# Molecular Imprinted Nylon-6 Stir Bar as A Novel Extraction Technique for Enantioseparation of Amino Acids

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**ABSTRACT:** The flexibility and simplicity of stir bar sorption extraction (SBSE) have been combined with the selectivity of molecularly imprinted polymers (MIP). Stir bars were coated reproducible with a 160-μm film formed from a formic acid solution of Nylon-6 polymer either non-imprinted or imprinted with L-glutamine. Time sorption profiles were measured for the extraction of L-glutamine at the concentration of 20–500 μmol $L^{-1}$ levels with both types of films in order to compare sorption characteristics. The results indicated that the MIP coated layer showed remarkable high affinity towards L-glutamine and equilibrium adsorption was attained rapidly (60 min) in contrast to conventional bulk molecularly imprinted polymer in which equilibrium adsorption was normally attained after 16–24 h. The imprinted stir bar was also shown a high degree of selectivity toward L-glutamine compared to its isomer and analogues, while a reference stir bar prepared without the imprinting molecule was not shown any selectivity towards the enantiomers. Evidence was also presented by FT-IR analysis that the amide-hydrogen-bonding interaction between L-glutamine and amide groups in MIP-film was originated for L-glutamine recognition. The scanning electron microscope photographs showed that visible pores structure could be detected in the L-glutamine imprinted Nylon-6 film. Compared with traditional MIP and SBSE, the MIP-coated film showed not only the high selectivity, but also the rapid equilibrium adsorption. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2665–2670, 2008

**Key words:** molecularly imprinted technique; stir bar sorption extraction; L-glutamine; enantioseparation

## INTRODUCTION

Molecular imprinting techniques are becoming a powerful tool for preparing polymeric materials, which can recognize target molecules through their templated cavities.[1–3] Molecularly imprinted polymers (MIPs) have been used as chiral stationary phases[4] in liquid chromatography and capillary electrophoresis, as selective materials[5] for biosensors and membranes, as materials for affinity chromatography,[6] and also as antibody mimics.[7] Typical procedures for preparation of such MIPs are the formation of a complex of a template molecule with one or more functional monomers, which is then polymerized with a crosslinker to obtain a resin. Upon removal of the template molecule, the cavities that can recognize the template molecule are produced. For their molecular imprinting methods, there are two main approaches such as covalent[8] and noncovalent imprinting methods[9] adopted by using crosslinked polymers. Another simple way of generating artifical macromolecular receptors is through the molecular imprinting of synthetic polymers.[10] Synthetic polyamides such as Nylon-6, which are commercially available polymers have high strength nature and possess desired functionality in practical applications.[11] It is worth noting that Nylon-6 has inter- and intramolecular hydrogen bonding through its amide group (—NH···O=C—) (as shown in Fig. 1). Thus, the ability of hydrogen bonding in polyamide is capable of using for MIP. The interactions of hydrogen bonding between the template and active sites of amide groups in Nylon-6 can subsequently drive the specific molecular recognition process. Moreover, this Nylon-6 MIPs differed from the typical MIP system in that the MIPs were cast from polymer solutions of the template molecule rather than polymerized from monomers in the presence of the template molecule.

Recently, a novel solventless and simple technique for preconcentration of organic solutes from aqueous matrices, namely stir bar sorptive extraction (SBSE), was developed by Sandra and coworkers.[12] SBSE approaches or equals the high enrichment factors of packed sorptive beds but with the application range and simplicity of SPME.[13–15] In conventional SBSE, a slice of special polydimethylsiloxane (PDMS) tubing, which covers on a glass tube with a magnetic core,

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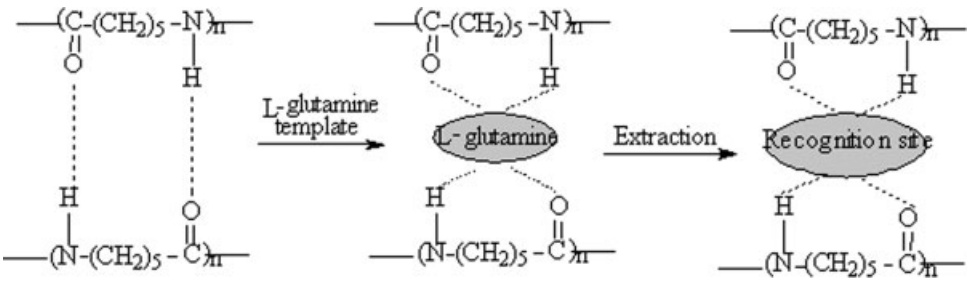


**Figure 1** Schematic presentation of hydrogen bonding interactions between Nylon-6 and L-glutamine.

has been used as the extraction phase. However, only few coatings are commercially available, other and more selective coatings in SBSE have to be considered. On the other hand, the coating technology of extraction phase in sorptive extraction is vitally important to the performance of the device.

To date, static coating technology and sol–gel coating technology are commonly used in SE.[16] Compared to static coating technology, sol–gel technology may be much more suitable for the preparation of thick film. Recently, we reported that stir bars can be coated with monocrotophos imprinted Nylon-6 films by sol–gel technology and such stir bar can be applied for the preconcentration of some polar organophosphorus pesticides in organic solvent.[17] In the present article, we described the preparation of the imprinted Nylon-6 stir bar using L-glutamine as the template molecule and its properties of enantioseparation of amino acids.

## EXPERIMENTAL

### Materials

Nylon-6 having molecular weight of 18,000 was produced by Yixing Chemical (Jiangsu, China). The amino acid standards were purchased from Shanghai biochemistry reagent factory and used as substrates without further purification. The following reagents: *o*-phthalaldehyde (OPA) and 3-mercaptopropionic acid (3-MPA) were obtained from Dikma (Beijing, China). Acetonitrile and methanol were obtained from Tedia (Beijing, China). All other chemicals were of analytical grade, and solvents were of HPLC quality. Ultrapure water used for sample preparation was obtained from a MILLI-R04 purification system, (Millipore, Germany).

### Instrumentation and chromatographic conditions

Chromatographic evaluation was performed on an Agilent 1100 series high performance liquid chromatography equipped with 1312A Binary Gradient Pump, 1313A Thermostatted Autosampler, G1316A column oven, G1321A Fluorescence Detector and G1319A Chemstation. Chromatographic separation was carried out with an Agilent XDB-$C_{18}$ column (150 mm $\times$ 4.6 mm i.d., Particle size 5 μm).

For elution, a Binary gradient was used. Mobile phase A was 20 mmol $L^{-1}$ sodium acetate adjusted to pH 7.2 with 1% acetic acid and completed with 0.2% of THF. Mobile phase B was 20 mmol $L^{-1}$ sodium acetate (pH 7.2) : methanol (1 : 4, v/v). The gradient was as follows: 0 min at 100% mobile phase A; 10 min at up to 50% mobile phase A; 15 min at 50% mobile phase A. For separation of the OPA-3-MPA derivatives, a flow rate of 0.45 mL $min^{-1}$ and a temperature of 40°C were applied. Excitation and emission wavelengths were 340 and 450 nm, respectively.

OPA-3-MPA solution: OPA (50mg) was dissolved in 1 mL of methanol, and then 3-MPA (50 μL) was added, followed by borate buffer (pH 10.4, 8.95 mL). This reagent must be stored in amber glass vials at 4°C, and freshly prepared daily.

### Preparation of molecular imprinting Nylon-6 stir bar

The stir bar coated with film of Nylon-6 polymer imprinted with L-glutamine (MIP-films) was prepared by phase inversion method according to Reddy et al.:[18] 30 wt % of Nylon-6 and 3 wt % of L-glutamine were mixed in formic acid (67 wt %). Solutions were placed into 50-mL flasks, closed and vortexed thoroughly at room temperature until the mixture became a clear colloid solution (about 20 h). A stir bar consisting of a 30-mm magnetic core coated with 200 mg PDMS was used. The 30-mm stir bar was submerged into the polymer colloid solution. Then, the stir bar covered by polymer colloid solution was put into with 100 mL purified water. After the gelation of the Nylon-6 in water (10 min), the stir bar was taken out and well washed with 10% (v/v) acetic acid/methanol solution to remove the solvent and the template molecule from the polymer. The blank stir bar coated with films of Nylon-6 polymer nonimprinted (NMIP-films) was also prepared in the similar manner covered with 30 wt % of Nylon-6-formic acid solution.

FTIR spectroscopy (MAGNA-IR 750 spectrometer (Nicolet, USA)) was adopted for characterization of the imprinted Nylon-6 before and after template extraction. A JEOL model JSM-6700F Scanning electron microscope (SEM) was used to visualize the surface features of the film. A thin layer of gold was coated before the SEM analysis.

### Recognition experiments of amino acids by the imprinted stir bar

Binding experiments of amino acids to the imprinted stir bar were performed in 10 mL of amino acids aqueous solution having 20–500 μmol $L^{-1}$ concentrations at 20°C for 1 h. The changes of amino acids concentrations in the solution were determined by reversed-phase HPLC. The amounts of amino acids bound to the polymer covered onto the surface of stir bar, $Q$, were calculated by following equation:

$$Q = (C_0 - C_t)V/W$$

where, $C_0$ and $C_t$ were the amino acids concentration (μmol $L^{-1}$), measured at initial and after interval time (10 min) for equilibrium, $Q$ (μmol $g^{-1}$polymer) was saturation binding amount of amino acids. Symbols $V$ and $W$ were the volume of the amino acid solution (10 mL) and the weight of dry polymer used for the binding experiment, respectively. Each experiment was carried out in triplicate.

## RESULTS AND DISCUSSION

### Binding of L-glutamine to molecularly imprinted Nylon-6 stir bar

To study the recognition characteristics of the L-glutamine imprinted Nylon-6 stir bar, the binding experiments were performed with various L-glutamine concentrations in the range of 10–500 μmol $L^{-1}$. The values of $Q$ increased with time and became constant at longer time than 60 min. One of the serious drawbacks of the conventional bulk imprinted polymers is the slowness in attaining the equilibrium (16∼24 h), which in fact limits their application.[18,19] In the present case, this MIP system was unique in that the template molecule was incorporated into the polymer matrix in solution, and the film was cast from this solution. That is, the polymerization step is eliminated. Therefore, the reintroduction equilibrium was attained rapidly (∼ 60 min). The saturation behavior of the L-glutamine binding indicated that the binding sites of the imprinted polymer were filled with L-glutamine. While the imprinted stir bar could highly bind L-glutamine, the stir bar prepared without molecularly imprinted treatment showed little binding of the L-glutamine.

**TABLE I**
**Effect of Solvents on L-Glutamine Binding to Imprinted and Nonimprinted Nylon-6 Stir Bar**

| Solvent | $Q$ (μmol $g^{-1}$) | |
|---|---|---|
| | MIP | NMIP |
| Water | 1.687 | 0.037 |
| Methanol | 0.752 | 0.035 |
| Acetonitrile | –[a] | – |
| Acetic acid/methanol (10%, v/v) | 0 | 0 |

[a] Meant that L-glutamine had denaturalization in acetonitrile.

This meant that the nonimprinted Nylon-6 stir bar had no binding sites of L-glutamine.

The binding properties of molecularly imprinted polymers are influenced by the type of solvent.[20] For determination of the specific binding properties of MIP, imprinted and nonimprinted Nylon-6 stir bars were equilibrated with L-glutamine (100 μmol $L^{-1}$) in water, methanol, acetonitrile and acetic acid/methanol at 20°C for 60 min. The change of L-glutamine concentrations in the solution was determined by reversed-phase HPLC. The results were shown in Table II. It indicated that the MIP stir bar exhibited high affinity to MCP in methanol and water. On the other hand, the nonspecific binding of MCP to the nonimprinted stir bar was very low. It is well known that the molecular recognition principle of most of MIPs is based on the hydrogen binding between the target and the polymer functional groups.[21] In this MIP system, the template (L-glutamine) could hydrogen bond strongly with amide group of Nylon-6, which cannot be disturbed in methanol and water (as shown in Table I). Hydrogen bonding has been shown to be very effective in the creation of recognition sites.[22] These bonds are used to create the recognition sites that later recognize the template molecule during reintroduction. But in higher acidic condition such as 10% acetic acid/methanol, the hydrogen bonding between L-glutamine and Nylon-6 was restrained and the molecularly imprinted Nylon-6 couldn't recognize the template molecule. Therefore, 10% acetic acid/methanol could be used as elution solvent. The results indicated that stirring with 10% (v/v) acetic acid/methanol solution for 60 min could completely remove the template from the imprinted Nylon-6 stir bar.

### Selectivity of L-glutamine imprinted Nylon-6 stir bar

The interaction of L-glutamine and Nylon-6 was characterized by IR spectroscopy. The FT-IR spectra provided information on the interaction between the imprinted polymer and template molecule via hydrogen bonding. According to Reddy et al.,[23] the

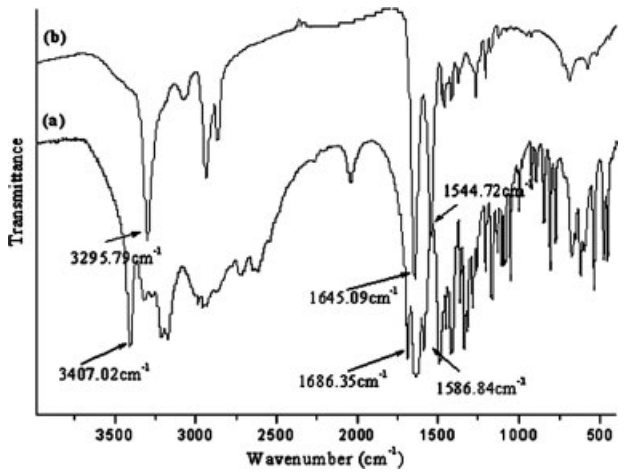


**Figure 2** FTIR spectra of (a) L-glutamine and (b) L-glutamine imprinted Nylon-6.

IR band of amide-carbonyl region at 500–1700 $cm^{-1}$ was analyzed to verify the hydrogen bonding between Nylon-6 polymer chains. In present paper, the IR bands of L-glutamine region at 500–4000 $cm^{-1}$ were measured to evaluate the hydrogen bonding between L-glutamine and Nylon-6 polymer chain.

Figure 2 showed the FTIR spectra of (a) L-glutamine and (b) L-glutamine imprinted Nylon-6 before the template molecule extraction. In the IR spectra, absorption of N-H stretching vibration at 3407.02 $cm^{-1}$ was shifted to lower wave number side of 3295.79 $cm^{-1}$ after the imprinted process. The broad band of medium intensity between 3214.02 and 3174.25 $cm^{-1}$, which may depend on the hydrogen-bonds among L-glutamine, was diminished after the imprinted process. On the other hand, absorption of amide-I and amide-II of L-glutamine were appeared near 1686 and 1587 $cm^{-1}$, which were assigned to C=O stretching vibration (amide-I) and N—H deformation vibration (amide-II), were shifted to lower wave number side of 1645 and 1544 $cm^{-1}$, respectively.[24] These changes indicated that the hydrogen bonding formation between L-glutamine and networks of the Nylon-6.

## Morphology characterization of MCP imprinted Nylon-6 film

As previously reported, the hydrogen bonding interaction between L-glutamine and amide group of Nylon-6 was characterized by IR spectroscopy. Here, we confirmed the morphology of the L-glutamine imprinted Nylon-6 by scanning electron micrography (SEM). Figure 3 depicted SEM pictures of the structures of the L-glutamine imprinted and nonimprinted Nylon-6. It showed that the sol–gel L-glutamine imprinted Nylon-6 film possessed porous structure, while the nonimprinted Nylon-6 film appeared to have smooth surface and few pore structures. M. Shibata et al. reported previously[25] that the imprinted Nylon-6 membranes prepared with phase inversion process had porous structure, which was in agreement with our result. The image implied that the pores were in the range of about 1–2 μm [as shown in Fig. 3(a)]. Therefore, their porous nature of the imprinted Nylon-6 enabled L-glutamine to permeate through the film and enhanced the surface area for solute-solid phase interactions in the polymer cavities and the extraction rate.

Nylon-6 consisted of regular hydrogen-bonding networks in the crystalline polyamides. Nylon-6 MIPs differed from the typical MIP system in that the films were cast from polymer solutions of the template molecule rather than polymerized from monomers in the presence of the template mole-

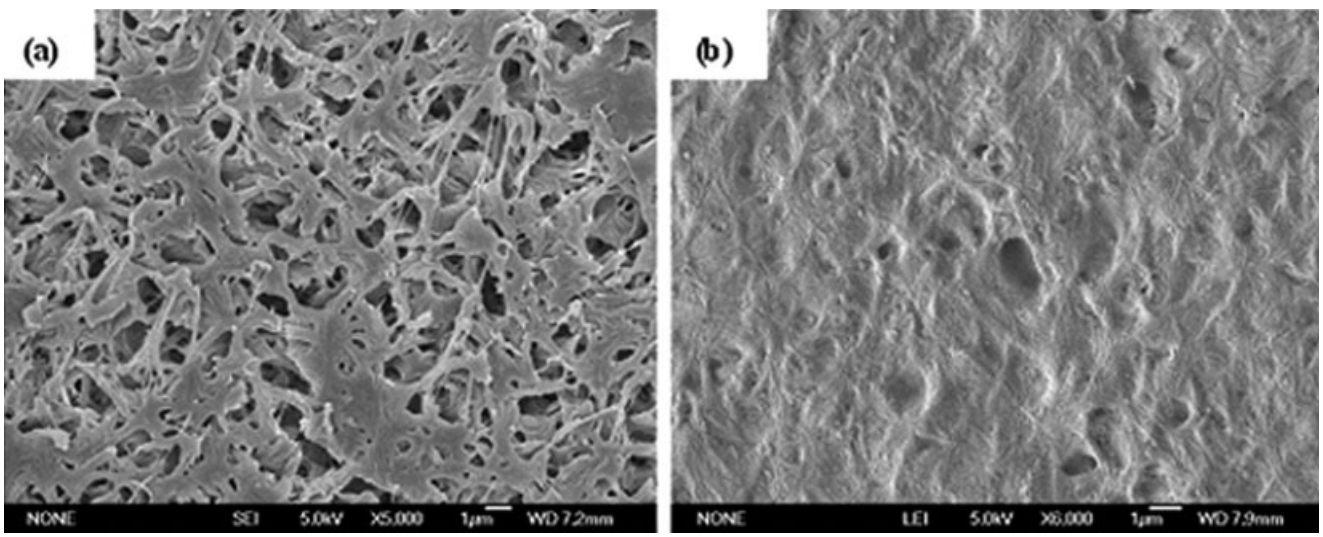


**Figure 3** SEM image of (a) L-glutamine imprinted Nylon-6 membrane and (b) nonimprinted Nylon-6 membrane.

**TABLE II**
**The Changes of Amino Acids Concentrations in the MIP-Film and NMIP-Film Stir Bar Extract Solution**

| Concentration (μmol $L^{-1}$) | L-Asparagine | L-Glutamic acid | D-glutamic acid | L-Glutamine | D-Glutamine |
|---|---|---|---|---|---|
| Standard solution | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| MIP-film stir bar extract solution | 18.7 | 24.0 | 23.9 | 9.64 | 24.1 |
| NMIP-film stir bar extract solution | 24.5 | 24.6 | 24.4 | 24.3 | 24.7 |

cule.[26] In formic acid solution, the nylon-6 crystalline polymer was separated into elongated chains. When the template molecule was added to Nylon-6 formic acid solution, it would hydrogen bond with the nylon chains forming linked linear chains. Spin coating removed the solvent, forming a film that was a network of L-glutamine and self-linked mixture in the crystalline and amorphous nylon. The extraction process removed L-glutamine, but the cavity within the polymer network remained.

To assess the stability of the homemade coating (MIP and NIP), the coated stir bars were exposed to various solvents and the films peeled from the stir bars were placed under a microscope to measure its thickness. With SEM, it was shown that the thickness of dry coated films was $\sim$ 160 μm. The reproducibility of the film preparation was good as fluctuations in coating thickness were within 10%. In addition, the stir bars could be used for up to 100 extractions with significant reduction of extraction performance.

### Selectivity of L-glutamine imprinted Nylon-6 stir bar

To evaluate selectivity of the MIP stir bar against its isomer and analogues, five amino acids (L-glutamine, D-glutamine, L-glutamic acid, D-glutamic acid and L-asparagine) were selected to test the binding characteristic of MIP stir bar. A total of 10 mL of a mixture aqueous solution of 25 μmol $L^{-1}$ of each amino acid was applied to similar binding experiments using the L-glutamine Nylon-6 stir bar and the blank star bar. After extraction, the stir bar was removed from the standard solution and the changes of amino acids concentrations in the solution were determined by reversed-phase HPLC.

The results were showed in Table II. It can be seen that the nonimprinted Nylon-6 stir bar showed little sorption of the amino acids, almost all of the amino acids were retained in the extract solution. However, a different result was observed for the L-glutamine imprinted stir bar. Besides the template molecule L-glutamine, L-asparagine could bind to the MIP stir bar. The result showed that the MIP stir bar exhibited highly selective sorption affinity for L-glutamine and L-asparagine, and demonstrated the adsorption of the two amino acids was due to imprinted sorption sites and not due to nonspecific sorption while other amino acids showed less or no sorption. The left amino acids could not be recognized by the MIP stir bar and separated from amino acids mixture.

To verify the adsorption of amino acids, each amino acid with the concentration of 100 μmol $L^{-1}$ was applied to similar binding experiments using the L-glutamine Nylon-6 stir bar and the blank star bar. Figure 4 presented the saturation binding amounts of five amino acids. Similarly, the values of $Q$ for the other amino acids increased with time as well as that for L-glutamine. And the MIP Nylon-6 stir bar bound somewhat other amino acids, especially L-asparagine. However, the values of $Q$ for the other amino acids were lower that measured for L-glutamine. This could be easily explained by their structural homology to L-glutamine (as shown in Fig. 5). Though there are similar function groups in the structures of these molecules, which can interact with amide group of Nylon-6 (as shown in Fig. 1). It can be seen that there are some differences between

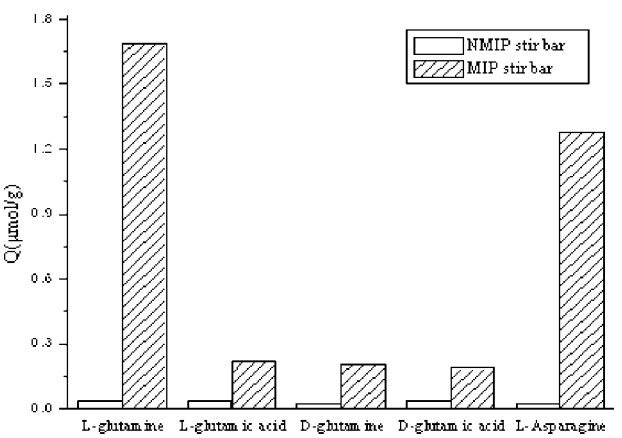


**Figure 4** The saturation binding amounts of five amino acids binding to the L-glutamine imprinted Nylon-6 stir bar and nonimprinted Nylon-6 stir bar. The concentration of five amino acids was 100μmol/L. The binding experiments were carried out at 20°C.

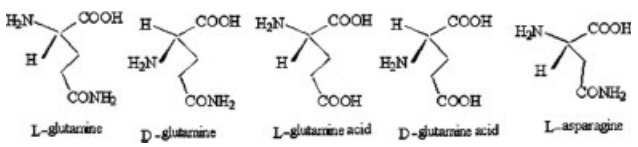


**Figure 5** Chemical structure of L-glutamine and its similar.

the structure of L-glutamine and other amino acids. For D-type amino acid, the most difference is $—NH_2$ in the different direction from chiral $—C^*$ position. For L-glutamic acid, the structural difference is a —COOH instead of $–CONH_2$. For L-asparagine, the only difference is lost a $—CH_2$ in framework, which shows no influence on function groups. According to the molecular recognition principle, the imprinting is not only based on the interaction of the functional groups of the analyte with those binding sites in the polymer cavities but also based on the combined effect of shape and position complementarily.[27] Therefore, not only the MIP stir bars have higher affinity for the template as compared with the respective blank stir bars, but they also could be used as a powerful tool for the selective enantioseparation of amino acids.

## CONCLUSIONS

This investigation showed the preparation of molecular imprinted film coated on stir bar and its performance for selective enantioseparation of amino acids. The MIP-film was prepared by precipitation of the polymer in the presence of the template molecule and coated onto the surface of stir bar immersed in water by the so L-gel imprinting technique. The preparation of the stir bar coated with MIP-film was simple and rapid. The extraction and reintroduction could be completed in less than 60 min. It has been demonstrated that both film preparation and extraction performance are reproducible as MIP- and NMIP- coated films were used throughout the study. The extraction showed that the MIP-film exhibited high affinity to L-glutamine in methanol and water, which wasn't disturbed in polar solvent. Evidence for the recognition was supported by FT-IR that the imprinting was performed in the hydrogen bonding networks of the MIP. The SEM photographs showed that active site structure could be detected in the MIP-film. This molecular imprinted Nylon-6 stir bar was found to be applicable successfully for selective enantioseparation of amino acids. This combination of MIP film and SBSE can extend the application molecular imprinting technology in bioanalysis conveniently.

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