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RESEARCH ARTICLE

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Preparation, characterization and properties studies of quinine-imprinted polymer in the aqueous phase

Abstract The uniform-sized spherical molecularly imprinted polymers were successfully prepared through molecular imprinting technology by two-step seed swelling and mini-emulsion polymerization in the aqueous condition using quinine as template molecules and methacrylic acid (MAA) as functional monomer. The polymers were characterized by IR spectra, thermal-weight analysis, scanning electron microscope and laser particle size analysis. The properties of imprinted polymers were investigated in different organic phases and aqueous media. In the organic media, results suggested that polar interactions (hydrogen bonding, ionic interactions) between acidic monomer/polymer and template molecules are mainly responsible for the binding and recognition; whereas in the aqueous medium, a considerable recognition effect was also obtained where the ionic (electrostatic) interaction and hydrophobic interaction play an important role. The experiments of binding different substrates indicated that the MIPs possessed an excellent rebinding ability and inherent selectivity to quinine.

Keywords template, two-step seed swelling, mini-emulsi on polymerization, molecular imprinting technology

1 Introduction

A high selective molecular recognition system is important in living organisms, such as the interactions between antibody–antigen, enzyme–substrate, protein–DNA and so on. The design, synthesis and evaluation of novel receptor-mimicking materials possessing specific recognition ability are of obvious importance in the chemical and biological field in recent years. Molecularly imprinted polymers (MIPs), as a kind of man-made acceptor, contain “prearranged” structure that can be utilized for specific recognition towards the target molecule. They have also been studied extensively in many different fields such as chromatographic separation [1–3], chemical sensors [4], reaction catalyst [5], enzyme mimics [6] and in particular, as solid-phase extraction adsorbents [7–9]. The recognition and formation of self-assembly complexes between the imprinting species and functional monomer were crucial in preparing MIPs based on the weak non-covalent interaction such as hydrogen bonds, ionic bonds, π-π and hydrophobic interaction, etc. In order not to destroy the binding strengths, the best imprinting porogens are very low polar organic solvents such as chloroform, dichloromethane and toluene. However, in nature, water plays an important role as the surrounding medium in most recognition systems, like enzyme and substrates, antibody and antigen. Exploring the preparation and subsequent recognition of MIPs in an aqueous-phase environment possesses important scientific and applicative value. Moreover, up to now, a molecularly imprinted polymer has been usually prepared in monolithic form by bulk polymerization, and then ground and sieved to the desired size. As a result, the ground particles tend to have extremely broad size distribution and shape—a distribution that appear unsuitable for chromatographic applications.

In this paper, to overcome the limitations described above, a series of uniform-sized spherical molecularly imprinted polymers have been successfully prepared by two-step seed swelling and mini-emulsion polymerization in the aqueous condition using quinine as template molecules and methacrylic acid (MAA) as functional monomers. A cross-linked polystyrene seed was first prepared by free-emulsifier emulsion polymerization for subsequent two-step swelling. The morphology and size of

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MIPs were carefully optimized by varying polymerization condition such as the type of surfactant, the amount of swelling reagent and polystyrene seed as well as the ratio of functional monomer to template, etc. In addition, in order to gain more insight into the origin of the recognition properties of the imprinted polymer, the imprinting effects of the polymers were examined by binding experiment and quantified by UV absorption.

2 Experimental

2.1 Materials

Ethylene glycol dimethacrylate (EDMA) was purchased from Jiangshu Anli Chemical Reagent Institute. It was washed with 10% NaOH, dried over $MgSO_4$, and then distilled prior to use. Methacrylic acid (MAA) was obtained from Shanghai Chemical Reagent Plant, and was distilled under reduced pressure before use. Styrene, dibutylphthalate (DBP), sodium dodecylsulfate (SDS) and 2,2′-azobisisobutyronitrile (AIBN) were from Guangzhou Chemical Reagent Co.. The DBP and styrene were purified by general distillation, and AIBN was purified by recrystallization in methanol. Quinine and cinchonine were purchased from Shanghai pharmacy company, and polyvinyl alcohol (PVA, polymerization degrees 1,700, hydrolyzation degrees 88%) was obtained from Shanxi Chemical Industry. Other reagents and solvents were of analytical grade, and used without further purification.

2.2 Preparation of spherical MIPs by two-step swelling

2.2.1 Preparation of polystyrene (PS) emulsion

Styrene (10 ml), potassium peroxidisulfate (0.1 g) and alcohol (10 ml) were added to 90 ml of distilled water. The polymerization was carried out under nitrogen atmosphere at 70 ^{o}C for 15 h with mechanical stirring at 200 rpm. The resulting emulsion was purged with nitrogen stream for 20 min to remove the residual styrene before further use. The concentration of the emulsion was measured by evaporating the water to obtain a solid particle concentration of 0.0557 g/ml.

2.2.2 Two-step swelling and polymerization

We describe here the synthetic procedure for the preparation of sample P2. An emulsion was prepared from 2 ml of DBP, 100 mg AIBN dissolved in 2 ml of toluene, 30 mg of SDS and 20 ml of distilled water by sonication for 5 min. It was then added to another emulsion prepared from 10 ml of polystyrene seed emulsion, 30 mg SDS and 20 ml of distilled water. The mixed solution was carried out for the first-step swelling at room temperature for 24 h. In the second step, the self-assembly solution was first prepared with 324 mg (1 mmol) of quinine, 1.0331 g (12 mmol) of MAA and 8 ml of toluene, then mixed with the emulsion prepared from 5.9466 g (30 mmol) of EDMA and 20 ml of distilled water containing 0.7g PVA as a dispersion stabilizer. The resulting emulsion was added to the first emulsion under stirring and further swelled at room temperature for 24h. After the second swelling was completed, the solution was poured into a 250 ml three-necked flask, and diluted with 80 ml distilled water containing 2.8 g PVA. The polymerization procedure was started at 75^{o}C under a nitrogen atmosphere with mechanical stirring at 200 rpm for 12 h. As a control, the non-imprinted polymers (NIPs) without the template were prepared in an identical manner. Table 1 shows the formulas for preparation of polymers.

Table 1 The proportion of material for preparation of polymers

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
PS(ml)	10	10	10	10	10	2	4	8	10	10
Water	140	140	140	140	140	140	140	140	140	140
SDS(g)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	-	3.06
PVA(g)	3.5	3.5	3.5	3.5	3.5	3.5	3.5	4.9	-	?
MAA(mmol)	4	12	18	12	12	12	12	12	12	12
EDMA(mmol)	30	30	30	30	30	30	30	30	30	30
DBP (ml)	2	2	2	5	10	2	2	2	2	2
Quinine (g)	0.324	0.324	0.324	0.324	0.324	0.324	0.324	0.324	0.324	0.324

2.2.3 Extraction of template molecules in polymers

The resultant polymer emulsion was boiled and purged by adding a small amount of NaCl. Subsequently, the emulsion was centrifuged and the upper supernatant was discarded. The obtained polymer beads were washed with both ether and methanol, and were then re-dispersed into 300 ml of boiling water and centrifuged again. This procedure was repeated three times to completely remove the surface stabilizer. Finally, the particles were washed one by one with methanol–acetic acid (9:1, v/v) and methanol in a Soxhlet apparatus for 24 h, followed by drying under vacuum at 80 °C. The non-imprinted polymers were treated using the same method.

2.3 Adsorption ability and selectivity of polymer beads

Dried polymer beads (30 mg) were suspended in 3 ml of quinine (2 mmol/l), cinchonine (2 mmol/l) and Triazolam (2 mmol/l) solution in a 10ml conical flask sealed by polyethylene film. The flasks were oscillated in a constant temperature bath oscillator at room temperature for 7 h. After the binding process was completed, the mixture was filtrated by 0.45 μm film. The supernatant (1 ml) was transferred to a 10ml test tube and then diluted with a known volume of chloroform. The concentration of free substrates in the supernatant was determined by using a spectrophotometer at maximum wavelength of every substrate. The amount of substrates bound to the polymers (Q) was calculated by subtracting the amount of free substrates from the initial concentration. The static distribution coefficient K_D values of the substrates on polymers were calculated as the ratio of the concentration of substrate on polymer to that in solution [10]. The relative factor α was calculated as the K_D of substrate on MIP to that on a NIP.

2.4 Characteristics of MIPs

All binding experiments were performed using a UV-2100 spectrophotometer (Unico, Shanghai). The surface morphology of MIPs was observed by a ZSM-6300 field emission scanning electron microscope (SEM, Japan), and the thermal stability of MIPs was evaluated with TGA-50 thermal-weight analytical equipment (Shimadzu, Japan). Meanwhile, the particle size and distribution range of MIPs were measured by a Mastersizer-2000 Laser Analyzer (Malvern, England), and the structure of MIPs was characterized by an FT-IR spectra on a VECTOR-IR instrument (Brucker, Germany).

3 Results and discussion

3.1 Characteristic of MIP micro-spheres

3.1.1 IR-spectra

Figure 1 shows the FT-IR spectra of (**a**) non-imprinted polymer and (**b**) extracted imprinted polymer measured by KBr pellets. It can be seen that the characteristic IR absorptions of hydroxyl and carboxyl groups exist in MIP and NIP, and the shape and position of all peaks in both IR spectra are exhibited similarity. This suggests that the majority of the template molecules have been successfully extracted from the imprinted polymer. In the infrared spectra, the peaks at 3,443 cm^{-1}, 2,956cm^{-1} and 1,726 cm^{-1} should correspond to absorption bands of –OH, $-CH_2$- or $-CH_3$ and –C=O stretching vibration, respectively. The symmetric and dissymmetry stretching vibration of –C–O–C of ester appeared near 1,261cm^{-1} and 1,161 cm^{-1}. The other absorption peak near 1,458 cm^{-1} should contribute to the bend vibration of $-CH_3$ or forficiform vibration of $-CH_2$-. The absorption peak at 1,633 cm^{-1} should be assigned to non-polymerized double bonds.

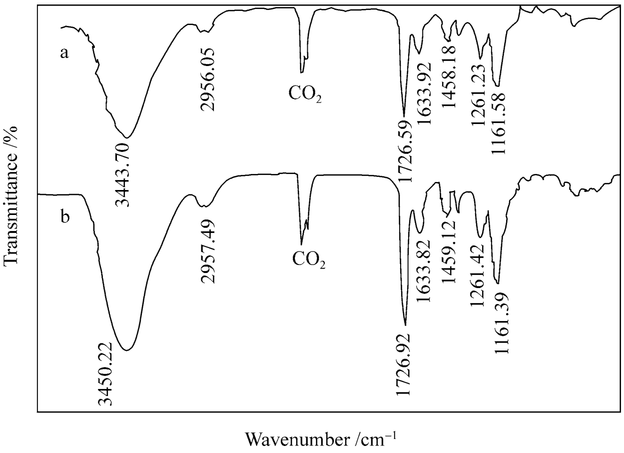

Fig. 1 The IR spectra of extracted imprinted polymer (**a**) and non-imprinted polymer (**b**).

3.1.2 Thermal-weight analysis

The thermal stability of MIPs was evaluated using TGA-50 thermal-weight analytical equipment. Heating rate: 10 °C/min. Recording temperature: 25°C ~ 730°C. Gas atmosphere: air. The result measured indicated this type of polymers have perfect thermal stability. Taking P2 as an example, the range of its decomposition temperature is from 259°C to 633°C, where the fastest decomposition temperature is at about 396 °C.

3.2 Factors affecting morphology of imprinted beads

3.2.1 Surfactant

The size and morphology of imprinted beads were affected by the following factors: the stirring speed, the amount of monomer, the water-to-oil ratio, the type and amount of porogen and the swelling reagent, etc. In this study, the stirring speed and the amount of water were fixed at about 200 rpm and 140 ml, respectively, with only the amount and type of surfactants being altered. The particles of products were observed by an electronic microscope (Fig. 2). When SDS was utilized as an emulsifier, the resulting polymer particles formed a gel with a number of fine beads and a broad particle size distribution. Although the fine beads could be easily avoided by using simplex PVA instead of SDS, the amount of PVA used for emulsifying was higher. This is considered unfavorable for the extraction of surface stabilizer. As an alterative, the morphology of the particles was improved by using PVA together with a little SDS as dispersants. Under these conditions, the spherical polymer beads were prepared with higher yields as well as a good regular form.

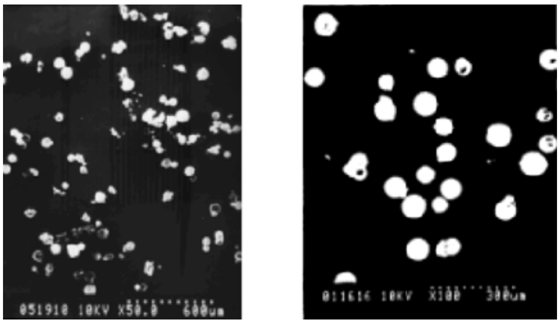

Fig. 2 Scanning electron micrograph of quinine-imprinted polymer beads; (**a**) beads were prepared using SDS as emulsifier; (**b**) beads were prepared using PVA and SDS as combined emulsifier

3.2.2 The amount of swelling reagent

In order to obtain mono-dispersed spherical beads, it is necessary to make the oil-dissolved initiator and monomers to go into the cavity of the polystyrene seed. Therefore, we utilized dibutylphthalate as a swelling reagent to activate the polystyrene seed. Based on the results of the prepared beads, it can be seen that the specific morphology and particle size of the polymers are affected by the amount of DBP. When 2 ml of DBP was added into 10 ml of polystyrene seed emulsion for swelling, the ultimate products showed a rather narrow size distribution in diameter with good mechanical capability. However, when the amount of DBP was increased to 10 ml in the same volume of polystyrene seed emulsion, a lot of broken particles with a wider particle size range appeared. This phenomenon is not surprising because the volume of polymer beads expands, and the density rapidly decreases when we increase the amount of the swelling reagent. The obtained beads easily lead to a smash under mechanical stirring. On the other hand, the particles were incapable of enduring high pressure due to lower mechanical capability and greater swelling ratios; thus, they are not suitable for the stationary phase of HPLC.

3.2.3 The amount of seed emulsion

According to the mechanism of seed swelling and suspension polymerization, the particle size of produced polymer beads is related to the particle size of the initial seed and the amount of seed and monomers used for polymerization. The related equation is defined as follows:

$$\frac{(d_P)^3}{(d_S)^3} = K \times \left(\frac{m_M}{m_S} \times \frac{\rho_S}{\rho_M} + 1\right) \qquad (1)$$

Where d_P and d_S are the particle size of imprinted polymer and seed beads, respectively. m_M and m_S are the mass of monomer EDMA and seed polystyrene, respectively. ρ_S and ρ_M are the density of seed beads and monomer, respectively. K is the proportional coefficient related with stirring rate, water–oil ratios and dispersant, etc. In this experiment, the particle size of the seed beads prepared by emulsifier-free emulsion polymerization was ca. 3~5 μm in diameter with a narrow size distribution range. The amount of seed emulsion was altered while the other parameters affecting the particle size of the polymer beads were kept constant. The particle size of the obtained polymer beads at different $\frac{m_M}{m_S}$ values were measured by laser particle scanning and are shown in Fig. 3. It shows that the average particle size of the polymer beads tends to decrease with an increase in the amount of seed. This result may be simply explained as: the higher the concentration of the seeds serving as core shell in the polymerization solution, the less the monomers can afford polymerization to be distributed on every core shell, leading to the ultimate particle size of the polymer beads. This represents an inverse proportional relation with the amount of seed used in the polymerization.

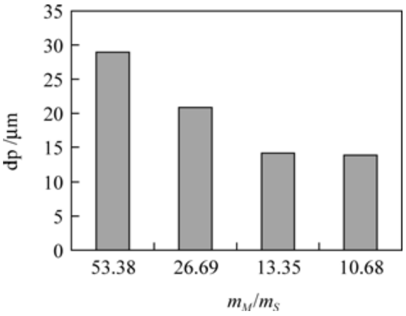

Fig. 3 The average particle size of MIPs varies with m_M/m_S

3.3 The optimization of functional monomer

MAA acts not only as a proton donor but also as a proton acceptor. The polymers prepared based on MAA as functional monomer have been reported by two-step or multi-step seed swelling [10–13] or suspension polymerization [14]. Quinine template as an alkaloid compound used in the research contains two amino groups and one hydroxyl group, and could form self-assembly complexes with acidic MAA through hydrogen bonding or ionic interaction. Generally, the greater the concentrations of functional monomer, the more stable the formed complexes. But if the amounts of functional monomer are too many, the excessive monomers are possibly distributed randomly on the surface of the polymer, resulting in a rapid increase of non-specific adsorption. To overcome the bond-breaking effects of water and improve the concentration of self-assembly complexes formed between the template and functional monomer via hydrogen bonding and ionic interaction, the amount of MAA used in the two-step swelling process was much more than traditional bulk polymerization. In the oil/water two-phase system, the formed complexes disperse among the emulsion containing the organic phase, and then enter the hydrophobic polystyrene seed by swelling. The dissociative template may renewably associate with free MAA to form complexes in the micro-cavity of the polystyrene seed. The high specific recognition ability of MIPs was obtained as a result of a greater number of recognition sites formed in the imprinted polymer. In order to test the influence of the molar amount of MAA on imprinting effect of MIPs, MAA-quinine (M/Q) ratio was well studied and carefully optimized. In this experiment, the ratio of cross-linker to functional monomer was fixed at 7.5:1, and the ratio of M/Q was just adjusted for 4:1, 12:1 and 18:1. The adsorption amounts of MIP or NIP towards the template were investigated by batch equilibrium analysis experiment. The results indicated that polymers prepared with a ratio of 4:1 shows little specific adsorption toward the template molecule, while MIPs prepared at an M/Q ratio 12:1 show good specific adsorption towards the template with relative factors (α) 2.72. On the other hand, when the M/Q ratio was increased to 18:1, the non-specific binding simultaneously increased and the relative factors dramatically decreased to 2.20.

3.4 Effects of solvents for adsorption capability

The recognition of the imprinted polymer towards the imprinting species often occurs in a less polar organic phase. But in some biotechnological applications, water-poor phases are prerequisites to the success of the process such as the recognition of enzyme–substrate, antibody–antigen, etc. Therefore, studies on the preparation and subsequent recognition of MIPs in the aqueous phase are of obvious importance.

To investigate the influence of solution media on adsorption capability of MIPs, the adsorption experiment of the quinine-imprinted polymer was carried out in various polar recognition media, including chloroform, acetonitrile, methanol and acetonitrile/water. The adsorptive datum of polymer in these solvent systems have been measured and listed in Table 2, from which it can be seen that the adsorptive capacity of both MIP and NIP beads in methanol were obviously less than that of polymers in chloroform. This is because the methanol is a protonic polar solvent, and it may participate in competing for either the recognition sites or the non-recognition of the polymers via hydrogen bonding, leading to a synchronous restraining in both specific and non-specific adsorption. In contrast, the adsorptive capacity of MIPs appears higher in moderate polar acetonitrile. The reason for this phenomenon is that acetonitrile, as a non-protonic solvent, does not interfere with the interaction between the template and the binding sites. The ionic interaction between the acidic carboxyl group of the polymer and two amino groups in quinine molecules is strengthed resulting in a higher total adsorption amount. To test the applied scope of MIP, the molecularly imprinted materials were investigated in a water-containing solution. A remarkable dependence on the ratio of water in organic solvent was recorded. An obvious decrease on the recognition properties was observed with 30% proportions of water in acetonitrile. On the other hand, a 50% proportion of water in acetonitrile led to an increase of total adsorption capacity. This is because the hydrogen bond between MIP and substrates is weakened with the addition of water into the organic phase, but at the same time, the ionic interaction and hydrophobic interaction is strengthened. The recognition mechanism of MIP towards substrates turns out to be controlled by the ionic-exchange together with hydrophobic interaction from initial hydrogen bonding. In contrast, the specific recognition stays nearly the same as that in acetonitrile.

Table 2 The adsorption amounts (μmol • g^{-1}) of polymer in different solutions

Solvent	MIPs（μmol • g^{-1}）	NIPs（μmol • g^{-1}）
Chloroform	63.03	28.93
Methanol	22.51	14.28
Acetonitrile	69.16	47.76
Acetonitrile/water (70:30, v/v)	53.72	26.58
Acetonitrile/water (50:50, v/v)	79.15	56.04

Binding time: 7 h; concentration of substrates: 2 mmol/l; binding temperature: 25 ^{o}C

3.5 Selectivity of imprinted polymer

The selectivity test of polymer was carried out using quinine, cinchonine and Triazolam as substrates. The selectivity was evaluated by the static distribution coefficient K_D value of three different substrates on MIPs and NIPs (see Table 3). Table 3 shows that the K_D value of quinine on MIPs is the highest among the three substrates, and is also higher than the K_D of non-imprinting polymer. This difference can be attributed to the recognizing cavities being created in resultant MIPs because of the addition of template molecules during polymerization, where orientation of the functional group inside the cavities and the shape of the cavities highly match the template molecules. Moreover, the interaction intensity between the carboxyl acid of the functional group on the MIPs and imprinting species can possibly get stronger in the imprinting environment. Consequently, MIPs exhibited excellent selectivity toward the template molecules. Cinchonine molecules only lost a $-OCH_3$ group in the quinoline structure in comparison with quinine, and there is also an access to the imprinting cavities of quinine imprinted polymers due to the similar structure. In addition, the amino group and the hydroxyl group of the side-chain in the quinoline ring of cinchonine molecules are consistent with that of quinine, and can result in the same interaction with the ligand of MIPs as quinine do. Thus, P (CA) could also give the definite cross-selectivity with a considerable K_D value for cinchonine. For triazolam, owing to the limitation of the spatial structure, the micro-cavities cannot accommodate the triazolam molecule. The intensity of the formed hydrogen bond is very weak with the absence of the recognizing cavities. So P (CA) would relatively poorly bind Triazolam molecules and shows the lowest K_D.

Table 3 The K_D values of different substrates on MIPs and NIPs

Polymer	Quinine	Cinchonine	Triazolam
MIPs	46.04	36.0	6.2
NIPs	16.91	19.65	8.5

4 Conclusions

Through a series of optimizations aimed at the type of surfactant, the amount of swelling reagent and polystyrene seed, a uniform-sized spherical molecularly imprinted polymer has been successfully prepared by two-step seed swelling and mini-emulsion polymerization in the aqueous condition using quinine as template molecule and methacrylic acid (MAA) as functional monomer. When the molar ratio of MAA to template is 12:1, the prepared MIPs show good recognition towards the template molecules in comparison with non-imprinted polymer. The recognition ability of MIP is strongly influenced by the polarity of the recognition media. Hydrogen bonding was the most important driving force for the recognition of MIP in the non-polar solvent. However, ionic bonding and hydrophobic interaction play an important role in rebinding in the aqueous phase. The experiments of binding different substrates indicated that MIPs possess an excellent rebinding ability and inherent selectivity to quinine. This study demonstrates that it is possible to develop a method to synthesize MIP in the aqueous phase and further utilize materials for chromatographic separation or solid extraction of drug molecules in the aqueous environment.

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References

1. Glad M., Reinholdsson P. and Mosbach K., Molecularly imprinted composite polymers based on trimethylolpropane trimethacrylate particles for efficient enantiomeric separations, React. Polym., 1995, 25: 47–54
2. Hwang C. C. and Lee W. C., Chromatographic resolution of the enantiomers of phenyl- propanolamine by using molecularly imprinted polymer as the stationary phase, J. Chromatogr., B, 2001, 765: 45–53
3. Lu Y., Li C., Zhang H. et al., Study on the mechanism of chiral recognition with molecularly imprinted polymers, Anal. Chim. Acta, 2003, 489: 33–43
4. Kazuyoshi Y. and Isao K., Molecularly imprinted polymers for biosensor application, Trends Anal. Chem., 1999, 18: 199–204
5. Bruggemann O., Chemical reaction engineering using molecularly imprinted polymeric catalysts, Anal. Chim. Acta, 2001, 435: 197–207
6. Ye L. and Mosbach K., Molecularly imprinted micro-spheres as antibody binding mimics. React. Funct. Polym., 2001, 48: 149–157
7. Baggiani C., Giovannoli C., Anfossi L. et al., Molecularly imprinted solid-phase extraction sorbent for the clean-up chlorinated phenoxyacids from aqueous samples, J. Chromatogr., A, 2001, 938: 35–44
8. Brambilla G., Fiori M., Rizzo B. et al., Use of molecularly imprinted polymers in the solid-phase extraction of clenbuterol from animal feeds and biological matrices, J. Chromatogr., B, 2001, 759: 27–32
9. Sergeyeva T. A., Matuschewski H., Piletsky S. A. et al., Molecularly imprinted polymer membranes for substance-selective solid-phase extraction from water by surface photo-grafting polymerization, J. Chromatogr., A, 2001, 907: 89–99
10. Zhang L. Y., Cheng G. X. and Fu C., Molecular selectivity of tyrosine-imprinted polymer prepared by seed swelling and suspension polymerization, Polym. Int., 2002, 51: 687–692
11. Vaihinger D., Landfester K., Krauter I. et al., Molecularly imprinted polymer nanospheres as synthetic affinity receptors obtained by mini-emulsion polymerization, Macromol. Chem. Phys., 2002, 203: 1965–1973
12. Hanginaka J. and Sakai Y., Uniform-sized molecularly imprinted polymer material for (S)-propranolol. J. Pharm. Biomed. Anal., 2000, 22: 899–907
13. Masci G., Aulenta F. and Crescenzi V., Uniform-sized clenbuterol molecularly imprinted polymers prepared with methacrylic acid or acrylamide as an interacting monomer, J. Appl. Polym. Sci., 2002, 83: 2660–2668
14. Meng Z. H., Wang J. W., Zhou L. M. et al., Beaded molecular imprinted polymer for stereo isomer separation, Chinese J. Chromatogr., 1999, 17: 323–325