Preparation of Molecularly Imprinted Polymer Coatings with the Multiple Bulk Copolymerization Method for Solid-Phase Microextraction

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ABSTRACT: The multiple bulk copolymerization method, which was developed in our previous works, was further investigated with prometryn, tetracycline, and propranolol as templates for the preparation of molecularly imprinted polymer (MIP) coatings on silicon fibers for solid-phase microextraction. The preparation conditions (e.g., the solvent, monomer, crosslinker, component proportions, polymerization time, and number of coating procedures) were investigated systemically in an effort to enhance the coating thickness, surface morphology, and reproducibility. The methodology was examined, and some common specialties were explored in the preparation of three MIP-coated fibers. Even after the coating procedure was repeated 10 times, the prometryn, tetracycline, and propranolol MIP-coated fibers were prepared reproducibly with coating-thickness relative standard deviations of 2.6, 3.0, and 5.1%, respectively; they were highly homogeneous, and a compact morphological structure was obtained. The extraction capacities of prometryn, tetracycline, and propranolol with corresponding MIP-coated fibers were approximately 10.4, 3.9, and 3.3 times as much as those with the nonimprinted polymer (NIP)-coated fibers, respectively, and the selectivity factors of prometryn, tetracycline, and propranolol MIP coatings for the template molecules and structural analogues were 2.2–10.4, 2.2–3.9, and 1.3–3.3, respectively, in comparison with the corresponding NIP coatings. In comparison with commercial polydimethylsiloxane/divinylbenzene coatings that were approximately 3 times thicker, the extracted amounts of prometryn, tetracycline, and propranolol were 4.2, 12.3, and 7.7 times higher with prometryn, tetracycline, and propranolol MIP coatings, respectively. J Appl Polym Sci 120: 1266–1277, 2011

Key words: coatings; fibers; molecular imprinting; separation techniques

INTRODUCTION

With the characteristics of specific selectivity, good chemical stability, and easy preparation, molecularly imprinted polymers (MIPs) have been used as recognition materials in various fields, such as chromatography, solid-phase extraction, and chemical sensing.[1–3] Recently, the application of MIPs to solid-phase microextraction (SPME) has been found to be attractive.[4–8] SPME is a simple, time-efficient, and solvent-free sample pretreatment technique based on the partitioning of analytes between the sample matrix and the polymer film coating on a silica fiber.[9] Consequently, the adoption of antibody-mimicking MIPs as SPME coatings could combine their advantages and achieve remarkable enhancements of the trace monitoring of analytes in complex samples.

However, for the MIP coatings on inorganic silica fibers, the conventional physical deposition method cannot guarantee the firm attachment of organic MIP coatings. To solve this problem, Koster et al.[4] employed a silane coupling agent to link inorganic fibers to MIP coatings through chemical bonding in bulk polymerization. Recently, a novel strategy without silica fibers was reported for the preparation of MIP monolithic fibers:[5] a silica capillary was applied as the mold and was removed or melted after bulk polymerization. However, MIP monolithic fibers were weaker with respect to thickness flexibility and mechanical strength than a silica fiber substrate. In our previous works,[6–8] a multiple bulk copolymerization method was developed to solve these problems and to achieve a controllable coating thickness by repeated coating procedures on a single silica fiber. However, the MIP coating thickness, surface

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morphology, and preparation repeatability were affected greatly by many conditions, such as the solvent, monomer, crosslinker, component proportions, and polymerization time. Moreover, these effects were amplified with repeated coating procedures, and it was unacceptable for the preparation of MIP-coated fibers with the coating procedure repeated approximately 10 times.

As we know, the optimization of various preparation conditions is crucial for MIP materials with specific selectivity, and comprehensive investigations have been performed for various factors governing the performance of MIP materials, such as the solvent, monomer, initiator, polymerization temperature, pressure, and polymerization time.[10–18] Consequently, with the aim of improving the preparation feasibility and repeatability, an investigation of the methodology is primarily needed for the preparation of MIP-coated fibers with the multiple bulk copolymerization method, and the common specialties could be explored and used for the further guidance of the preparation of MIP-coated fibers.

In this study, the preparation conditions in multiple bulk copolymerization were investigated systemically to improve the thickness, surface morphology, and preparation repeatability of MIP coatings with prometryn, tetracycline, or propranolol as the template. The characteristics of three MIP-coated fibers were investigated, and the extraction performances were validated by comparison with commercial polyacrylate (PA), polydimethylsiloxane (PDMS), and PDMS/divinylbenzene (DVB) SPME coatings.

EXPERIMENTAL

Chemicals and reagents

Triazine herbicides (prometryn, propazine, atrazine, simetryn, ametryn, terbuthylazine, and terbutryn) were kindly provided by Bingzhou Pesticide Plant (Shandong, China). Tetracycline, oxytetracycline hydrochloride, and doxycycline hyclate were purchased from Fluka (Buchs, Switzerland). Chlortetracycline hydrochloride and *R,S*-propranolol hydrochloride were purchased from Acros (Morris Plains, NJ). Alprenolol hydrochloride, (*R*)-(+)-atenolol, and pindolol were purchased from Sigma–Aldrich (St. Louis, MO).

Acrylamide (AA) and 4-vinylpyridine (4-VP) were purchased from Sigma–Aldrich. Methacrylic acid (MAA) and azobisisobutyronitrile were purchased from Damao Reagent Plant (Tianjin, China). Trimethylolpropane trimethacrylate (TRIM) and ethylene glycol dimethacrylate (EGDMA) were purchased from Corel Chemical Plant (Shanghai, China). 3-(Methacryloxy)propyl-trimethoxysilane was obtained from Shengda Fine Chemical Industry Corp. (Beijing, China). Methanol and acetonitrile [high-performance liquid chromatography (HPLC) grade] were purchased from Sigma–Aldrich and Merck (Darmstadt, Germany), respectively. Water was doubly distilled. All other reagents were analytical-grade. Silica fibers were kindly provided by FiberHome Telecommunication Technologies (Wuhan, China). The commercial SPME fibers with PA, PDMS, or PDMS/DVB coatings were purchased from Supelco (Bellefonte, PA).

MIP-coated fiber preparation

The chemical pretreatment and the silylation of silica fibers were performed according to the method reported in our previous works.[6–8] The template and the monomer were dissolved together in the polymerization solvent to prepare the prepolymer solution. This solution was swirled for 12 h in an oscillator at room temperature, and then azobisisobutyronitrile and the crosslinker were added and dissolved adequately. Some of the mixture (1.5 mL) was transferred into a small glass tube and was deoxygenized with a stream of nitrogen for 5 min. Subsequently, a silylated fiber was inserted into the tube. The tube was sealed immediately with a rubber stopper and inserted into a nitrogen evaporator (Quandao, Shanghai, China) so that copolymerization could occur at 60°C. Some time later, the solid polymer was achieved with the fiber immobilized in it. Then, the fiber was pulled out cautiously, and a thin layer of the MIP coating was obtained on the fiber surface. This MIP-coated fiber was placed in another empty tube filled with N_2 and was heated at 60°C for 24 h. Finally, for the removal of the template molecules, the fiber was soaked in 5 mL of a 10% (v/v) acetic acid solution in methanol for 30 min. This procedure was performed repetitiously until the template could not be detected by HPLC in the soaking solution. The nonimprinted polymer (NIP) coated fibers were prepared simultaneously according to the same procedures mentioned previously, but without the addition of the template. The polymerization conditions for the preparation of the three MIP-coated fibers are provided in detail in the supporting information. The final coatings of the MIP- and NIP-coated fibers were slightly different in length ($\sim$ 2.0 cm). To obtain a uniform length of 10 mm, the unwanted coating was scraped from the top with a penknife. Then, the uncoated end of the fiber was stuck to a 10-cm-long hollow stainless steel tube (ca. 550-μm external diameter) for the subsequent application.

Investigation of the preparation conditions

The investigation of the preparation conditions was performed with the prometryn, tetracycline, and

propranolol MIP-coated (once) fibers. For the study of the number of coating procedures, the MIP-coated (once) fibers were immersed again in fresh polymerization solutions and were repeatedly coated onto the surface of a previous MIP layer with the identical copolymerization method.

Characterization of the MIP coatings

For the investigation of the preparation conditions, an evaluation of the uniformity and surface morphology of prometryn, tetracycline, and propranolol MIP coatings was performed with an XSG-409L biological microscope (Hanjin, Shanghai, China) under magnifications of 200× and 400×. The coating thickness was measured with a scale eyepiece that was calibrated with a 0.01-mm slide micrometer.

The morphological evaluation of the MIP-coated fibers was performed with an XL-30 scanning electron microscope (Philips, Eindhoven, the Netherlands) or a JSM-6330F field emission scanning electron microscope (JEOL, Tokyo, Japan). The infrared absorption spectrum between 400 and 4000 cm^{-1} was obtained with a Prestige 21 Fourier transform infrared spectrometer (Shimadzu, Kyoto, Japan). The thermogravimetric analysis was performed with an STA-409 PC thermogravimetric analyzer (Netzsch, Selb/Bavaria, Germany) over the temperature range of 50–800°C (heating rate = 10°C/min).

MIP-coated fiber performance

For the prometryn, tetracycline, and propranolol MIP-coated fibers prepared under the optimized conditions, investigations of the extraction capacity and selectivity and comparisons with PDMS/DVB, PDMS, and PA SPME coatings were performed according to the method provided in detail in the supporting information. The HPLC conditions for the prometryn, tetracycline, and propranolol analysis are also provided in the supporting information.

RESULTS AND DISCUSSION

Multiple bulk copolymerization method

For the preparation of commercial SPME coatings on silica fibers, the physical deposition method is normally applied. However, the cracking of coatings is incidental during fiber applications, and the solvent-resistant capabilities of coatings are not satisfactory,[19] so their life spans are limited. It is thought that an ideal MIP coating for SPME fibers should possess the physical characteristics of fine uniformity, firm attachment, and good solvent resistance. This depends mainly on the coating properties. Simultaneously, the coating preparation method also plays a crucial role.

A schematic representation of the multiple bulk copolymerization method is shown in Figure 1. The silica fiber is first pretreated with a silane coupling reagent containing vinyl groups [Fig. 1(a)]. Then, the silylated fiber can participate in MIP copolymerization through the vinyl groups and can be coated firmly with the MIP through chemical bonding [Fig. 1(b)]. To achieve a satisfactory coating thickness, the MIP-coated fiber is immersed again into the fresh polymerization solution and is coated repeatedly onto the surface of a previous MIP layer by the same copolymerization method until the wanted thickness is obtained [Fig. 1(c)]. Therefore, the coating thickness, uniformity, and surface morphology of the MIP coating are mainly affected by the copolymerization conditions, such as the solvent, monomer, crosslinker, component proportions, polymerization time, and number of coating procedures. To investigate the methodology and explore the common specialties, we selected three MIP preparation systems with prometryn, tetracycline, and propranolol as the templates for the systemic investigation of these preparation conditions.

Silica fiber silylation

The silylation of silica fibers was crucial for the preparation of the MIP-coated fibers. When nonsilylated fibers were used, no prometryn, tetracycline, or propranolol MIP coatings formed on the fiber surface. In contrast, silylated fibers with surface unsaturated bonds could participate in the bulk copolymerization and ensure firm chemical bonding of the MIP coatings. Therefore, even after they were used more than 80 times, the prometryn, tetracycline, and propranolol MIP coatings prepared under the optimized conditions retained good integrity, and no cracking occurred.

Polymerization solvents

For the investigation of polymerization solvents, the first consideration should be the solubility of the template[20] because a large number of template molecules are needed in bulk polymerization. Prometryn has good solubility in nonpolar and polar solvents, so toluene, benzene, ethyl acetate, chloroform, acetone, and acetonitrile were selected for the investigation. In contrast, the solubility of tetracycline or propranolol in nonpolar solvents could not meet the requirements of MIP preparation, so only four polar solvents were adopted. It is well known that a polymerization solvent with a low polarity is advantageous for the enhancement of MIP selectivity.[21,22] Simultaneously, the structure and morphology of an

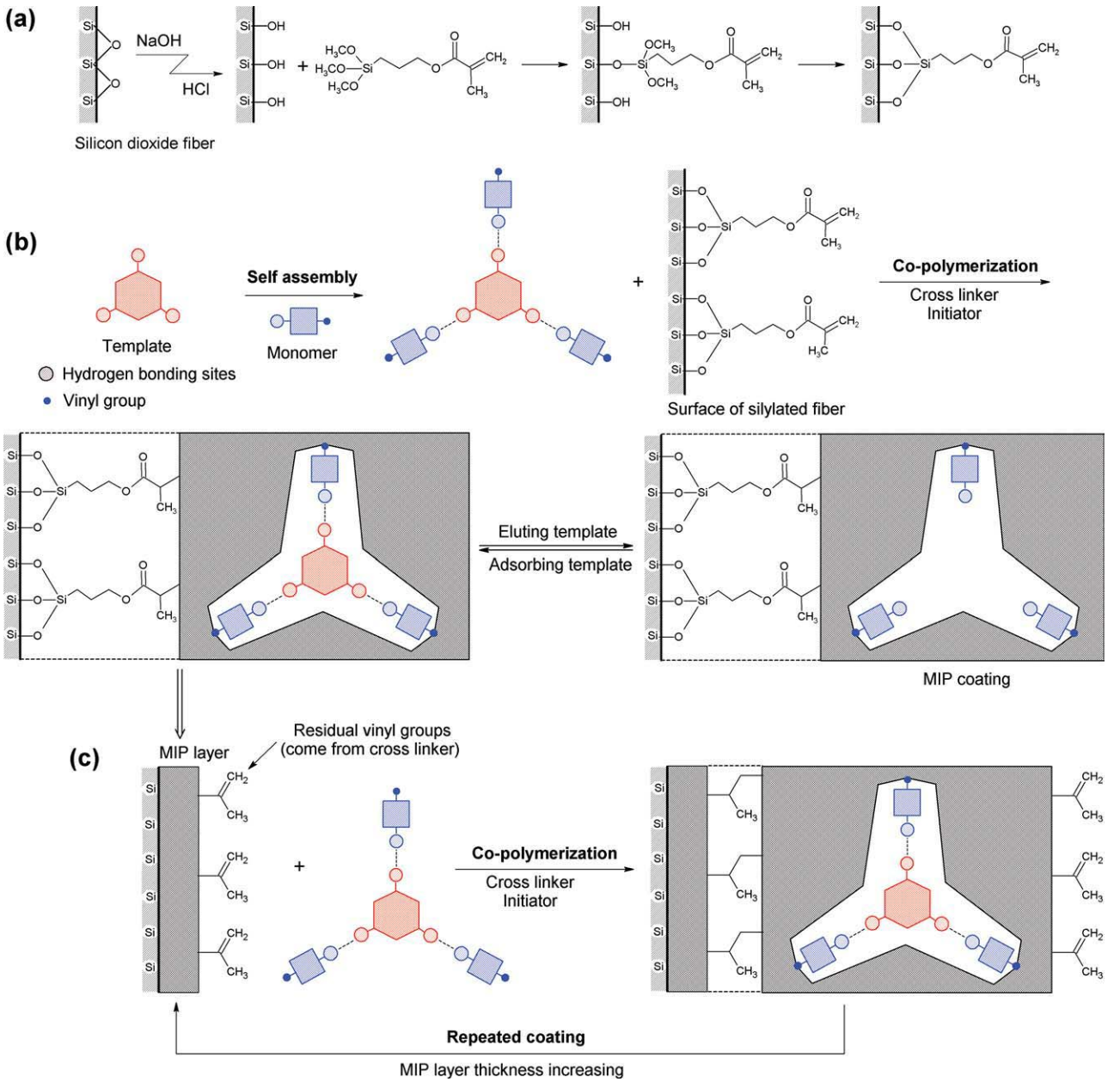

Figure 1 Schematic representation of the multiple bulk copolymerization method: (a) silica dioxide fiber silylation, (b) MIP-coated fiber preparation, and (c) repeated coating procedure. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MIP material are affected by the polymerization solvent.[23,24] The results of the investigation of polymerization solvents are shown in Table I. For tetracycline and propranolol, only polar solvents could be used, but marked differences were still observed among the four polar solvents, and the optimal solvents were acetone and acetonitrile, respectively. However, for prometryn, when polar ethyl acetate, acetonitrile, chloroform, and acetone were used, MIP coatings were achieved with a bad surface morphology and thin thicknesses of less than 1.0 μm, or even no MIP coating was formed. In contrast, the adoption of toluene or benzene resulted in marked enhancements of the coating thickness, uniformity, and morphology. With the guarantee of sufficient solubility for the template, nonpolar solvents such as benzene and toluene were suggested.

Volume ratio of the monomer plus the crosslinker to the polymerization solvent

The volume proportion of the polymerization solvent is also important for MIP preparation. The total concentration of the monomer and crosslinker, presented as the volume ratio of the monomer plus the crosslinker to the polymerization solvent, will affect

TABLE I
Effects of the Polymerization Solvents on the Thickness, Uniformity, and Surface Morphology of Prometryn, Tetracycline, and Propranolol MIP Coatings

	Prometryn			Tetracycline			Propranolol		
Solvent	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology
Toluene	2.6	++	++	N/A			N/A		
Benzene	2.1	+	+	N/A			N/A		
Tetrahydrofuran	N/A			0.4	++	+	N/A		
Ethyl acetate	0.9	−	−	N/A			N/A		
Chloroform	NC			NC			0.9	−	+
Acetone	NC			1.3	++	++	0.3	−	+
Acetonitrile	0.4	−	−	N/A			3.1	+++	++
Dimethyl sulfoxide	N/A			0.9	++	+	1.0	+++	++

The uniformity was estimated with the interfacial straightness of the MIP coating, the surface morphology was estimated by the integration of the roughness, cracking, coverage, and bulky grain presence of the MIP coating. − = bad; + = normal; ++ = good; +++ = excellent; N/A = not applicable; NC = no coating formed.

the crosslinking degree and subsequent coating properties. Consequently, the conventionally applied monomer MAA and the crosslinker TRIM in a molar ratio of 1 : 1 were chosen simultaneously for the preparation of prometryn, tetracycline, and propranolol MIP coatings, and then the volume ratio was investigated within the range of 1 : 1–1 : 30, as shown in Table II. The preparation feasibility, morphology, and thickness were affected markedly. The optimal volume ratio was approximately 1 : 6–1 : 9. When the volume ratio was larger than this range, an increase in the total concentration of the monomer and crosslinker resulted in the acceleration of polymerization. For the preparation of prometryn MIP coatings with a volume ratio of 1 : 2, the time needed for polymerization was only 30 min, but there was no MIP coating on the silylated fiber because there were two different polymerization processes: the reaction on the fiber surface and the reaction in solution. The matching of these two rates was probably the critical factor determining the film growth. Consequently, a much faster reaction in solution resulted in coating failure, and no MIP film was found. In contrast, when the volume ratio was lower than the aforementioned optimal range, an increase in the solvent percentage resulted in some problems, such as bulky grains, bad uniformity, and cracking. When the volume ratio of 1 : 30 was used, the concentration of the monomer plus the crosslinker was too low for the copolymerization to be carried out for the synthesis of tetracycline and propranolol MIPs.

Monomers

The main function of the monomer in MIP preparation is to provide scheduled multiple recognition sites to template molecules, as shown in Figure 1. Therefore, the structure of the template molecule should be considered during monomer selection, and another consideration is the effect of the monomer on the thickness, uniformity, and surface morphology of the MIP coating.[25] MAA, AA, and 4-VP, three extensively applied monomers, were

TABLE II
Effects of the Volume Ratios of the Monomer and Crosslinker to the Polymerization Solvent on the Thickness, Uniformity, and Surface Morphology of Prometryn, Tetracycline, and Propranolol MIP Coatings

	Prometryn			Tetracycline			Propranolol		
Volume ratio	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology
1 : 1	NC			N/A			N/A		
1 : 2	NC			N/A			N/A		
1 : 4	4.1	+	++	N/A			N/A		
1 : 6	4.3	++	+++	1.3	++	+	2.9	++	++
1 : 9	4.1	++	++	1.5	++	++	2.0	++	++
1 : 15	N/A			1.3	++	−	2.3	−	+
1 : 30	N/A			NC			NC		

The symbols are the same as those used in Table I.

TABLE III
Effects of the Monomers on the Thickness, Uniformity, and Surface Morphology of Prometryn, Tetracycline, and Propranolol MIP Coatings

	Prometryn			Tetracycline			Propranolol		
Monomer	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology
MAA	3.6	+++	+++	2.3	++	+	2.7	+++	++
AA	3.0	++	++	3.2	++	+++	2.2	++	+
4-VP	1.5	++	−	0.5	++	++	2.4	++	++

The symbols are the same as those used in Table I.

investigated for the preparation of three different MIP-coated fibers. As shown in Table III, the results indicated that in the preparation of prometryn and tetracycline MIP coatings, the thickness with MAA or AA as the monomer was obviously greater than the thickness with 4-VP. For propranolol MIP coatings, the thickness difference among the three monomers was not remarkable. The optimal monomers for prometryn, tetracycline, and propranolol were MAA, AA, and MAA, respectively.

The molar ratio of the template to the monomer is important for sufficient self-assembly through intermolecular interactions. Excess monomer results in an increase in the number of residual monomer groups with random orientation in the MIP material and subsequent enhancement of nonselective adsorption, whereas excess template heightens the difficulty of complete template elution. Therefore, the ratios of 1 : 4 and 1 : 8 were applied extensively for MIP preparation. However, except for changes in the arrangement and orientation of the monomer through intermolecular interactions, the template did not participate in the copolymerization and should not have affected the physical properties of the MIP coatings. The propranolol MIP coating was selected for the investigation of the template–monomer molar ratio in the range of 1 : 1–1 : 16. The results indicated that there was no marked difference in the coating thickness, uniformity, or surface morphology. The ratio of 1 : 8 was adopted for the preparation of prometryn, tetracycline, and propranolol MIP coatings.

Crosslinker

EGDMA and TRIM, crosslinkers extensively used in MIP preparation, were investigated for the preparation of three different MIP-coated fibers. As shown in Table IV, the results indicated that no matter what MIP system was studied, there were marked improvements in the MIP coating thickness, uniformity, and surface morphology with TRIM versus EGDMA. This occurred because TRIM with three vinyl groups is a ternary crosslinker, and EGDMA has only two vinyl groups. Under the same conditions, the use of TRIM is advantageous for the formation of three-dimensional structures and the enhancement of the coating thickness and morphology.

Molar ratio of the monomer to the crosslinker

The molar ratio of the monomer to the crosslinker determines the MIP coating composition and has an influence on the coating thickness and morphology. This influence was investigated with the optimal monomer and crosslinker mentioned previously, and the molar ratio range of 1 : 4–6 : 1 was selected. As shown in Table V, the results indicated that an increase in the ratio led to an obvious decrease in the thicknesses of prometryn, tetracycline, and propranolol MIP coatings, and the coating uniformity and surface morphology changed gradually for the worse. This was caused by the reduction of the

TABLE IV
Effects of the Crosslinkers on the Thickness, Uniformity, and Surface Morphology of Prometryn, Tetracycline, and Propranolol MIP Coatings

	Prometryn			Tetracycline			Propranolol		
Crosslinker	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology
EGDMA	3.6	+	+	1.7	++	−	1.4	−	+
TRIM	4.3	+++	+++	2.5	++	+++	3.0	+++	++

The symbols are the same as those used in Table I.

TABLE V
Effects of the Molar Ratios of the Monomer to the Crosslinker on the Thickness, Uniformity, and Surface Morphology of Prometryn, Tetracycline, and Propranolol MIP Coatings

	Prometryn			Tetracycline			Propranolol		
Molar ratio	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology	Thickness (μm)	Uniformity	Surface morphology
1 : 4	3.6	−	++	2.5	+++	++	3.1	+++	++
1 : 2	5.1	++	+	1.6	++	+	3.0	++	+
1 : 1	4.3	+++	++	1.5	++	−	2.5	++	+
2 : 1	3.8	+	−	1.0	+	+	2.3	++	+
4 : 1	4.1	+	−	0.9	+	−	2.0	++	−
6 : 1	3.0	+	−	0.6	+	−	1.5	+	−

The symbols are the same as those used in Table I.

crosslinker proportion in the polymerization solution. When the ratio was 4 : 1 or higher, cracking was found easily on the coating surface. Consequently, the function of the crosslinker as the main framework of the MIP coating could be validated, and molar ratios higher than 2 : 1 would be not accepted for the preparation of MIP coatings. For prometryn, tetracycline, and propranolol MIP coatings, the optimal molar ratios of the monomer to the crosslinker were 1 : 1, 1 : 4, and 1 : 4, respectively.

Polymerization time

During the preparation of MIP coatings, the crosslinking degree is enhanced gradually with the polymerization time increasing. When fibers are drawn after different times, differences in the MIP coating morphology and thickness should be observed. Consequently, the effect of the polymerization time on prometryn, tetracycline, and propranolol MIP coatings was investigated. Because of the consideration of the effective polymerization time for silicon fibers in the solidified polymer, it was counted only after the solidification of the polymerization solution. All results indicated that a polymerization time less than 2 h resulted in bad coating uniformity and coverage, and with increasing time, these problems were solved. However, the prolonged time simultaneously caused an enhancement of MIP rigidity; moreover, it was difficult to pull the fibers from the solid polymer, or the fibers could not be pulled out or broke when they were pulled. As for the coating thickness, it increased with the polymerization time in the beginning, and then it reached a stable value. Consequently, a time should be selected on the basis of the fiber security, coating uniformity, and thickness. Finally, 6, 3, and 3 h were proven to be optimal for the preparation of prometryn, tetracycline, and propranolol MIPs, respectively.

Number of coating procedures

These investigations of the polymerization conditions produced marked improvements in the uniformity, surface morphology, and thickness of prometryn, tetracycline, and propranolol MIP coatings. The preparation feasibility and repeatability were enhanced remarkably. However, coating thicknesses of approximately 2–4 μm were too thin to achieve a satisfactory binding capacity. This problem could be effectively solved with the multiple bulk copolymerization method, in which an MIP was repeatedly coated onto one silicon fiber. Investigations of the effects of the number of coating procedures on prometryn, tetracycline, and propranolol MIP coatings were performed. As shown in Figure 2, the results demonstrated that the thicknesses of the three MIP coatings increased along with the number of coating procedures, and there was good linearity between them with correlation coefficients of 0.9962–

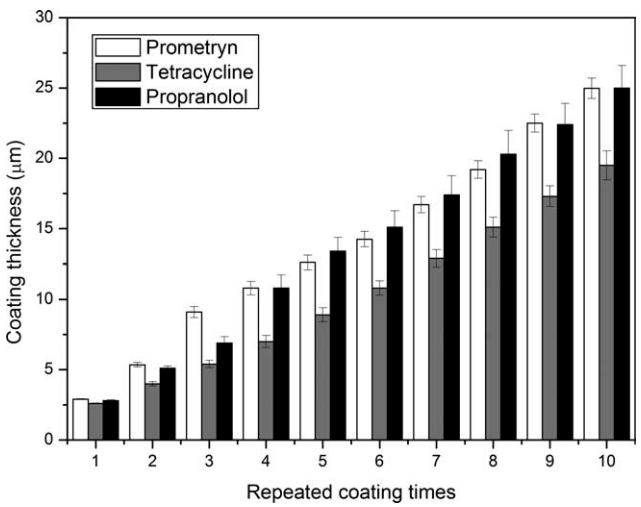

Figure 2 Effect of the number of coating procedures on the thickness of prometryn, tetracycline, and propranolol MIP coatings.

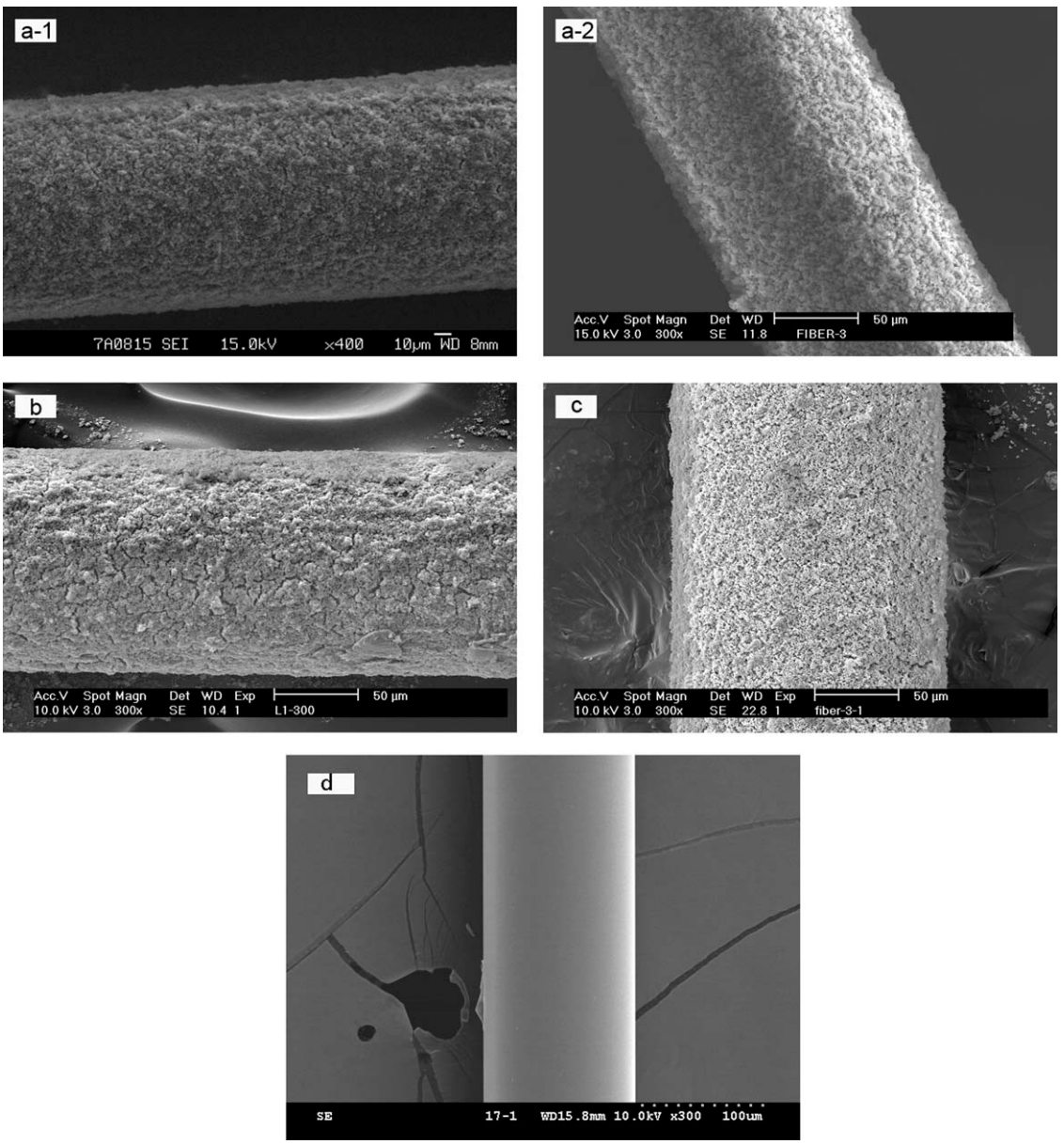

Figure 3 Scanning electron micrographs of (a) prometryn, (b) tetracycline, and (c) propranolol MIP-coated fibers and (d) an uncoated silicon fiber at a magnification of (a1) 400× or (a2–d) 300×.

0.9983. One addition to the number of coating procedures resulted in the thickness increasing by approximately 1.9–2.5 μm, and simultaneously, there were no marked changes in the coating uniformity or surface morphology. However, when the number of coating procedures was higher than 10, the coating uniformity became degressive. Consequently, 10 repetitions of the coating procedure were selected. As a result, the coating thicknesses were improved to 24.8, 20.2, and 25.8 μm, respectively.

For the preparation of MIP-coated fibers with the multiple bulk copolymerization method, some common ground was found through the investigations of the preparation conditions for the three different MIP systems. As for the solvent, its selection should be supported mainly by a low polarity. However, the requirement of satisfactory solubility for the template often precluded the adoption of a nonpolar solvent. The volume percentage of the solvent in the polymerization solution was very important to the thickness and morphology of the MIP coatings, and the range of 1 : 6–1 : 9 (volume ratio of the monomer plus the crosslinker to the solvent) was proven to be optimal. Monomer selection was based on the functional groups of the template molecule, and MAA and AA with a suitable molar ratio to the template of 4 : 1–8 : 1 were used for most of the MIP preparation. Without question, TRIM was the better

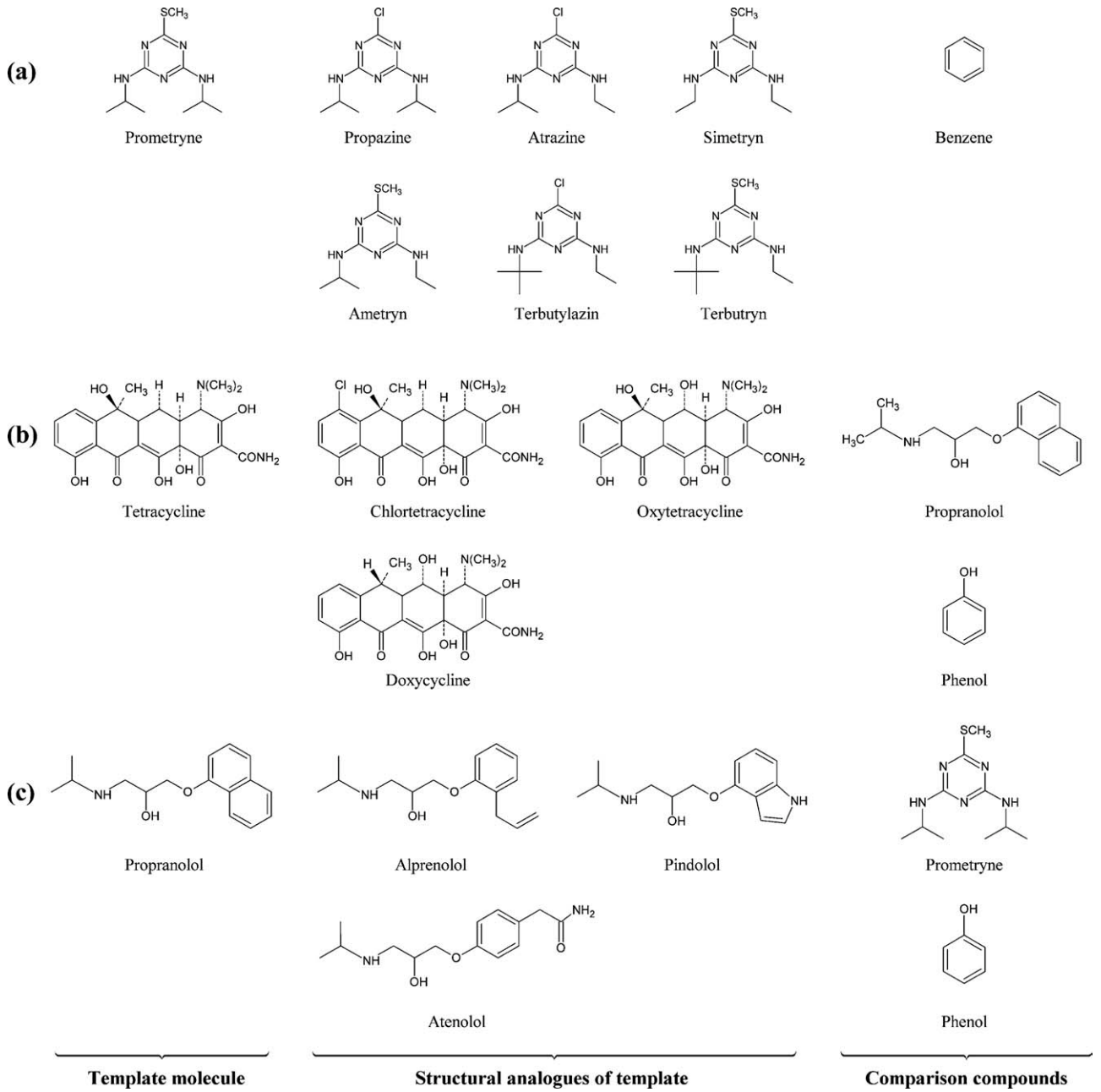

Figure 4 Chemical structures of the template molecule, its structural analogues, and comparison compounds for the selectivity investigation of (a) prometryn, (b) tetracycline, and (c) propranolol MIP-coated fibers.

crosslinker choice than EGDMA for the enhancement of the coating uniformity and thickness, so it was adopted with a moderate molar ratio to the monomer of 1 : 1 or 1 : 4 for the preparation of the three MIP coatings. The polymerization time was decided on the basis of the coating thickness, morphology, and fiber safety; 3 or 6 h was selected. The number of coating procedures was mainly limited by the decrease in the coating uniformity and repeatability when it was higher than 10, so 10 repetitions of the coating procedure were finally selected for the preparation of the three MIP coatings.

Preparation reproducibility

The preparation reproducibility of MIP-coated fibers with the multiple bulk copolymerization method was investigated with different numbers of coatings. When prometryn, tetracycline, and propranolol MIP-coated fibers were coated only once, the relative standard deviations (RSDs) of the coating thickness were only 1.0, 1.4, and 1.8% ($n = 10$), respectively. These results were much better than those reported for the preparation of clenbuterol MIP coatings (RSD $\approx$ 10%).[4] RSDs of the thickness increased with repeated coatings, and they reached 2.6, 3.0, and

5.1%, respectively, when the coating procedure was repeated 10 times. Simultaneously, the batch repeatability of the preparation of MIP-coated fibers was also studied for three MIP systems with 10 coating procedures. RSDs of the coating thickness between two batches of MIP-coated fibers were only 4.8, 2.7, and 2.9%, respectively. This satisfactory reproducibility was mainly due to the improvement in the coating uniformity based on the preparation-condition optimization mentioned previously. Consequently, the practicability and applicability of the multiple bulk copolymerization method could be enhanced.

MIP-coating characterization

Scanning electron micrographs displaying the morphological structures of the prometryn, tetracycline, and propranolol MIP-coated fibers are shown in Figure 3(a1,b,c), respectively. For comparison, a scanning electron micrograph of an uncoated silicon fiber is shown in Figure 3(d). The results indicated that, because of the systemic preparation-condition optimization mentioned previously, a highly homogeneous surface was obtained for all MIP coatings despite 10 coating procedures. In comparison with the micrograph in Figure 3(a2), which was taken from another batch of prometryn MIP-coated fibers reported in a previous work,[6] the highly similar surface morphology indicated that good batch reproducibility could be achieved with the multiple bulk copolymerization method.

Through an infrared spectroscopy study (see Fig. S2 in the supporting information), the chemical structure of the MIP coating could be confirmed to be the copolymerization product of the monomer and the crosslinker. The characteristic infrared absorption peaks were found at 3477–3564 (hydroxyl or amino groups of the MAA or AA monomer), 2961–2974 (methyl groups), 1735–1732 (carbonyl groups), and 1389–1398 cm^{-1} (methyl groups). Significantly, the minor peaks at 1602–1638 cm^{-1}, which were attributed to the residual C=C bond, were found for all the MIP coatings. This indicated that there were still unsaturated bonds in the MIP coatings, which were used to form the chemical bonding between the previous and next MIP layers in the repeated copolymerization procedure, as shown in Figure 1(c).

The thermal stability of the three MIP coatings was investigated with thermogravimetric analysis. As shown in Figure S2 in the supporting information, the results indicated that all MIP coatings were stable at temperatures below 250–270°C.

The reusability of the MIP-coated fibers was investigated. Extraction could be repeated approximately 100, 100, and 80 times with prometryn, tetracycline, and propranolol MIP-coated fibers, respectively, and there was not a marked decline in the extraction performance or morphology of the MIP coatings. However, hindered by the breakage of fragile silica fibers, further validation of the reusability was difficult, and the real lifespan was frequently shortened.

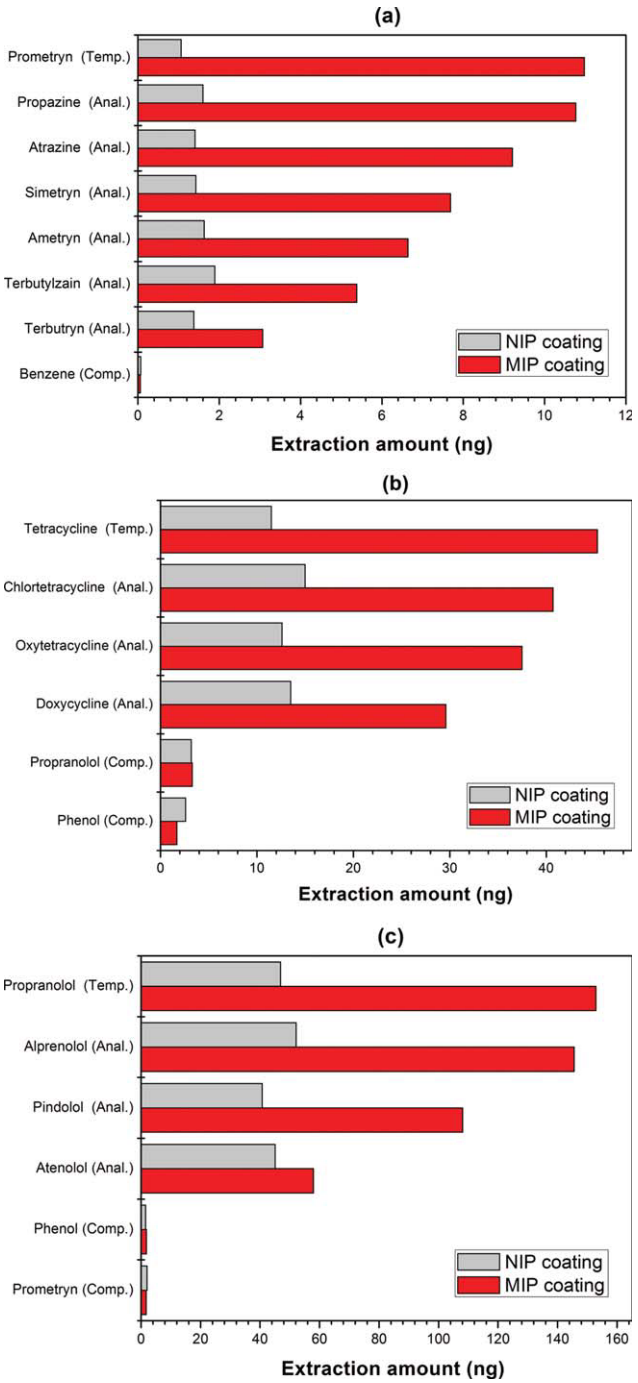

Figure 5 Extracted amounts of the template molecule, its structural analogues, and comparison compounds with (a) prometryn, (b) tetracycline, and (c) propranolol MIP-coated fibers and corresponding NIP-coated fibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Finally, good chemical stability was observed with the MIP coatings. The MIP-coated fibers were immersed into methanol, acetonitrile, acetone, chloroform, ethyl acetate, tetrahydrofuran, benzene, toluene, 10% (v/v) acetic acid in methanol, and acetonitrile for 24 h. All the MIP coatings retained good surface quality without cracking.

Fiber-extraction performance

With NIP-coated fibers used for comparison, we selected template molecules with a series of concentrations to investigate the binding performance of prometryn, tetracycline, and propranolol MIP-coated fibers. The results were in accord with our expectation that all MIP-coated fibers would have a much higher template-binding capacity than the corresponding NIP-coated fibers. When binding equilibrium was achieved, the binding capacity of prometryn, tetracycline, and propranolol MIP-coated fibers was approximately 10.4, 3.9, and 3.3 times as much as that of the NIP-coated fibers, respectively.

The selectivity investigations of prometryn, tetracycline, and propranolol MIP-coated fibers were all performed with template molecules, structural analogues of the template, and comparison compounds (see Fig. 4). It was obvious that in comparison with the NIP coatings, specific selectivities for the template and its structural analogues were obtained for all MIP coatings, as shown in Figure 5. In contrast, for the comparison compounds, there were not marked differences in their extracted amounts with MIP and NIP coatings. Defined as the ratio of the extracted amount of the analyte with the MIP coating to the amount with the NIP coating, the selectivity factors of the template and its analogues were 2.2–10.4, 2.2–3.9, and 1.3–3.3 for prometryn, tetracycline, and propranolol MIP-coated fibers, respectively. There was an obvious selectivity difference between the template and its analogues in each MIP system. The selectivity for the template was always highest, and a positive relationship between the selectivity for the analogues and the similarity in their template structure (see Fig. 4) was observed. The more similar structure generally resulted in higher selectivity. It was indicated that along with the improved coating uniformity and preparation reproducibility, good binding capacities and

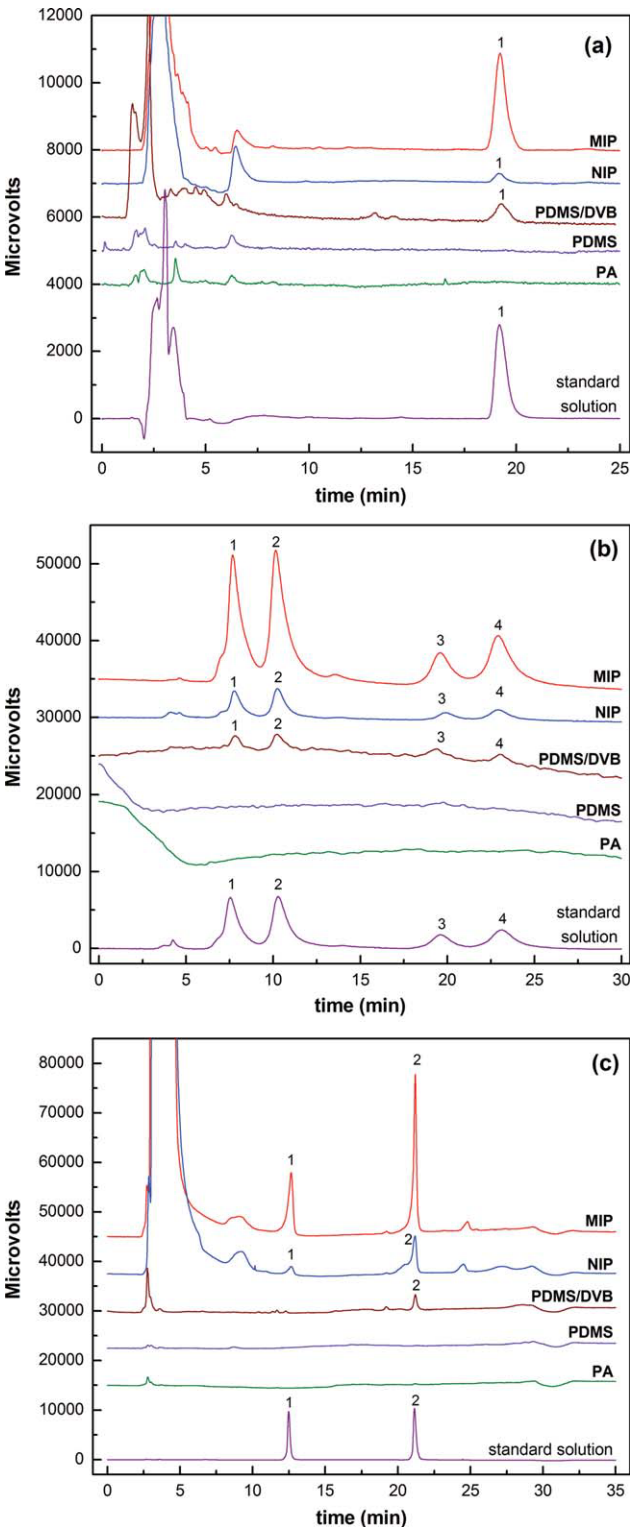

Figure 6 (a) Chromatograms of a 1.00 mg/L prometryn standard solution and extracts of a 0.500 μg/L prometryn solution with MIP and NIP coatings and commercial PMDS/DVB, PDMS, and PA coatings: (1) prometryn. (b) Chromatograms of a 1.00 mg/L tetracycline (TC) mixed standard solution and extracts of a 500 μg/L TC mixed solution with MIP and NIP coatings and commercial PMDS/DVB, PDMS, and PA coatings: (1) oxytetracycline, (2) tetracycline, (3) doxycycline, and (4) chlortetracycline. (c) Chromatograms of a 2.00 mg/L propranolol/pindolol mixed solution and extracts of a 500 μg/L propranolol/pindolol mixed solution with MIP and NIP coatings and commercial PMDS/DVB, PDMS, and PA coatings: (1) pindolol and (2) propranolol. The injection volume for the direct HPLC analysis of the standard solutions was 10 μL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

selectivity were found simultaneously with these MIP-coated fibers. The fiber-to-fiber reproducibility was investigated, and RSDs in the range of 3.3–9.6% for the extracted amounts were achieved for these fibers.

For further investigation, commercial SPME fibers with PDMS/DVB, PDMS, or PA coatings were used for comparison. The extraction conditions for the commercial coatings are provided in detail in the supporting information. To ensure that the comparison was authentic and effective, water as the extraction solvent and a 60-min extraction time [much longer than the time (30 min) for the MIP and NIP coatings] were used for these commercial coatings. As shown in Figure 6, the results indicated that prometryn, four tetracycline antibiotics, and two β-blockers could not be extracted with commercial PDMS and PA coatings, but they could be extracted with PDMS/DVB coatings; however, the amounts were only 24%, 2.9–30%, and 13% of those achieved with prometryn, tetracycline, and propranolol MIP coatings, respectively. Compared with the three commercial coatings with thicknesses of 60–100 μm, the MIP coatings with thicknesses of only approximately 20.2–25.8 μm had better extraction capacities for the template molecules and their structural analogues.

CONCLUSIONS

The multiple bulk copolymerization methodology was investigated with prometryn, tetracycline, and propranolol MIP coatings as the template systems. Significantly, even after 10 coating procedures, good coating uniformity and preparation reproducibility were obtained with prometryn, tetracycline, and propranolol MIP-coated fibers, and the coating thicknesses were 24.8, 20.2, and 25.8 μm with RSDs of 2.6, 3.0, and 5.1%, respectively. Simultaneously, good coating characteristics and extraction performance were achieved. The extraction capacities of the prometryn, tetracycline, and propranolol MIP-coated fibers were approximately 10.4, 3.9, and 3.3 times as much as those of the NIP-coated fibers, respectively. Good selectivities with factors of 2.2–10.4, 2.2–3.9, and 1.3–3.3 were found with the prometryn, tetracycline, and propranolol MIP-coated fibers, respectively. In comparison with commercial PDMS/DVB, PDMS, and PA SPME coatings that were approximately 3 times thicker, remarkably higher extraction capacities were still observed with the three MIP coatings. This indicates that improved multiple bulk copolymerization is suitable and valuable for the development of MIP-coated SPME fibers with uniform surfaces, controllable thickness, and specific selectivity.

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