Preparation and Evaluation of a Molecularly Imprinted Polymer for Tolazoline

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ABSTRACT: A uniformly sized molecularly imprinted polymer for the peripheral vasodilator drug tolazoline (T-MIP) was prepared, and a nonimprinted polymer (NIP) was also synthesized in the same way but in the absence of the template. The T-MIP was prepared with methylacrylic acid as functional monomer and ethylene glycol dimethacrylate as crosslinker by a multistep swelling and polymerization method. These imprinted materials were characterized by scanning electron microscopy, nitrogen adsorption, and static adsorption experiments. Binding studies were also performed to evaluate the uptake of T-MIP and NIP with the results that T-MIP had a significantly higher binding capacity for tolazoline (T) than did NIP. The maximum static adsorption capacities of T-MIP and NIP for T were 78.9 and 38.8 μmol/g, respectively. The T-MIPs and NIPs were used as stationary phases of solid-phase extraction (SPE), and a relative selectivity coefficient (k') value of 5.21 was obtained, which showed that the T-MIP sorbent had higher selectivity than the NIP sorbent. The method was applied to the determination of T in urine samples. The prepared polymer sorbent showed promise for SPE for gas chromatography determination of T in urine samples.
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Key words: molecular imprinting; polystyrene; step-growth polymerization

INTRODUCTION

Molecular imprinting is a technique for synthesizing organic polymers that contain recognition sites for small molecules.[1] It is a versatile tool for the preparation of separation materials with predetermined selectivities. The molecularly imprinted polymers (MIPs) approach has already been used successfully for mimicking natural receptors and for the synthesis of polymers carrying binding sites with high affinities toward drugs, small analytes, peptides, and proteins.[2] The advantages that MIPs holds over natural receptors, such as their stability at extremes of pH and temperature, high mechanical strength, low cost, and reusability, have led to the development of various MIP applications, including chromatography, artificial antibodies, chemical sensors, and solid-phase extraction (SPE).[3–12] In recent years, there has been an increase in the application of highly selective MIPs in the assays of drugs.[13] In addition, molecularly imprinted solid-phase extractions (MISPEs) are a valid method for determining trace analytes, particularly in complex pharmaceutical and biomedical samples.[14–16]

Molecular imprinting in organic polymer matrices has received an increasing amount of attention in recent years.[17,18] To date, imprinted polymers in the form of particles are reportedly made by bulk, suspension, emulsion, multistep swelling, and precipitation polymerization methodologies, each of them developed to suit specific targets.[19] Among these methods, multistep swelling is good for the preparation of variously functional microspheres, which was applied to the synthesis of MIPs for tolazoline (T) recognition in this study.

T (Fig. 1) has been used extensively since 1939 for its histaminergic, α-adrenergic antagonist, and vasodilator activities.[20] T is administered frequently to newborn infants as a pulmonary vasodilator.[21] Because repeated administration to neonates has been associated with undesirable side effects, careful monitoring of the dosage regimen is necessary.[22] Several methods have been reported for the determination of T and other imidazoline derivatives, such as spectrophotometry, colorimetry, thin-layer chromatography, gas–liquid chromatography, and liquid column chromatography.[23]

MIPs used as tailored sorbents for molecular recognition based on SPE have many merits. First, MIPs are materials with features of predetermination

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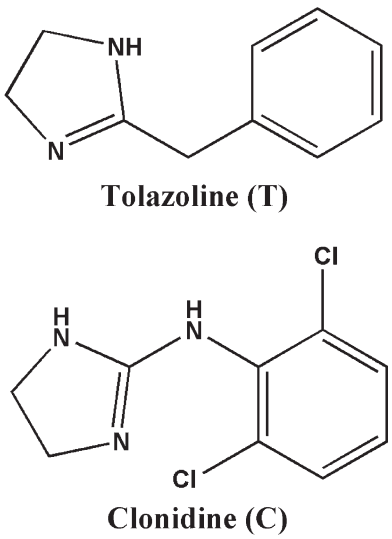

Figure 1 Structures of T and C.

and specific recognition.[24] Second, extraction with MIPs as a stationary phase is fast and cost effective, and the time of sample pretreatment is less than 2 h. Third, recoveries achieved with the MIPs are higher than 90%, and the clean extracts allow low detection levels with minimized matrix effects.[25] Fourth, MISPE not only can preconcentrate the analyte but also can remove the other compounds present in the sample matrix, which is important when the sample is complex and impurities can interfere with quantification.[26]

In this article, we report a new procedure for the separation of T by MIPs. MIP particles imprinted with T were prepared by multistep swelling polymerization, and its recognition characteristics were studied. Finally, it was preliminarily applied to SPE sorbents coupled with gas chromatography (GC) for T determination.

EXPERIMENTAL

Reagents and chemicals

Tolazoline hydrochloride [4,5-dihydro-2-(phenylmethyl)-1*H*-imidazole hydrochloride; 99%], methylacrylic acid (MAA), and ethylene glycol dimethacrylate (EDMA) were purchased from Acros Organics (Morris Plains, NJ). MAA and EDMA were purified by general distillation *in vacuo* to remove the polymerization inhibitor. Clonidine hydrochloride [2-(2,6-dichlorophenylamino)-2-im-idazoline hydrochloride; 99%] was purchased from Sigma-Aldrich Chemical, Ltd. (St. Louis, MO). α,α′-Azobisisobutyronitrile (AIBN) and potassium peroxydisulfate were purchased from Kelong Chemical Engineering Reagent Co. (Chengdu, Sichuan, China). AIBN was recrystallized before use. All reagents were analytical chemical (AC) grade. The structures of T and clonidine (C) are shown in Figure 1.

Preparation of free T and C from their hydrochlorides

T and C, as hydrochloride salts, were extracted with chloroform from an alkaline aqueous solution and finally isolated as free bases by evaporation of the solvent.[27]

Preparation of uniformly sized polystyrene (PS) seed particles

Uniformly sized PS seed particles (Fig. 2) used as the shape template were prepared by emulsion polymerization. They were synthesized by the addition of 10 mL of styrene, 100 mL of water, and 0.10 g of sodium chloride, which was used to adjust the ionic strength of the polymerization medium. The mixture was stirred at 350 rpm at room temperature while nitrogen was bubbled through the mixture for 20 min to remove oxygen. To the previous reactants, a degassed solution of 0.10 g of potassium peroxodisulfate was admixed. The polymerization was allowed to proceed for 24 h at a constant temperature. After the reaction was completed, the latex particles were purified through the removal of the remaining monomer and salts by repeated filtration and sonic redispersion. The resulting particles were redispersed again in water (0.497 g/mL) for use in further steps.

Multistep swelling and polymerization

The MIP for T was prepared by a multistep swelling and polymerization method. A nonimprinted polymer (NIP) was prepared for comparison.

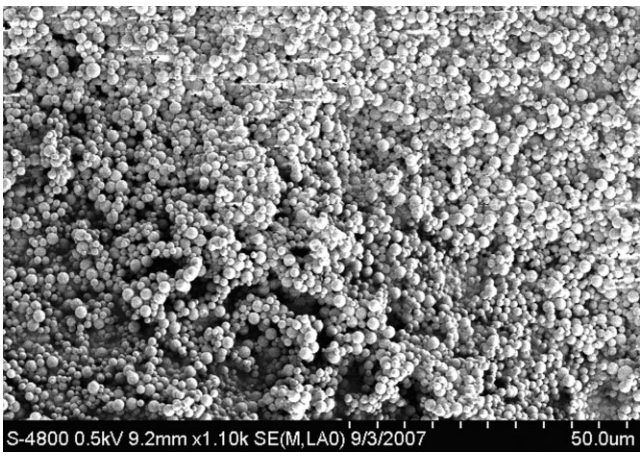

Figure 2 SEM micrograph of PS by an emulsion polymerization method.

A water dispersion of the PS seed particles (0.166 g/mL, 0.51 mL) was admixed with a microemulsion prepared from 0.48 mL of dibutylphthalate as an activating solvent, 0.02 g of sodium dodecyl sulfate, and 10 mL of distilled water by sonication. The first-step swelling was carried out at room temperature for 15 h with stirring at 250 rpm until the oil microemulsion disappeared. Another microemulsion was prepared from 0.375 g of AIBN, 5 mL of toluene, 12.5 mL of water, and 10 mL of a 4.8% poly(vinyl alcohol) solution and then added to the first-step swollen particles. The second-step swelling was carried out at room temperature for 2 h with stirring at 250 rpm. To the dispersion of the second-step swollen particles, a dispersion of 5 mL of EDMA, 6 mmol of MAA, 12.5 mL of water, and 10 mL of a 4.8% poly(vinyl alcohol) solution was added. The third-step swelling was carried out at room temperature for 2 h with stirring at 250 rpm. To the third-step swollen particles, 4 mmol of T and the monomers were admixed to polymerize for 24 h at 65°C under a nitrogen atmosphere.

Washing of the polymers

The dispersion of polymerized particles was poured into 250 mL of methanol, and the supernatant was discarded after sedimentation of the particles. The polymer particles were redispersed into methanol, and this procedure was repeated twice in methanol, once in water, and twice in tetrahydrofuran. They were then rinsed with tetrahydrofuran and then extracted by acetone in Soxhlet's for 24 h; the resulting polymer particles were collected with a standard analysis sieve.

Morphological characteristics of the MIPs and NIPs

The morphological characteristics, tested by pore analysis and scanning electron microscopy (SEM) analysis of the polymers, were also investigated in this experiment. The determinations of specific surface areas were performed with a AUTOSORB-1 gas sorption analyzer (Quantachrome, USA), on the basis of the nitrogen Brunauer–Emmett–Teller method. Microscopic analysis of the MIPs was carried out in an SEM (S-4800, Hitachi, Japan) at 15 kV.

Scatchard analysis

To investigate the binding capacity of the MIPs in alcoholic solutions, binding studies were performed to evaluate the uptake of the MIPs and NIPs. The polymers were washed until it was confirmed that no species were detected in the recovered solution with GC (GC-2010, Shimadzu, Kyoto, Japan). Alcoholic solutions of T were added to 10-mL vials containing 30 mg of polymer; we let these stand for 3 h at 4°C and subsequently centrifuged them for 10 min at 5000 rpm at ambient temperature. These procedures were repeated three times for each concentration of T solution. The concentration of unbound T was measured by GC. After that, binding isotherms of MIP and NIP were obtained.

The data of the static absorption experiment were further processed with the Scatchard equation to estimate the binding parameters of the MIPs:

$$Q/C_{\text{free}} = (Q_{\max} - Q)/K_D$$

where Q is the amount of T bound to the MIPs at equilibrium, $Q_{\max}$ is the maximum binding capacity, C_{free} is the equilibrium concentration of T, and K_D is the dissociation constant. Information on equilibrium was extracted by Scatchard analysis of the calibration curve, a tool already applied in MIP work.[28]

Comparison of the retention behavior of T between the NIP and the molecularly imprinted polymer for the peripheral vasodilator drug tolazoline (T-MIP) sorbents' SPE

The structurally similar drug C was chosen as a competitive species with T for the comparative recognition study. NIP (50 mg) and T-MIP (50 mg) were placed into three empty SPE cartridges matched with an SPE Waters apparatus (Milford, MA). After they were pretreated with 3 mL of alcohol, 3 mL of 7.23×10^{-3} mol/L T and the same concentration of C mixture solution were loaded onto the MIP–SPE column and the NIP column, respectively, at a speed of 2 mL/min. Then, the NIP–SPE column was eluted with 3 mL of alcohol solution, and the MIP–SPE column was eluted with 3 mL of alcohol–acetic acid (95:5 v/v) solution. The elution was analyzed by GC with electron-capture detection (ECD) at 300°C.

Determination of T in the spiked urine samples

The extraction of T was performed in a single procedure. A 1.0-mL urine sample was placed into a 100-mL, round-bottom flask along with 5.5 mL of a spiked T alcoholic solution at three concentration levels (6.36×10^{-5}, 6.36×10^{-4}, and 1.0×10^{-3} mol/L), 2.5 mL of extraction buffer (0.1 mol/L $KHCO_3$, 0.1 mol/L K_2CO_3, pH 10.0), and 50.0 mL of methylene chloride. The contents were ultrasonicated for 20 min and centrifuged for 5 min at 5000 rpm. The aqueous layer at the top was removed by aspiration. The organic layer was evaporated to dryness, and the dry residue was reconstituted by ultrasonication. According to the procedure in the section Comparison of the Retention Behavior of T Between the NIP

and the Molecularly Imprinted Polymer for the Peripheral Vasodilator Drug Tolazoline (T-MIP) Sorbents' SPE, the MISPE column was prepared for the determination of T in the urine samples. The urine samples were spiked with T at three concentration levels (6.36×10^{-5}, 6.36×10^{-4}, and 1.0×10^{-3} mol/L). These T solutions (2 mL) were introduced onto the MISPE columns at a flow rate of 2 mL/min. The fractions eluted from the SPE columns were analyzed by GC. The tests were carried out in a GC system that included a DB-1capillary column and an ECD detector.

RESULTS AND DISCUSSION

Preparation and characteristics of the PS particles and the MIPs

The morphology of the PS particles (see Fig. 2) and MIP particles were assessed by SEM (micrographs are shown in Fig. 3). For the preparation of PS particles, the size of the polymer particles was uniform. In the multistep swelling procedure, a near monodisperse population of particles was slightly irregular in shape, as expected.

The average specific surface areas from the nitrogen adsorption experiments were 0.5972 m^2/g for MIP and 0.5384 m^2/g for NIP (see Table I), respectively. The similar surface areas of MIP and NIP indicated that the selectivity of the MIP was due to a special imprinted recognition, as discussed later.

The PS particles were preferred for subsequent use as seeds in the formation of larger particles, as their volume could be increased up to almost 70 times when the swelling was accomplished with an appropriate emulsified solvent.[29] The main feature of the swelling technique was the initial activation of PS seed particles in an aqueous dispersion. As a result, the activated beads were capable of absorbing the monomer and crosslinker in an amount that far exceeded that of the pure polymer beads. Consequently, the MIP particles were larger than PS in size and irregular in shape (cf. Figs. 2 and 3).

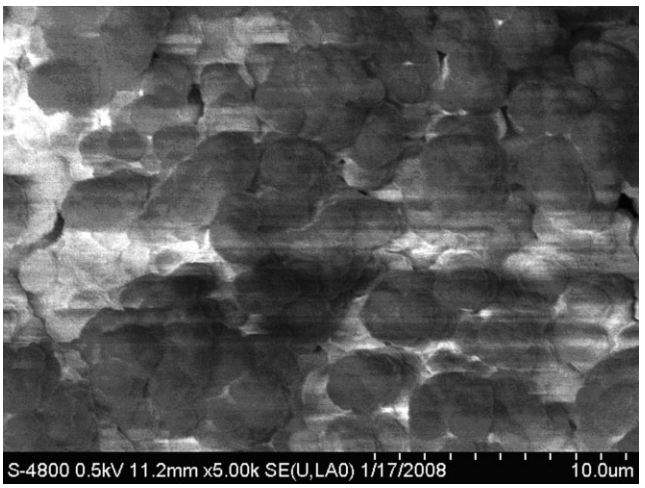

Figure 3 SEM image of the MIP particles by the multistep swelling and polymerization method.

TABLE I
Surface Characteristics of T-MIP and NIP

	T-MIP	NIP
Surface area (m^2/g)	0.5972	0.5384
Pore volume (mL/g)	0.04687	0.03116
Pore size (A)	18.91	13.77

An excess of functional monomer relative to the template is usually required to favor template-functional monomer complex formation and to maintain its integrity during polymerization. Therefore, a fraction of the functional monomers is randomly incorporated into the polymer matrix to form nonselective binding sites. The activation of the seeds results from the presence of a highly water-insoluble, low-molecular-weight compound (dibutylphthalate). An oil-soluble initiator must be added during the activation step to accomplish the final polymerization.

A second step of swelling is subsequently performed by the addition of the monomer, crosslinker, template, and porogen (which gives macroporous particles) in the form of an aqueous suspension to the activated seed particles.

Evaluation of the static adsorption and batch binding test for T-MIP

To measure the adsorption capacity, the binding isotherm was investigated. Figure 4 shows that the binding capacity of the T-MIP was larger than that of the NIP. This may have been due to the imprinted

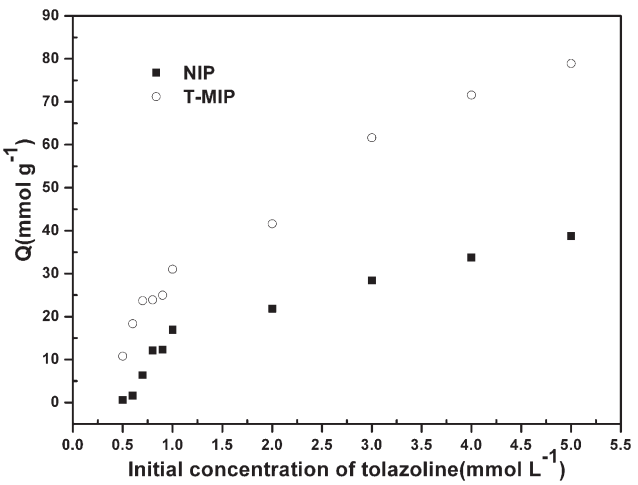

Figure 4 Loading isotherm of T on the T-MIP and NIP sorbents (each point in the isotherm is the average value of three replicates; the RSDs for all points were lower than 2.7%).

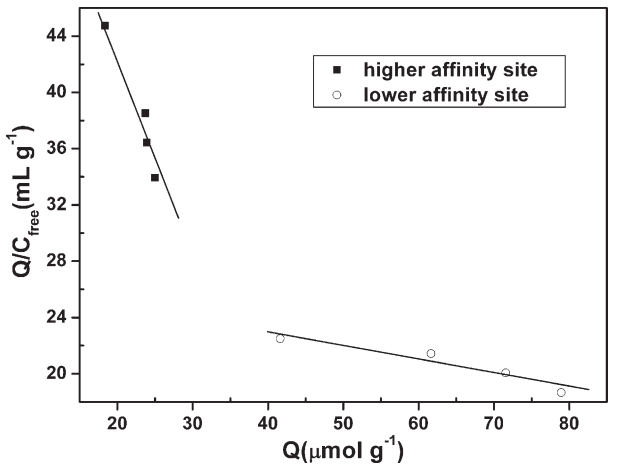

Figure 5 Scatchard analysis of the T-MIPs.

effect and the difference in the molecular interactions. During the preparation of the T-MIP sorbent, the template of T was incorporated into the organic network. After the removal of T, the imprinted cavities and specific binding sites in a predetermined orientation were formed, whereas the NIP sorbent had no such imprinted cavities or specific binding sites. As a result, T-MIP could recognize T selectively.

To estimate the affinity and the theoretical number of binding sites for T, T-MIP was subjected to batch binding tests, which were useful for characterizing the T-MIP as a sorbent material. Because MIPs have been applied to sorbent assays and chromatography, this characterization was important for assessing the usefulness of the imprinted polymers.[30] A Scatchard plot is shown in Figure 5, where the two distinct linear portions indicate that two types of binding sites existed in the imprinted polymer: one was of high selectivity or affinity with a high binding energy, and the other was of low affinity with a low binding energy. The K_D and Q_{max} values were calculated from the slopes and intercepts of the two linear portions of the Scatchard analysis, and the results are listed in Table II. The obtained values for Q_{max}, therefore, corresponded to 15 and 31% of the theoretical total binding sites derived from the amount of template used for the polymerization.

TABLE II
Results of the Scatchard Analysis

Binding site	Linear equation	K_d (mmol/L)	Q_{max} (μmol/g)
Higher affinity site	$Q/C_{free} = 72.655 - 1.506Q$	0.664	48.244
Lower affinity site	$Q/C_{free} = 26.898 - 0.099Q$	10.101	271.697

Selectivity of the MIP–SPE column

The selectivity of an MIP–SPE procedure depends on the sorbent/solvent combination used. Most methods reported to date have used C_{18}, C_8, silica, Florisil, or other chemically modified sorbents as the solid support.[3] In the most common technique, uncharged analytes are adsorbed on a hydrophobic sorbent. Enrichment and clean up of hydrophilic analytes is usually more difficult to obtain and lead to disturbances in the subsequent chromatographic analysis.

Because of the special selectivity for the target analyte, the MIPs proved to be better sorbents and gave higher adsorption values and more clear extracts than NIP (Table III). The distribution coefficient (K_d), selectivity coefficient of the sorbent (k), and k' were obtained in these comparative binding experiments. K_d suggests the character of a substance adsorbed by a sorbent, k suggests the otherness of two substances adsorbed by the same sorbent, and k' suggests the otherness of two different sorbents. These factors were calculated, as shown in eqs. (1)–(3) in Table III.[31]

TABLE III
Competitive Loadings of T and C by the T-MIP and NIP Sorbents

Sorbent	Initial solution (mmol/L)		Final solution (mmol/L)		K_d (mL/g)		k	
	T	C	T	C	T	C	T/C	k'
T-MIP	7.23	7.23	6.43	7.10	48	7.8	6.15	5.21
NIP	7.23	7.23	6.83	6.89	24	20.4	1.18	

$K_d = [(C_i - C_f)/C_f] \times [\text{Volume of solution (mL)}]/[\text{Mass of sorbents (g)}]$ (1)

$k = K_{d1}/K_{d2}$ (2)

$k' = k_{imprinted}/k_{nonimprinted}$ (3), where $k_{imprinted}$ and $k_{nonimprinted}$ are retention factors of a solute on an m2p and an n2p, respectively, K_d is the distribution coefficient; C_i and C_f represent the initial and final concentrations, respectively; k is the selectivity coefficient; and k' is the relative selectivity coefficient.

TABLE IV
Recovery (%) and RSD of T After C_8 and MISPE of the Spiked Urine Samples

T in the urine samples (mol/L)	Recovery (%)		RSD (%, $n = 3$)	
	MIP	C_8	MIP	C_8
6.36×10^{-5}	99.33	89.4	2.5	4.6
6.36×10^{-4}	103.5	84.3	2.1	3.0
1.0×10^{-3}	114.8	86.9	1.9	2.7

The K_d values of T and C were similar for the NIP sorbent. The adsorbed capacity of T-MIP for T was over six times than for C. The k value of the T-MIP sorbent (6.15) was more than fivefold that of the T-NIP sorbent (1.18), which indicated that the T-MIP sorbent had a high selectivity for T over the structurally similar compound C. The k' value was 5.21, which was greater than 1 and revealed that the MIP showed a higher selectivity for T than did NIP.

Evaluation of the usefulness of the MISPE

To obtain a higher recovery for T in the urine, elution solutions of alcohol and alcohol–acetic acid (90:10 v/v) were used to optimize the elution conditions. The results demonstrate that the use of alcohol–acetic acid (90:10 v/v) as an elute solution gave better recovery than the use of alcohol. During SPE tests, the specificities of the synthesized polymers and commercial C_8 adsorbents were tested. T was observed to be easily eluted from the C_8 column, which indicated that the C_8 column may have had a low affinity for T because of nonspecial interactions. The samples were extracted according to the section Comparison of the Retention Behavior of T Between the NIP and the Molecularly Imprinted Polymer for the Peripheral Vasodilator Drug Tolazoline (T-MIP) Sorbents' SPE. The recoveries and reproducibility of the method were calculated and are summarized in Table IV. As shown, the average recovery of the MISPE method was 105.9% at the studied levels, and the average recovery of the C_8 method was 86.9% with a high relative standard deviation (RSD) because of the nonspecial selectivity for the conducting target analyte isolation. These results suggest that the MIPs were better sorbents and gave higher recovery values than the C_8 sorbents.

CONCLUSIONS

In the study reported herein, a multistep swelling and thermal polymerization method was developed to synthesize T-MIP and NIP microbeads. The polymer was used as an SPE sorbent to determine T in urine samples, and the T-MIP sorbent had a high adsorption capacity and selectivity and good site accessibility for T. The MIP–SPE–GC method showed good recoveries and higher selectivities. The precision and accuracy of the method were satisfactory. The molecular imprinting technique combined with SPE–GC could have wide applicability for affinity-based extraction of a target drug in biological fluids.

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References

1. Hart, B. R.; Shea, K. J. J Am Chem Soc 2001, 123, 2072.
2. Yang, H. H.; Zhang, S. Q.; Tan, F.; Zhang, Z. X.; Wang, X. R. J Am Chem Soc 2005, 127, 1378.
3. Yan, H. Y.; Qiao, F. X.; Row, K. H. Anal Chem 2007, 79, 8242.
4. Wulff, G. Chem Rev 2002, 102, 1.
5. Wulff, G. Angew Chem Int Ed 1995, 34, 1812.
6. Mosbach, K.; Ramström, O. Biotechnology 1996, 14, 163.
7. Haupt, K.; Mosbach, K. Chem Rev 2000, 100, 2495.
8. Batra, D.; Shea, K. J. Curr Opin Chem Biol 2003, 7, 434.
9. Katz, A.; Davis, M. E. Nature 2000, 403, 286.
10. Bass, J. D.; Katz, A. Chem Mater 2003, 15, 2757.
11. Zimmerman, S. C.; Wendland, M. S.; Rakow, N. A.; Zharov, I.; Suslick, K. S. Nature 2002, 418, 399.
12. Mertz, E.; Zimmerman, S. C. J Am Chem Soc 2003, 125, 3424.
13. Alvarez-Lorenzo, C.; Concheiro, A. J Chromatogr B 2004, 804, 231.
14. Feng, S. Y.; Lai, E. P. C.; Dabek-Zlotorzynska, E.; Sadeghi, S. J Chromatogr A 2004, 1027, 155.
15. Caro, E.; Marcé, R. M.; Cormack, P. A. G.; Sherrington, D. C.; Borrull, F. J Chromatogr A 2003, 995, 233.
16. Hennion, M. C. J Chromatogr A 1999, 856, 3.
17. Alexander, C.; Andersson, H. S.; Andersson, L. I.; Ansell, R. J.; Kirsch, N.; Nicholls, I. A.; Mahony, J. O.; Whitcombe, M. J. J Mol Recogn 2006, 19, 106.
18. Molecularly Imprinted Polymers: Manmade Mimics of Antibodies and Their Applications in Analytical Chemistry; Sellergren, B., Ed.; Elsevier Science: Amsterdam, 2001; Vol. 23.
19. Pérez-Moral, N.; Mayes, A. G. Anal Chim Acta 2004, 504, 15.
20. Ahlquist, R. P.; Huggins, R. H.; Woodbury, R. A. J Pharm Exp Ther 1949, 89, 271.
21. Goetzman, B. W.; Sunshine, P.; Johnson, J. D.; Wennberg, R. P.; Hackel, A.; Merten, D. F.; Bartoletti, A. L.; Silverman, N. H. J. Pediatr 1976, 89, 617.
22. Aranda, J. V.; Portuguez-Malavasi, A.; Collinge, J.; Outerbridge, E. Pediatr Res 1978, 12, 422.
23. McLinden, V. J.; Stenhouse, A. M. Forensic Sci Int 1979, 13, 70.
24. Andersson, L. I. J Chromatogr B 2000, 739, 163.
25. Caro, E.; Marcé, R. M.; Borrull, F.; Cormack, P. A. G.; Sherrington, D. C. Trends Anal Chem 2006, 25, 143.
26. Molecularly Imprinted Materials: Science and Technology; Yan, M. D.; Ramström, O., Eds.; CRC: Boca Raton, FL, 2005; Part VI, p 603.
27. Sanbe, H.; Haginaka, J. Analyst 2003, 128, 594.
28. Fang, L.; Liu, Y. J.; Hu, J. M. Langmuir 2004, 20, 1788.
29. Smigol, V.; Svec, F.; Hosoya, K.; Wang, Q.; Fréchet, J. M. J. Angew Makromol Chem 1992, 195, 163.
30. Matsui, J.; Goji, S.; Murashima, T.; Miyoshi, D.; Komai, S.; Shigeyasu, A.; Kushida, T.; Miyazawa, T.; Yamada, T.; Tamaki, K.; Sugimoto, N. Anal Chem 2007, 79, 1754.
31. Han, D. M.; Fang, G. Z.; Yan, X. P. J Chromatogr A 2005, 1100, 135.