. The interaction of MIP4 with 17-β-estradiol might be destroyed by water since about 50% separation factor was lost while the mobile phase only contained 5% distilled water. Hence, it can be concluded that the binding force is a kind of noncovalent type, more specifically of hydrogen bonding. In addition, adsorption kinetic studies pointed out that MIP4 had better adsorption character. Therefore, it is highly feasible for the TFMAA-*co*-TRIM imprinted polymers to be suited as a core sensitive material for the specific recognition of 17-β-estradiol from other isomers.

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