Journal of Applied Polymer Science

Mixed Templates Molecularly Imprinted Solid-Phase Extraction for the Detection of Sulfonamides in Fish Farming Water

Shili Qin,[1] Liqiang Su,[2] Peng Wang,[1] Sheng Deng[1]

[1]State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Huanghe Road, Nangang District, Harbin, China

[2]Key Laboratory of Applied Chemistry, School of Chemistry and Chemical Engineering, Qiqihar University, Wenghua Street, Jianhua District, Qiqihar, China

Correspondence to: L. Su (E-mail: slq202@163.com) and P. Wang (E-mail: pwang73@hit.edu.cn)

ABSTRACT: Highly selective molecularly imprinted polymers (MIPs) that absorb sulfonamides (SAs) are prepared using two types of SAs as mixed templates, 2-vinylpyridine as the functional monomer and ethylene glycol dimethacrylate as the crosslinker. The optimum combination of the mixed templates, their adsorption effect and the imprinting mechanism are evaluated based on SPE recoveries of a family of analytes, equilibrium binding, BET surface area analysis and UV. The results indicate that the mixed templates not only optimize the cavities of the MIPs but also improve the MIPs selectivity and adsorption capacity for the target analytes in aqueous solution. Therefore, MIPs are used for the quantitative analysis of SAs in fish farming water using off-line SPE coupled to HPLC/DAD. The recovery and RSD were 84.16–101.19 and 1.98–7.10%, respectively. Four SAs analytes were detected in four types of water samples in the range of 8.49–74.60 ng L^{-1}. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41491.

KEYWORDS: adsorption; molecular recognition; separation techniques

Received 19 April 2014; accepted 2 September 2014
DOI: 10.1002/app.41491

INTRODUCTION

Sulfonamides (SAs) are among the most widely used antibacterial agents in the world because of their low cost, low toxicity and broad spectrum of activity against common bacterial diseases.[1,2] However, these agents' increased indiscriminate use causes SAs to be released into natural ecosystems in large amounts and ingested through the food chain. It has been reported that up to 95% of the administered dose of human or veterinary drugs can be excreted un-metabolized and discharged into the environment directly through the treatment of people and animals and indirectly via the urine or feces.[3–5] There is a global concern for the veterinary applications of SAs due to the escalating spread of pathogen resistance.[6,7] Over the past few years, the fate and occurrence of SAs in the environment has become concerning.[8–12] However, it is very difficult to analyze practical environmental samples because of the low concentrations of target analytes, the complex background matrix, expensive laboratory materials, etc.[13–17]

Molecularly imprinted solid-phase extraction (MISPE) is a new type of extraction method that not only retains some advantages of SPE, such as high recovery, low solvent consumption and simple operation, but also has specific selectivity and recognition ability toward the molecular templates of MIPs.[18–22] Additionally, MISPE coupled with HPLC/HPLC–MS is more suitable for accurate quantitative analysis of trace concentrations in complex matrices.[23–26] In theory, the imprinted sites of MIPs that use single molecular templates closely match the template so that these materials cannot exhibit high affinity or selectivity for co-generic analytes.[27,28] However, in practical sample analysis, the determination of only one analyte does not accord with the actual requirements of reduced analytical time and low cost. To overcome this problem, mixed template MIPs using more than one compound as templates were prepared.[29–34] Our novel mixed templates molecularly imprinted polymers have more binding sites and stronger recognition ability compared with those of most compounds in the target family of analytes. Meanwhile interference compounds could be removed from complicated matrix effectively. Although this technique is more important for a variety of SAs residue analyses in the aquatic environment, there are few publications on the mixed templates MISPE-HPLC analysis of a family of SAs. So the research of mixed templates imprinted polymer which is more suitable for application in actual analysis is necessary.

In this research, mixed templates molecularly imprinted polymers (MMIPs) were synthesized by template-directed molecular

Table I. Preparation and Evaluation of MMIPs

Polymers	Analytes	Q_{MIP} (mg g^{-1})	Q_{NIP} (mg g^{-1})	K_{MIP} (mL g^{-1})	K_{NIP} (mL g^{-1})	k'
SMR + SMZ – MIP1	SMX	15.31	5.04	600.31	156.74	3.43
	SMZ	19.53	3.94	634.03	134.90	4.70
	SMR	21.31	4.51	649.34	168.66	3.85
SMZ + SMX – MIP2	SMX	29.27	5.04	907.52	156.74	5.79
	SMZ	21.15	3.94	723.06	134.90	5.36
	SMR	19.70	4.51	654.40	168.66	3.88
SMR + SMX – MIP3	SMX	28.71	5.04	891.85	156.74	5.69
	SMZ	13.83	3.94	474.85	134.90	3.52
	SMR	20.63	4.51	747.16	168.66	4.43
	SMX	14.10	5.04	409.09	156.74	2.61
SMR – MIP4	SMZ	14.80	3.94	391.21	134.90	2.90
	SMR	16.80	4.51	558.26	168.66	3.31
	SMX	8.98	5.04	188.09	156.74	1.20
	SMZ	17.44	3.94	284.64	134.90	2.11
SMZ – MIP5	SMR	10.97	4.51	263.11	168.66	1.56
	SMX	19.22	5.04	413.79	156.74	2.64
SMX – MIP6	SMZ	9.09	3.94	148.39	134.90	1.10
	SMR	9.43	4.51	227.61	168.66	1.35

imprinting using a noncovalent imprinting method. To expand the multiselective abilities of MMIPs (SAs), pairs of the SAs sulfamerazine (SMR), sulfamethazine (SMZ), and sulfamethoxazole (SMX) were combined to form mixed templates. The bulk MMIPs were copolymerized via covalent bonding with a functional monomer (2-vinyl pyridine; 2-Vpy), a crosslinking agent (ethylene glycol dimethacrylate; EGDMA) and an initiator (azo-*bis*-isobutyronitrile; AIBN). Furthermore, the optimal combination of molecular templates, adsorption properties and mechanism analysis were evaluated using SPE, ultraviolet spectrophotometry (UV) and Brunauer–Emmett–Teller (BET) surface area analysis. The MMIPs were used as sorbents for MISPE-HPLC to determine seven types of trace SAs in practical fish farming water, and the SPE conditions, including pH, metal ion content and elution solvent, were optimized.

EXPERIMENTAL

Reagents and Chemicals

Sulfathiazole (STZ), sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfisoxazole (SIX), SMR, SMZ, SMX, cefradine (CED), cefotamixe (CTX), trimethoprim (TMP) and formic acid (FAc) were purchased from Sigma-Aldrich (Steinheim, Germany). AIBN and *N*, *N*-dimethylformamide (DMF) were purchased from Kermel (Tianjin, China). 2-Vpy and EGDMA were purchased from Acros Organics (Beel, Belgium). HPLC-grade methanol and acetonitrile (ACN) were purchased from Kermel (Tianjin, China). All other reagents are of analytical grade. Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Standard stock solutions of SAs (1 mg mL^{-1}) were prepared in methanol and diluted to the required concentration with ultrapure water. All solutions were stored at 4°C.

Preparation of MMIPs

The synthesis of the MMIPs involved a single bulk polymerization process. The mixed templates consisted of any two pairs of the SAs SMR, SMZ, and SMX and were dissolved with a functional monomer (2-Vpy) in a solvent (DMF) in a molar ratio of 1 : 4. The mixture was prepolymerized for 8 h at room temperature. Then, it was mixed with a mixture of 3.8 mL (20 mmol) EGDMA and 60 mg (0.36 mmol) AIBN and was transferred into an ampoule tube that was purged with nitrogen for 30 min after evacuation. Polymerization was initiated at 60°C, and the reaction was allowed to continue for 24 h at the same temperature.

Upon completion of the synthesis, the bulk polymers were manually pulverized in a mortar. The powder was washed twice with methanol/formic acid (90/10, v/v) for 12 h and washed again with pure methanol for 24 h using Soxhlet extraction method. Meanwhile dipping process (6 h) and repeated washing were after each Soxhlet extraction process. Meanwhile in order to remove templates from polymer completely, the elution in Soxhlet extractor was detected by HPLC until the templates was not detected. Finally, fine particles were filtered out using a griddle with a 200-mesh sieve and precipitated to remove tiny particles. The particles were dried at 60°C under vacuum in an oven for 24 h. Mixed nonimprinted polymer (MNIPs) particles were prepared analogously in the absence of a template.

Evaluation and Characterization of MMIPs

Adsorption Test. The adsorption capacity was used to evaluate the adsorption performance of the MMIPs and MNIPs. Approximately 10 mg of the MMIPs was mixed with 10 mL of an aqueous solution of SMR, SMZ, and SMX at different concentrations (1–50 μg mL^{-1}). The mixture was then shaken evenly

Table II. Recoveries of Seven Kinds of SAs from a 0.01 μg mL^{-1} Solution with MMIPs and MNIPs

	Recovery (%)									
Polymers	STZ	SDZ	SMR	SMZ	SMX	SIX	SDM	TMP	CED	CTX
SMR + SMZ – MIP1	80.09	81.55	101.22	107.21	79.84	76.01	76.27	35.78	12.21	20.11
SMZ + SMX – MIP2	87.63	83.59	88.19	96.77	101.17	89.09	87.00	43.89	32.56	26.11
SMR + SMX – MIP3	81.96	82.66	95.34	84.33	98.66	87.58	79.43	43.66	41.09	29.43
MNIP	15.21	20.91	14.70	11.71	11.37	10.90	14.22	15.20	18.08	18.67

and incubated for 12 h. HPLC was used to measure the concentration of the analytes before and after adsorption. The adsorption capacity (Q), the partition coefficients (K), and the selectivity coefficients (k') were calculated according to the following equations:

$$Q = (C_i - C_f)V/W \quad (1)$$

$$K = Q/C_f \quad (2)$$

$$k' = K_{\mathrm{MMIPs}}/K_{\mathrm{MNIPs}} \quad (3)$$

where C_i and C_f are the initial and final concentrations of the analytes in the aqueous solution, respectively, V and W are the volume of the solution and the mass of the polymer, respectively, and Q_{max} is the maximum adsorption capacity. Curves of Q and C_i were then plotted.

MISPE Test. SPE tubes (3 mL) were padded uniformly with 60 mg of MMIPs or MNIPs and fixed with two PTFE frits. The tubes were balanced washing with 1 mL methanol and 1 mL water before sample loading. A 5 mL standard SAs solution in blank raw water was loaded into the preconditioned tube and washed with 5% methanol. An eluting step was performed using 3 mL elution solvent, and elutes were dried under a gentle nitrogen stream. The residue was dissolved with 1 mL of the mobile phase for subsequent HPLC analysis.

Brunauer–Emmett–Teller (BET) Analysis. The pore size distribution and surface area of the polymers were measured using a group point N_2 adsorption NOVA 2000e analyzer (Quantachrome). A 100 mg sample of the dried polymer was used for analysis. All samples were degassed at room temperature for 12 h under nitrogen flow prior to measurement. Nitrogen adsorption/desorption isotherms were recorded at 285 K. The BJH method was applied to calculate the pore size distribution from the analysis of the adsorption and desorption branches of the isotherms.

HPLC Analysis

Chromatographic analysis was carried out with an Agilent1100 HPLC system, which was equipped with a diode array detector (DAD). Seven types of SAs analytes were separated using a Diamosil C18 column (4.6 × 250 mm) packed with 5 μm diameter particles. A gradient program was used with the mobile phase by combining (A) 0.5% v/v formic acid in water and (B) methanol as follows: the mobile phase (A) was varied from 90% at $t = 0.0$ min to 80% at $t = 5.0$ min and switched to 60% at 5.0–12.0 min. Then, (A) was varied from 60 to 35% for 8.0 min. Finally, the mobile phase (A) was recycled to 90% for 5.0 min and equilibrated for 5.0 min. The flow rate during the separation gradient was 1 mL min^{-1}. The LC column temperature was set to 35°C. The injection volume for all LC experiments was 100 μL. The DAD system was set to 270 nm according to the maximum adsorption wavelengths of the SAs.

Mechanism of Action Between the Molecular Template and Functional Monomer

The molecular templates (SMZ, SMX, SMZ, and SMX) and the functional monomers were mixed in different proportions (1 :

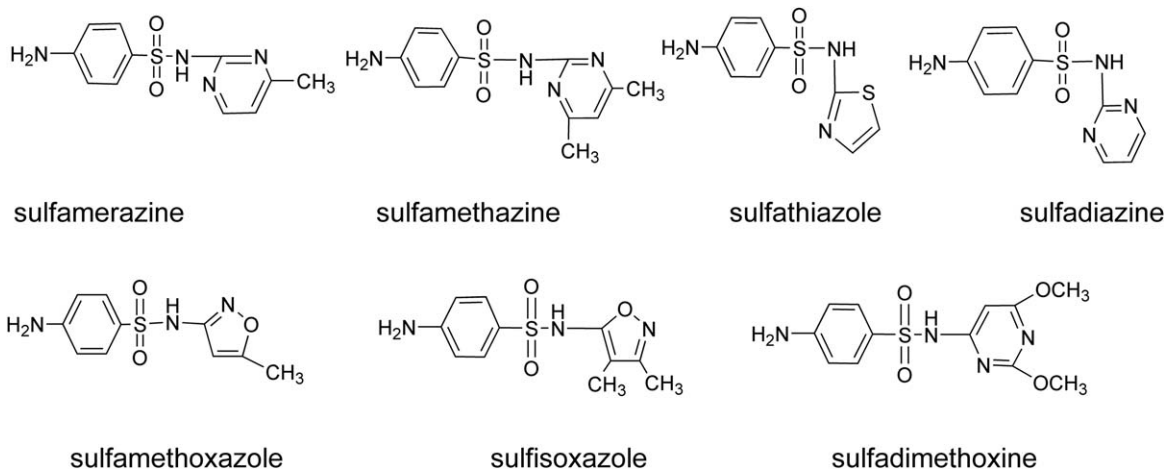


Figure 1. Structures of seven kinds of SAs.

Table III. The Pore Structure Characters of the Mixed-Templates MIPs

Type	a_s, BET (m^{-2} g^{-1})[a]	Total pore volume (cm^{-3} g^{-1})	Average pore diameter (nm)
SMR + SMZ – MIP1	127.57	0.46	4.81
SMZ + SMX – MIP2	155.82	0.69	5.82
SMR + SMX – MIP3	135.79	0.54	4.99
MNIP	35.96	0.28	4.72

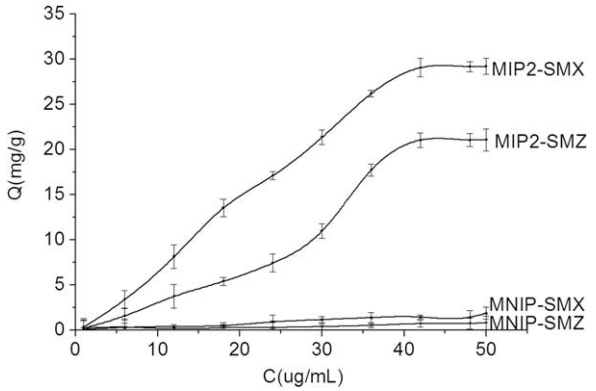


Figure 2. Adsorption equilibrium isotherm of the MIP2 and MNIP for SMX and SMZ.

1–1 : 5) in 10 mL DMF solvent. The concentration of the molecular templates was 0.1 mol L^{-1}. Then, the prepared solution was allowed to rest for 12 h at room temperature. The absorbance values of the different solution were measured and recorded at 270 nm by an ultraviolet–visible spectrophotometer (Evolution 201).

Practical Sample Application

The method employed was validated before practical sample application. An external standard method was used for quantitative analysis. A series of working standards of SAs analytes were diluted with ultrapure water to seven concentrations ranging from 10 to 2000 ng mL^{-1}. Precision and recovery experiments were performed by analyzing the water sample spiked with a standard solution six consecutive times at 50, 100, and 2000 ng L^{-1}. Additionally, the limits of detection (LOD) and enrichment factor (EF) for this method were calculated using the noise of the HPLC profile and the ratio of the slopes of the calibration curves.

Fish farming water samples from four sites of the quality supervision and inspection center of the fishery environment and from the aquatic products of the Agriculture Ministry in Harbin, including raw water, aquarium water, hatching water and overwintering water, were collected in 2.5-L rinsed amber glass bottles in May of 2013 and stored at 4°C until the measurements were performed. The samples were filtered through a 0.45-μm filter (Navigator, Lab Instrument) to remove suspended matter, and the pH was adjusted. Afterward, 500 mL of water was pretreated using homemade MISPE cartridges packed with 500 mg of the MMIPs that were prepared as described in "MISPE Test". Finally, the column packing was eluted with 5 × 1 mL of elution solvent. The elution solution was dried under N_2 and redissolved with 1 mL of the mobile phase for HPLC analysis.

RESULTS AND DISCUSSION

Preparation of MMIPs

The selectivity and recognition ability of MIP cavities toward molecular templates and their analogs mainly depends on the shape and size of the molecular template and the functional groups of the monomer. Compared to single-template MIPs, double imprinted templates introduced an additional degree of freedom in material design.[34] More appropriate cavities for the recombination and release of the analytes were applied using the mixed molecular templates.[33] These cavities provide a channel through which the target analytes can be rapidly captured.[35]

To increase the MIPs' adsorption performance and selectivity in this experiment, three types of MMIPs were synthesized in mixed molecular template pair combinations using SMR, SMZ, and SMX and using alkaline and hydrophobic 2-Vpy as the functional monomers according to prior research.[36] The mixed template combinations were optimized using the trough Q_{max}, K, k' and the recoveries (α) of the target analytes.[37,38]

K and k' represent the ratio of the bound molecular target to the residual amount in the solution phase and the ratio of the partition coefficient of the imprinted materials to that of the nonimprinted ones, respectively. According to the Q_{max}, the initial concentration was defined as 100 μg mL^{-1}. As shown in Table I, the Q_{max} and K of the MMIPs were significantly higher than those of the triploid MNIPs. Samples in which different MMIPs were incorporated into their templates presented similarly higher values of Q_{max} and K than those of the other nonimprinted molecular templates. For example, the Q_{max} and K were lower for MIP1, which used SMZ and SMR as the mixed templates, than for the samples incorporating SMX (15.31 mg g^{-1}, 600.31 mL g^{-1}), SMZ

Table IV. Langmuir and Freundlich Isotherm Parameters for Adsorption of SMX and SMZ

	Langmuir		Freundlich	
Sample	Equation (1)	R^2	Equation (2)	R^2
MIP2 - SMZ	$y^b = 0.01x^a + 3.04$	0.591	$y^d = 1.260x^c + 1.91$	0.975
MIP2 - SMX	$y^b = 0.009x^a + 0.40$	0.237	$y^d = 1.571x^c + 1.69$	0.956

[a] The equilibrium adsorption concentration of SMZ or SMX.
[b] The molar ratio of the equilibrium adsorption capacity/the equilibrium adsorption concentration of SMZ or SMX.
[c] The logarithm of equilibrium adsorption concentration of SMZ or SMX.
[d] The logarithm of equilibrium adsorption capacity.

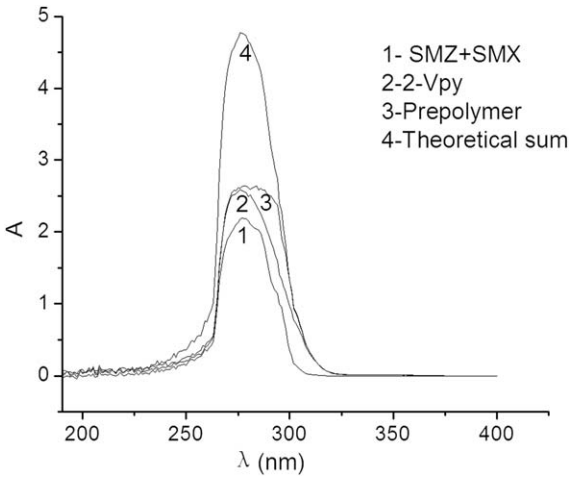


Figure 3. UV spectra of the mixture of mixed templates and 2-Vpy in DMF solution.

(19.53 mg g^{-1}, 634.03 mL g^{-1}), and SMR (21.31 mg g^{-1}, 649.34 mL g^{-1}). These data demonstrated that the imprinted cavities and the active sites left by the molecular template determined the affinity and specific recognition ability toward their molecular templates in the MMIPs. Additionally compared with the prior research of single template MIPs (MIP4–MIP7), mixed templates MIPs performed better on Q_{max}, K, and k' of SAs[36] in Table I. The results stated that double templates provided more cavities for SAs and enhanced the imprinted performance of the polymer. Furthermore, MIP2 exhibited the maximum k' value of the three different types of MMIPs, which displayed the best imprinted effects and adsorption capacities.

MMIPs would be used as SPE packing in practical sample analysis; thus, α was highly necessary for assessing the multiselective adsorption of the seven types of SAs for the MMIPs. The data presented in Table II suggested that the highest recoveries were obtained from the three MMIPs with their respective templates (95.34–107.21%), and the recoveries of the family of SAs were lower (76.01–88.19%). This result was observed because the imprinted molecular template was sized and shaped to fit into the cavities, and when combined at the top speed, fewer cavities remained to readsorb other structural analogs.

As indicated by the recovery data shown in Table II, the cavities of MIP1 were not beneficial to absorb SMX and SIX because of the noticeable structural difference between mixed templates (SMR and SMZ) and the target analytes (SMX and SIX). Moreover the O of SMX or S of SIX atoms in heterocyclic structures as strong hydrogen bond acceptors could easily form intramolecular hydrogen bonds with —NH— (Figure 1). So that the interaction was weaken between target analytes (SMX and SIX) and special recognized sites of MIP1. Furthermore SDM recovery of MIP3 was lower than that of MIP2. This result was obtained because the molecular size of SDM, which was calculated by Gaussian equation, was larger than that of the cavities formed by mixed templates (SMX and SMR) in MIP3. The recovery of data in Table II manifested that SMZ and SMX were the best combination of mixed templates due to form different sizes and structures cavities in MIP2. In conclusion, MIP2 was the most excellent MMIP and was selected for further experiments.

Furthermore the cross reactivity of MMIPs in MISPE experiment also was studied. The recoveries of TMP, CTX and CED were in range from 12.21 to 43.89%, which were less than other seven SAs. It demonstrated that MMIPs not only showed "group selectivity" for SAs but also eliminated the impurities disturbance in complicated matrix.

BET Method Analysis

The specific surface areas and pore size distributions of the MIPs have a direct effect on the adsorption capacity of the MIPs. At larger specific surface areas and pore volumes, a higher capacity for molecular combination is observed. For four polymers, the specific surface areas and pore volumes of the different mixed templates forming the MMIPs were greater than those of the MNIPs, and those of MIP2 were the highest, as shown in Table III. It may be that that after eluting templates, the number of imprinted cavities for template molecular circulating were retained in MMIPs so that the adsorption capacity was greater than those in MNIPs.[39] Additionally, the mixed SMZ and SMX templates interacted with the functional monomer easily and also formed more stable prepolymers. Thus, more cavities were formed in MIP2, which were more beneficial to substance exchange between the target analytes and recognition sites. And the adsorption speed of the target molecular was

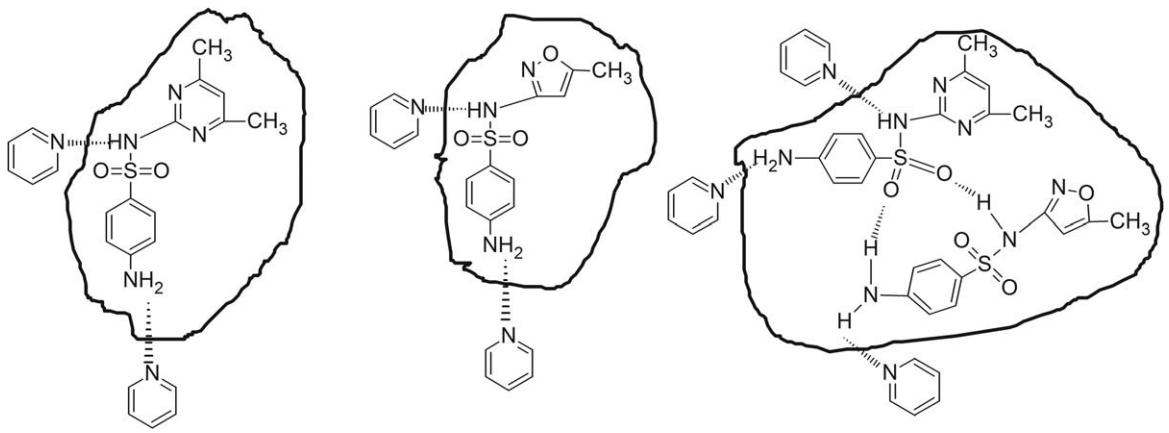


Figure 4. Effect protocol for mixed templates and 2-Vpy.

Table V. Linear Regression Equation and Correlation Coefficient of $\Delta A/b_0^n - \Delta A$

Template	SMZ			SMX			SMZ+SMX		
	$\Delta A/b_0^n - \Delta A$	R^2	k	$\Delta A/b_0^n - \Delta A$	R^2	k	$\Delta A/b_0^n - \Delta A$	R^2	k
$n = 1$	$y^b = -1.777x^a + 3.56$	0.641	1.777	$y = -0.873x + 6.989$	0.775	0.873	$y = -4.487x + 9.666$	0.958	4.487
$n = 2$	$y = -43.4x + 40.44$	0.980	43.42	$y = -16.49x + 48.01$	0.996	26.49	$y = -81.41x + 122.0$	0.994	81.41
$n = 3$	$y = -184.7x + 399.0$	0.892	184.7	$y = -107.2x + 262.2$	0.964	107.2	$y = -238.1x + 355.4$	0.995	238.1
$n = 4$	$y = -4913x + 3894$	0.861	4913	$y = -585.0x + 1340$	0.917	585.0	$y = -7864x + 10630$	0.758	7864
$n = 5$	$y = -48997x + 38304$	0.822	48997	$y = -3016x + 6699$	0.872	3016	$y = -78042x + 10466$	0.723	78042

[a] The ΔA.
[b] The ratio of $\Delta A/b_0^n$.

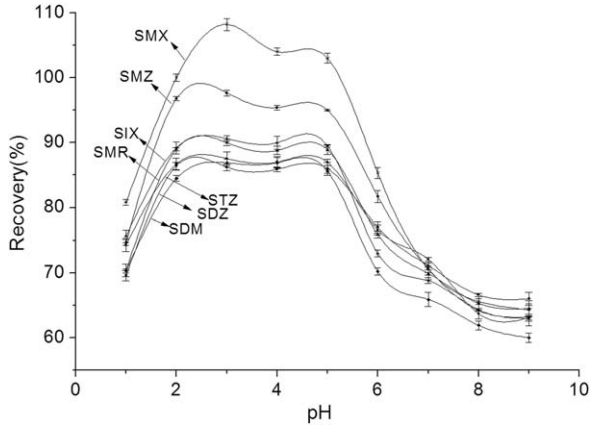


Figure 5. Influence of sample pH (1–9) for MISPE recovery.

enhanced. These results are consistent with the conclusion drawn in "Preparation of MMIPs".

Static Adsorption Analysis

The MMIPs, which were synthesized using mixtures of the SMZ and SMX templates in this study, consisted of two or multiple binding sites for a group of SAs. To evaluate the adsorption properties of MIP2 samples with respect to their templates and to describe the adsorption mode active in these samples, a saturation adsorption experiment and adsorption isotherms were carried out as shown in Figure 2 and Table IV. Figure 2 showed that the adsorption capacity of the MMIPs increased with an increase in the concentrations of SMZ and SMX and was closer to equilibrium at higher concentrations. Compared with that of the MNIPs, the amount of the templates of the MMIPs was much higher. This finding suggested that because of noncovalent effects between the template and functional monomer, the cavities with selective recognition and specific adsorption properties developed during MMIP synthesis. Furthermore, Langmuir, and Freundlich models were used to fit the adsorption data and were expressed, respectively, as follows:

$$C_e/Q_e = C_e/Q_{\max} + 1/k\,Q_{\max} \quad (4)$$

$$lg\,Q_e = lg\,C_e/n + lg\,Q_{\max} \quad (5)$$

where Q_e is the equilibrium adsorption capacity (mg g^{-1}), C_e is the equilibrium adsorption concentration of SMZ or

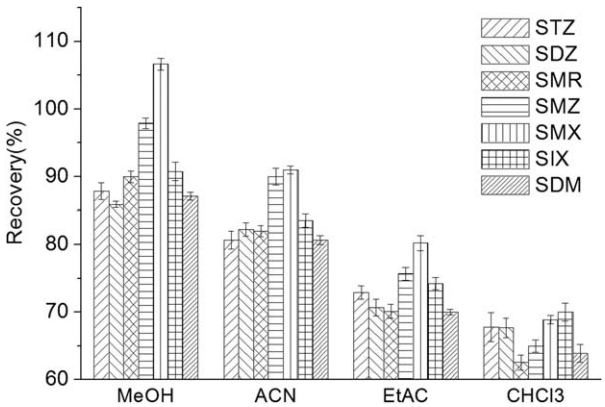


Figure 6. Influence of elution solvent types for MISPE recovery.

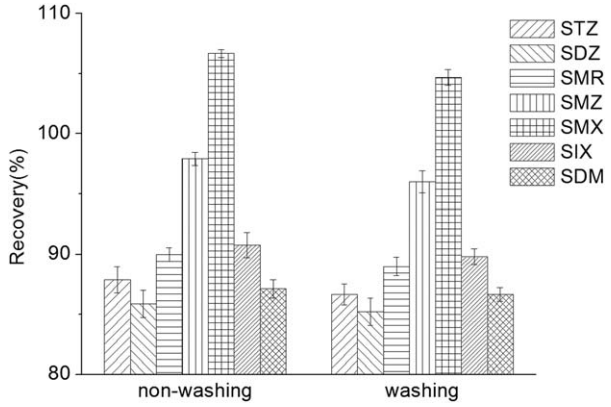


Figure 7. Influence of washing and nonwashing for MISPE recovery.

SMX (mg L^{-1}), Q_{max} is the maximum adsorption capacity (mg g^{-1}), k is the Langmuir adsorption equilibrium constant, and n is the Freundlich adsorption index.

From the linearly dependent coefficient of the fitted equations in Table IV, the Freundlich isotherm model was determined to fit the equilibrium data significantly better than the Langmuir model, which meant that multi sites adsorption occurred in the imprinted polymer. It is known that the Freundlich model is a continuous distribution model. The affinity distribution curve of the binding points (N), which changes with the binding constant (K), is continuous, and each K value has a corresponding N value. The Langmuir model, which is a discrete distribution model, is based on the assumption that MMIPs are relatively homogeneous during the adsorption of a monolayer and contain a single binding site.[40] During the synthesis of the MIP2 samples, the various binding sites that were formed between two classes of templates and functional monomer appeared to be nonhomogeneous and exhibited different affinities. Thus, the Freundlich model was more applicable to most of the noncovalently imprinted polymers, and more accurate relevant parameters were obtained.

Mechanism of Action

UV spectroscopy is a technique capable of determining the modification of the maximum absorption of a molecular template. The imprinting process begins with a complexation between functional monomers and templates via hydrogen bonding such that the formation of this bond can be readily identified using this technique.[41] As shown in Figure 3, the absorption value of prepolymer (3) was clearly less than that of the theoretical sum (4) of the templates (1) and functional monomer (2). Additionally, a slight red shift of the absorption value of the prepolymer (3) could be observed. These results indicated that there were certain interactions between the templates and the functional monomers, and the prepolymer was also formed in the following crosslinking polymerization reaction.

To analyze the binding constants and the chemical coordination numbers of the templates and functional monomers, eq. (6) was applied to describe the interactions between the receptor and ligand[42]:

$$\Delta A / b_0^n = -k\Delta A + k\Delta\varepsilon a_0 l \quad (6)$$

where ΔA is the absorbance difference before and after the interaction between the template and the functional monomer, n is the chemical coordination number, b_0 and a_0 are the initial concentrations of the functional monomer and the template, respectively, $\Delta\varepsilon$ is the absorbance coefficient and k is the binding constant. When any positive integer ($n = 1, 2, 3\ldots$) is used in plotting $\Delta A / b_0^n$ against ΔA, the relationship between $\Delta A / b_0^n$ and ΔA is linear when the value of n is reasonable. As shown in Table V, the relationship between $\Delta A / b_0^n$ and ΔA was linear at $n = 2$ when SMZ or SMX was used as the single template. Similarly, $\Delta A / b_0^n$ and ΔA showed good linear relationships at $n = 2$ and 3 when SMZ and SMX were used as mixed templates. According to chemical coordination ratios of 1 : 2 and 1 : 2–1 : 3, single molecular templates and mixed templates, respectively, formed host–guest complex with 2-Vpy through noncovalent interactions (Figure 4). Additionally, the binding constant of the mixed templates was greater than that of the single template when the values of n were equivalent, as shown in Table V. These results were obtained because during the prepolymerization process, the molecular template and excessive functional monomers were liable to complete the high coordination numbers. The interaction between double molecular templates could have generated nonhomogeneity in the MIPs and increased the number of binding affinity sites.[40] Thus, it was advantageous for multicavities to be formed for the adsorption of the SAs.

Table VI. Recoveries of Seven Kinds of SAs Spiked at Three Concentration Levels

	50 ng L^{-1}		100 ng L^{-1}		2000 ng L^{-1}	
Analyte	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
STZ	84.16	7.10	87.63	5.23	87.49	3.92
SDZ	83.29	2.57	83.59	4.69	86.59	1.78
SMR	89.90	4.45	88.19	6.47	88.79	3.56
SMZ	96.09	3.95	96.77	3.26	96.96	3.42
SMX	98.19	2.91	101.17	4.44	101.19	4.01
SIX	90.99	2.44	89.09	4.91	90.90	3.56
SDM	87.11	1.98	87.00	3.89	86.67	2.44

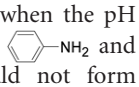
Table VII. Calibration Curve, LOD, and EF of Seven Kinds of SAs

Analyte	Calibration curve[c]	R^2	LOD (ng L^{-1})	Calibration curve[d]	EF
STZ	$y^b = 308.2x^a + 0.267$	0.9993	7.77	$y = 0.7281x - 0.016$	423
SDZ	$y = 337.1x + 1.462$	0.9994	4.46	$y = 0.8143\ x + 0.002$	414
SMR	$y = 411.7\ x + 1.170$	0.9991	6.63	$y = 0.9508\ x + 0.010$	433
SMZ	$y = 401.1x - 0.263$	0.9993	10.41	$y = 0.7958x - 0.003$	504
SMX	$y = 564.5\ x + 0.141$	0.9994	12.34	$y = 1.079\ x + 0.016$	523
SIX	$y = 572.8x - 0.050$	0.9996	6.98	$y = 1.203x - 0.001$	476
SDM	$y = 530.4x - 0.227$	0.9997	9.97	$y = 1.275x - 0.014$	416

[a] The concentration of SAs.
[b] The chromatographic peak area.
[c] The calibration curve before enrichment.
[d] The calibration curve after enrichment.

Effects of pH and Metal Ion Concentration

In practical applications, regional disparities lead to the complexity of the environmental background of water bodies, which is not conductive to the detection of trace substances. A pretreatment experiment was conducted by regulating pH[43,44] and adding a chelating agent[45] to mask metal ions and to enrich and separate the target analytes of the optimum molecular form through SPE. Experimental fish farming water was sampled from ground water of the Hulan district, and pH and metal concentrations were determined using national standards. The results were as follows: (1) the raw water was alkalescent at pH 8.07, and (2) the contents of Ca^{2+} and Fe^{3+} were higher at $C_{Ca} = 68.98$ mg L^{-1} and $C_{Fe} = 0.44$ mg L^{-1}.

The effects of pH (2.0–5.0) and the concentrations of Ca^{2+} and Fe^{3+} (10.00–90.00 and 0.1–0.9 mg L^{-1}, respectively) on the SPE recoveries were studied. The recoveries were high when the pH was in the range of 2.0–5.0, as shown in Figure 5.  and –SO_2—NH– as the ionized groups of SAs could not form hydrogen bond with affinity sites of MIP2 so that the ability of recognition and bond between the absorbed molecular and MIP2 were weaken. Thus, the recoveries of the seven target analytes (60.00–80.80%) were lower when the pH was greater than 5.0 or less than 2.0. Additionally, the increase in the concentrations of Ca^{2+} and Fe^{3+} had only a slight effect on the recoveries of the seven SAs, the variations of recoveries were in range from 1.00 to 5.31%. This result was a manifestation of the SAs difficulty in forming complexes with bivalent and trivalent metal ions. Thus, MIP2, as the SPE packing for the target analytes, not only exhibited high recoveries over a wide pH range but also effectively eliminated the interferents and simplified the aquatic pretreatment. In the following experiments on practical water samples, the chelating agent (EDTA) of the masking metal ions was not added.

MISPE Analysis

SPE is the most common sample preparation method and is extremely important in the area of environmental analysis. According to the partition coefficient ($lg\ K_{ow}$), six of the SAs dissolved in organic solvent more easily than in aqueous solution; however, SDZ, which had a solubility that is approximately equal in the two types of solvent, did not. As shown in Figure 6, the recoveries of SAs by ACN and MeOH as the elution solvents were significantly higher than those of ethyl acetate and chloroform in four solvent tests. The stronger polar solvents could destroy the hydrogen bonds and cause the target analytes to desorb completely. Because of the low viscosity of ACN, the retention time was too short to enhance the interaction between the solvent and analytes. Thus, pure methanol was the best elution solvent.

The washing steps of SPE could remove impurities and improve the sensitivity and recoveries of the target analytes for water samples with a complex matrix. However, the results of Figure 7 showed that the washing steps had little effect on the

Table VIII. The Concrete Contents of Seven Kinds of SAs in Different Water Samples

Analyte	Overwintering pond water (ng L^{-1})	Pisciculture pond water (ng L^{-1})	Raw water (ng L^{-1})	Hatching pond water (ng L^{-1})
STZ	-[a]	-	-	-
SDZ	-	-	-	-
SMR	25.99	18.76	74.60	36.33
SMZ	18.40	23.00	15.58	-
SMX	-	-	-	-
SIX	8.49	15.10	14.47	14.82
SDM	14.89	20.90	58.79	33.42

[a] No residue detected.

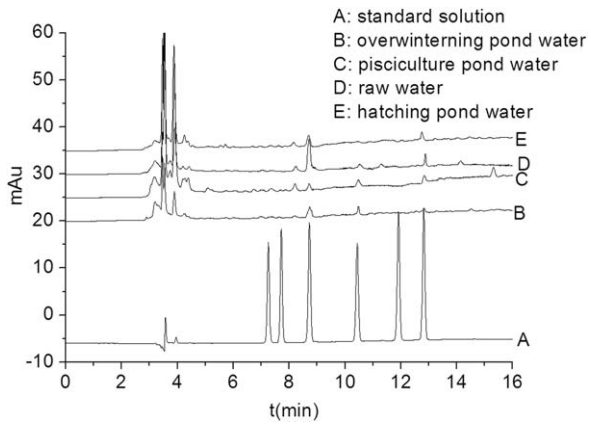


Figure 8. The chromatographic profiles at 270 nm from the analysis of the sample. 1:STZ;2:SDZ;3:SMR;4:SMZ;5:SMX;6:SIX;7:SDM.

recoveries of the seven SAs. Thus, the washing steps were omitted in the large-batch actual aquatic analysis. The molecular imprinting technique simplified the experimental process and reduced the pretreatment time for the analysis of large, actual water samples.

Practical Fish Farming Water Analysis

A rapid, accurate and less organic-solvent-consuming MISPE-HPLC method was established using MIP2 as the SPE packing for the detection of trace SAs in fish farming water. Seven SAs were quantitatively analyzed using extra-calibration curves over the wide range of 10.0–2000.0 ng mL^{-1}. The method exhibited a linear relationship between the extent of adsorption and the concentration of the adsorbing materials, and the coefficients of determination (R^2) were typically greater than 0.999. Based on the recoveries obtained via EF and RSD experiments, we demonstrated that the MISPE enrichment effects on the SAs were favorable. The overall recoveries of all target analytes ranged from 83.29 to 101.19%, with a relative standard deviation (RSD) less than 7.10%. The LOD and EF in wastewater samples were defined using the lowest concentration producing a signal-to-noise ratio (S/N) of 3, and the ratios of the slopes of the calibration curves before and after enrichment were in the ranges of 4.46–12.34 ng L^{-1} and 414–523, respectively. As shown in Tables VI, VII, VIII and Figure 8, the results illustrate that SMR, SMZ, SMX, and SDM were primarily detected, with their contents falling in the range of 8.49–74.60 ng L^{-1}.

CONCLUSIONS

In this study, we demonstrated the applicability of preparing novel MIPs using SMZ and SMX as mixed templates for preconcentrating a group of SAs. The characterization, recognition capacity and action mechanism between the templates and the functional monomers were analyzed and discussed. The results suggest that mixed-template MIPs exhibited better selectivity and affinity for a group of SAs than single-template MIPs. Thus, the optimized method included a MISPE procedure followed by HPLC with diode-array detection for fish farming water. The LOD and EF for the SAs were 4.46–12.34 ng L^{-1} and 414–523, respectively. Additionally, this method was proven to be suitable for the screening and analysis of SAs in various environmental water samples.

REFERENCES

1. Connor, E. E. *Primary Care Update OB/GYNS* **1998**, *5*, 32.
2. Baran, W.; Adamek, E.; Ziemiańska, J.; Sobczak, A. *J. Hazardous Mater.* **2011**, *196*, 1.
3. Jiang, S. M.; Chen, Y. J.; Sun, Q. X.; Hai, D.; Chin, J. *J. Nosocomiol.* **1995**, *5*, 42.
4. Kim, H.; Hong, Y.; Park, J.; Sharma, V. K.; Cho, S. *Chemosphere* **2013**, *91*, 888.
5. Maszkowska, J.; Kołodziejska, M.; Białk-Bielińska, A.; Mrozik, W.; Kumirska, J.; Stepnowski, P.; Palavinskas, R.; Krüger, O.; Kalbe, U. *J. Hazardous Mater.* **2013**, *260*, 468.
6. Białk-Bielińska, A.; Stolte, S.; Arning, J.; Uebers, U.; Böschen, A.; Stepnowski, P.; Matzke, M. *Chemosphere* **2011**, *85*, 928.
7. Göbel, A.; McArdell, C. S.; Joss, A.; Siegrist, H.; Giger, W. *Sci. Tot. Environ.* **2007**, *372*, 361.
8. Kasteel, R.; Mboh, CM; Unold, M.; Groeneweg, J.; Vanderborght, J.; Vereecken, H. *Environ. Sci. Technol.* **2010**, *44*, 4651.
9. Kim, H.; Hong, Y.; Park, J.; Sharma, V. K.; Cho, S. *Chemosphere* **2013**, *91*, 888.
10. Jesús García-Galán, M. J.; Silvia Díaz-Cruz, M. S.; Barceló, D. *Environ. Int.* **2011**, *37*, 462.
11. Murata, A.; Takada, H.; Mutoh, K.; Hosoda, H.; Harada, A.; Nakada, N. *Sci. Tot. Environ.* **2011**, *409*, 5305.
12. Gao, P.; Munir, M.; Xagoraraki, I. *Sci Tot. Environ.* **2012**, *421*, 173.
13. Białk-Bielińska, A.; Kumirska, J.; Palavinskas, R.; Stepnowski, P. *Talanta* **2009**, *80*, 947.
14. Sajid, M.; Na, N.; Safdar, M.; Lu, X.; Ma, L.; He, L.; Ouyang, J. *J. Chromatogr. A* **2013**, *1314*, 173.
15. Lu, K. H.; Chen, C. Y.; Lee, M. R. *Talanta* **2007**, *72*, 1082.
16. Lin, C. Y.; Huang, S. D. *Anal. Chim. Acta.* **2008**, *612*, 37.
17. Lillenberg, M.; Yurchenko, S.; Kipper, K.; Herodes, K.; Pihl, V.; Sepp, K.; Lõhmus, R.; Nei, L. *J. Chromatogr. A* **2009**, *1216*, 5949.
18. Wang, Y.; Wang, E.; Wu, Z.; Li, H.; Zhu, Z.; Zhu, X.; Dong, Y. *Carbohydr. Polym.* **2014**, *101*, 517.
19. Quesada-Molina, C.; Claude, B.; García-Campaña, A. M.; Olmo-Iruela, M.; del, Morin P. *Food Chem.* **2012**, *135*, 775.
20. He, C.; Long, Y.; Pan, J.; Li, K.; Liu, F. *J. Biochem. Biophys. Methods* **2007**, *70*, 133.
21. Puoci, F.; Garreffa, C.; Iemma, F.; Muzzalupo, R.; Spizzirri, U. G.; Picci, N. *Food Chem.* **2005**, *93*, 349.
22. Yin, J.; Wang, S.; Yang, G.; Yang, G.; Chen, Y. *J. Chromatogr. B* **2006**, *844*, 142.
23. Shi, Y.; Peng, D. D.; Shi, C. H.; Zhang, X.; Xie, Y. T.; Lu, B. *Food Chem.* **2011**, *126*, 1916.
24. Sun, H.; Qiao, F. *J. Chromatogr. A* **2008**, *1212*, 1.

25. Ebrahimzadeh, H.; Molaei, K.; Asgharinezhad, A. A.; Shekari, N.; Dehghani, Z. *Anal. Chim. Acta* **2013**, *767*, 155.
26. Shah, K. A.; Halquist, M. S.; Karnes, H. T. *J. Chromatogr. B* **2009**, *877*, 1575.
27. Chen, F. F.; Xie, X. Y.; Shi, Y. P. *J. Chromatogr. A* **2013**, *1300*, 112.
28. Urraca, J. L.; Carbajo, M. C.; Torralvo, M. J.; González-Vázquez, J.; Orellana, G.; Moreno-Bondi, M. C. *Biosens. Bioelectr.* **2008**, *24*, 155.
29. Liu, W.; Liu, X.; Yang, Y.; Zhang, Y.; Xu, B. *Fuel* **2014**, *117*, 184.
30. Duan, Y. P.; Dai, C. M.; Zhang, Y. L.; Chen, L. *Anal. Chim. Acta* **2013**, *758*, 93.
31. Dai, C. M.; Zhang, J.; Zhang, Y. L.; Zhou, X. F.; Duan, Y. P.; Liu, S. G. *Chem. Eng. J.* **2012**, *211*, 302.
32. Huang, C.; Tu, Z.; Shen, X. *J. Hazard Mater.* **2013**, *248*, 379.
33. Jing, T.; Wang, Y.; Dai, Q.; Xia, H.; Niu, J.; Hao, Q.; Mei, S.; Zhou, Y. *Biosens. Bioelectr.* **2010**, *25*, 2218.
34. Dickert, F. L.; Achatz, P.; Halikias, K.; Fresenius, J. *Anal. Chem.* **2001**, *371*, 11.
35. Prasad, B. B.; Rai, G. *Spectrochim. Acta A Mol. Biomol. Spectrosc* **2012**, *88*, 82.
36. Qin, S.; Deng, S.; Su, L.; Wang, P. *Anal. Methods* **2012**, *4*, 4278.
37. Xie, C.; Liu, B.; Wang, Z.; Gao, D.; Guan, G.; Zhang, Z. *Anal. Chem.* **2008**, *80*, 437.
38. Valtcheva, M.; Palma, B. S.; Schiller, M.; Steinfelda, U. *J. Hazard Mater.* **2009**, *170*, 722.
39. Tominaga, Y.; Kubo, T.; Yasuda, K.; Kato, K.; Hosoya, K. *Microporous Mesoporous Mater.* **2012**, *156*, 161.
40. Umpleby II, R. J.; Baxter, S. C.; Rampey, A. M.; Rushton, G. T.; Chen, Y.; Shimizu, K. D. *J. Chromatogr. B* **2004**, *804*, 141.
41. Karim, K.; Breton, F.; Rouillon, R.; Piletska, E. V.; Guerreiro, A.; Chianella, I.; Piletsky, S. A. *Adv. Drug Deliv. Rev.* **2005**, *57*, 1795.
42. Piletsky, S. A.; Panasyuk, T. L.; Piletskaya, E. V.; Nicholls, I. A.; Ulbricht, M. *J. Membr. Sci.* **1999**, *157*, 263.
43. McClure, E. L.; Wong, C. S. *J. Chromatogr. A* **2007**, *1169*, 53.
44. Białńska, A.; Kumirska, J.; Palaviska, R.; Stepnowski, P. *Talanta* **2009**, *80*, 947.
45. Chang, H.; Hu, J.; Asami, M.; Kunikane, S. *J. Chromatogr. A* **2008**, *1190*, 390.