Synthesis of Highly Efficient D-naproxen Imprinted Polymer and Investigation of Their Specific Performance

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ABSTRACT: D-naproxen was selected as the imprinted molecule. An original ultraviolet spectrum analysis method based on proof of solvent effects was designed to verify the realization of the noncovalent self-assembly between naproxen and functional monomers under the influence of the solvents with different nature. The facts proved that it is a more scientific way to effectively implement the pre-selection of functional monomer. Then, a series of molecular imprinted polymers (MIPs) were synthesized by different ratios of raw materials. These products were detected by infrared spectroscopy as well as adsorption experimentation to analyze their structure and performance. Meanwhile, the transmission electron microscopy (TEM) was first used to observe the morphology of MIPs, by which the pore-forming mechanism and the effect of the solvent (porogen) were investigated. The MIPs with morphology of different structure were also tested in the adsorption kinetics experiments to testify the influence of morphology factors on the materials' adsorption properties. Through the evaluation of the imprinting efficiency, the best condition accessing to the preparation of highly efficient D-naproxen imprinted polymer was obtained. The apparent imprinting efficiency of the ultimate product was as high as 90.39%. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1050–1062, 2009

Key words: molecular imprinted polymer; supramolecular self-assembly; porous material with stereo-mesh structure; imprinting efficiency; D-naproxen

INTRODUCTION

The original thought of molecular imprinting can be traced back to Fischer's "lock and key," Pauling's "antigen–antibody," as well as Dickey's "specific absorption." But the elements of modern molecular imprinting technology should own to Wulff[1,2] and Mösbach,[3,4] whose pioneering work have been making progresses in molecular imprinting technology by leaps and bounds. Molecular imprinting technique is an ingenious way based on "copy and memory," in which a skeleton of macromolecules with the nature of selective molecular recognition can be prepared. This technique provides a promising alternative way to design artificial recognition sites within a synthetic polymer network through the template polymerization process. In this process, polymerizable functional monomers are prearranged around a template molecule in porogenic solvent. The resultant pre-polymer complexes are copolymerized with an excess of crosslinking monomer in the presence of a free radical initiator under thermal or photochemical conditions. After the removal of the template by extracting, binding sites complementary to the template molecule both in shape and chemical functionality, are left within the polymer matrices that allow rebinding of the template with high specificity. This copolymer product is known as molecular imprinted polymer (MIP), and the template as imprinted molecule.

MIP is a kind of porous material of highly crosslinked polymer based on three-dimensionally molecular recognition. It has been exploited extensively in many different applications including their use as separation materials, chemical sensors, reaction catalysts, enzyme mimics and so on.[5–9] In particular, the practical application of a matrix or medium material for enantiomeric separation of chiral drugs has become a spectacular subject.[10–12]

The specific properties of molecular imprinted polymer material lies on several factors: First, the interaction force between imprinting molecule and functional monomer to form the host–guest complex is the core factor for characteristic functionality of the synthetic materials. There are two cardinal matching modes including the preorganization process relies on the role of covalent bond and the self-assembly process relies on noncovalent binding.

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They are mainly determined by the imprintogenicity of target molecule as well as the characteristic groups of functional monomer.[13] The interaction force is able to affect the spatial orientation and stability of the complex, so as to determine the imprinting efficiency. Second, crosslinking agent and synthetic conditions make an important guarantee for the materials to have an excellent performance. The crosslinking agent is the main reactant for the synthesis. Its species (rigid elements) and amount (crosslinking degrees) would influence on every aspect of properties of MIPs. At the same time, the initiation method and environmental conditions, in the synthesis process of radical polymerization mechanism, could certainly make an impact on the final performance of materials.[14] In addition, solvents and other factors will also have effects on the modality and property of MIPs, which cannot be ignored. As an important part of the raw materials, solvent plays two major roles in the synthetic system during the entire preparation process. The one is to provide a reaction field, and the other is to act a pore-forming function. Solvent, which can influence the imprinting efficiency and morphology of the product, is also a determinant element for the material properties. But the research work in this field has not been enough till now; this study has done an intensive analysis on this aspect.

The self-assembly process of host–guest complex in noncovalent interaction belongs to category of supramolecular chemistry. With advantages of great variety, easy-to-implement, and closer to the natural characteristics of the molecular recognition system, it has become research focus in the field of molecular imprinting technology. In this study, the choice of D-naproxen, which is a kind of nonsteroidal anti-inflammatory drug for antipyretic analgesics, has the potential of the supramolecular complex in the molecular structure and, therefore, has good imprintogenicity. In this study, an ultraviolet spectrum analysis method was designed to verify the realization of naproxen compound with functional monomers in the control of the solvents effects. Facts have proved that this is a more scientific way to effectively implement the preselection of functional monomer. In this experiment, a series of MIPs synthesized by different ratios of raw materials were tested by infrared spectroscopy and adsorptive experimentation to analyze their structure and performance. Through the evaluation of the imprinted efficiency, the best condition accessing to the preparation of highly efficient D-naproxen imprinted polymer was obtained. Meanwhile, the most important research was that transmission electron microscope (TEM) was used, for the first time, to observe the morphology of molecular imprinted polymers, by which the pore-forming mechanism and the effect of the solvent (porogen) were explored. The MIPs with morphology of different structure were also measured in the adsorption kinetics experiments to validate the influence of morphology factors on the materials' adsorption properties.

Naproxen, who has a pair of optically active enantiomers, is a kind of chiral drug. Clinical studies have shown that the drug-effect of S-(+)-2-(6′-methoxy-2′-naphthyl) propionate is about 28 times than its enantiomeric configuration of R-(−)-2-(6′-methoxy-2′-naphthyl) propionate. To reduce the dosage of drugs as well as avoid toxic and side effects on the human body, the medicine on sale must be in the form of a pure isomer, which is chiral separated from the racemate. In our work, a novel material of molecular imprinted polymer with the specific selectivity of recognition on D-naproxen both in three-dimensional structure and binding sites was prepared. It is expected to be used as a matrix or medium material for the chiral separation. This study laid a foundation for the further applied research and provided a new idea on enantioseparation of chiral drugs.

EXPERIMENTAL

Materials and reagents

D-Naproxen (Npx) was obtained from Zhejiang Charioteer Pharmaceutical Co., Ltd. (Xianju, Zhejiang Province, China, 99.5% ee). Acrylamide (AA) and methacrylic acid (MAA) were supplied by Tianjin Chemical Reagent Research Institute. Ethylene glycol dimethacrylate (EGDMA) was purchased from Shanghai Haiqu Chemical Co., Ltd. All of the monomers were purified by vacuum distillation or recrystallization to remove inhibitor before use. 2,2-Azo-bis-isobutyronitrile (AIBN) was product of Tianjin No.2 Chemical Reagent Factory and used as received. Chloroform, acetonitrile, methyl alcohol, acetic acid, anhydrous ethanol, and other chemicals were analytical grade and obtained from commercial sources.

Main apparatuses

All UV–vis spectra and adsorption experiments were performed using an UV-1200 ultraviolet-visible spectrophotometer (Beijing Rayleigh Analytical Instrument Corp., China). The infrared spectrum analyses were taken on a TENSOR37-Fourier Transform Infrared Spectrometer (Bruker Corporation, Germany). An H-7650 Transmission Electron Microscope (Hitachi, Ltd., Japan) was used to observe the morphology of imprinted polymer. To synthesize, prepare, and process the products, there are many other equipments were used. Including: WE-1 thermostat water bath oscillator (Tianjin Honour

TABLE I
The Chemical Composition and Initiation Method of Synthesis[a]

MIP	Mole ratio of raw materials	Solvent/Porogen (mL)	Initiation method
P1	Npx : AA : EGDMA = 1 : 4 : 20	$CHCl_3$(15)	Thermal
P2	Npx : AA : EGDMA = 1 : 4 : 20	$CHCl_3$(20)	Thermal
P3	Npx : AA : EGDMA = 1 : 4 : 20	$CHCl_3$(25)	Thermal
P4	Npx : AA : EGDMA = 1 : 4 : 10	$CHCl_3$(15)	Thermal
P5	Npx : AA : EGDMA = 1 : 4 : 30	$CHCl_3$(30)	Thermal
P6	Npx : AA : EGDMA = 1 : 4 : 10	$CHCl_3$(15)	UV
P7	Npx : AA : EGDMA = 1 : 4 : 20	$CHCl_3$(20)	UV

[a] The molar amounts of D-naproxen: 1 mmol. The dosage of initiator (AIBN): 1% of the weight of EGDMA.

Instruments Inc., China), SK5200 ultrasonic vibration generator (Shanghai Kudos Ultrasonic Instrument Co., Ltd., China), TG16-WS(1650D) supercentrifuge (Shanghai Luxiangyi Gentrifuge Instrument Co., Ltd., China.), long-wave (365 nm) ultraviolent lamp (Tianjin Zijin Special photo Source Co., Ltd.), Soxhlet extractor, etc.

UV spectrometry on the mixture solutions of D-naproxen and functional monomers

A series of solutions were prepared with a fixed concentration of Npx (0.5 mmol/L) and various amounts of AA in $CHCl_3$. The ratios of the molar concentration between Npx and AA among this set of solutions were as follows: 1 : 0, 1 : 1, 1 : 2, 1 : 3, 1 : 4, 1 : 5, 1 : 6, 1 : 8. Then, they were shaken for about 4–5 h aiming at sufficient interaction. The changes in absorbance and difference absorption spectra of these solutions were determined on UV-spectrometer with corresponding AA-$CHCl_3$ solution as reference. Another solvent (acetonitrile) was also applied on the identical method.

A compared study, replace AA with MAA as an alternative, was also carried out in the same way. These results were used for further analysis.

Synthesis of D-naproxen molecular imprinted polymers

To compare the properties of MIPs prepared by different polymerization conditions, a series of polymers were synthesized. The chemical composition and initiation method for making MIPs are shown in Table I. The synthetic procedure for the preparation of the standard polymer P1 was as follows: 0.23026 g (1 mmol) of D-naproxen (Npx) and 0.28432 g (4 mmol) of functional monomers (acrylamide, AA) were dissolved in chloroform in a 50-mL glass ampoule. After oscillating for 6 h, a certain amount of crosslinker (ethylene glycol dimethacrylate, EGDMA) and initiator (AIBN) were added. The solution was degassed in a sonicating bath, and deoxygenated with a stream of nitrogen for 10 min. Then, the ampoule was placed into an ice bath apparatus and sealed under vacuum. The polymerization was allowed to carry out in a constant water bath at 55°C or under ultraviolet lamp (365 nm) at 15°C for 24 h. The resultant rigid polymers were ground to pass through a 74-μm sieve. Fine particles were removed by decantation in acetone. The resulting particles were placed in a Soxhlet extractor and washed with 10%acetic acid-methanolic solution until the template could no longer be detected in the elution. Then, the particles were washed with pure methanol to remove residual acetic acid and dried to constant weight under vacuum at 80°C. As a control, the nonimprinted polymers (NIPs) in the absence of the template were prepared and treated by using the same method.

IR spectrometry on the polymers

The polymers were grinded into fine powder. After they were dried completely, made test samples in pressed disc method with KBr. Then scan to obtain their infrared spectra, respectively, using TENSON37-Fourier transform infrared spectrometer. And then the polymers of molecular structure were analyzed, comparatively.

TEM characterization of molecular imprinted polymers

Add the MIPs with a certain degree of fineness into ethanol to prepare suspension of an appropriate concentration and scatter for 15 min in the ultrasonic oscillator. Then employ the professional copper net to dredge powder samples, quickly, or just adopt a point sample method to make samples under test. After removing the volatile ethanol absolutely, they can be observed in the TEM.

The kinetics approach of polymers' adsorption on naproxen

AA solutions were prepared to 4 mmol/L at 10 mL, and adsorption experiment was performed adding

100 mg MIP, which removed the template. When the absorption went through different time (15, 30, 45, 60, 75, 90, 120, 150, 180, and 240 min), the mixture was separated by centrifugation, each moment. As the absorbency of upper clear liquid was determined with quantitative measurement of UV absorption spectroscopy, their concentration could be fixed according to the standard curve. Then the amounts of Npx bound to the imprinted polymer, Q (μmol/g), were calculated by the following eq. (1):

$$Q = (c_0 - c_t) \times V/m \quad (1)$$

where, c_0 and c_t were the Npx concentration (mmol/L), measured at initial and postinterval time (min) for equilibrium. Symbols V and m were the volume of the Npx solution (10 mL), and the weight of dry polymer (100 mg) used for the adsorption experiment, respectively.

The obtained data were used in drawing up kinetic curves reflecting the relationship between adsorption volume Q of polymers on Npx and adsorption time t.

The isothermal adsorption experiment of polymers

Ten equivalent portions of MIP for mass of 100 mg were put into a group of Npx solutions with gradient concentrations (0.25–7.00 mmol/L), respectively. After the equilibrium was attained for all MIPs, they were centrifuged, and the degree of adsorption was measured on the UV–vis spectrophotometer.

The MIPs and NIPs, after the adsorption to naproxen, could be eluted in accordance with the method of molecular imprinting removing off the template and dried for reusable.

RESULTS AND DISCUSSION

Verification of supramolecular complex by UV spectrum

It is critical to determine the possibility and stability of interaction between template molecule and functional monomers to form host–guest complex before the design of synthesis. In this study, the selected imprinted molecule (Npx) and functional monomer (AA) were expected to form a stable supramolecular complex through the noncovalent self-assembly process, which would impact electronic energy levels in the imprinted molecular. Ultraviolet–visible absorption spectra that are generated by the electron energy level transition in molecules depend on the electronic distribution and valence electron structure. So the various compounds of ultraviolet–visible absorption spectrum are the manifestation of characteristic in a variety of electronic transition between energy levels of molecules in the inherent law. Accordingly, the UV spectrum can be used to analyze the molecular structure before and after self-assembly and confirm the formation of supramolecular complex.

In addition, the solvent effect has a complicated impact on the ultraviolet spectrum. The most obvious is the different polarity of the solvent can cause changes in the shape of the band and shift of the maximum absorption peak location. This is also due to a variety of different solvents have different effects on the energy of electronic ground state and excited state. Therefore, to verify the noncovalent self-assembly between imprinting molecule (Npx) and functional monomer (AA), the mixture of Npx and AA was tested using the ultraviolet spectrum analysis method in the control of the solvents effects. In these experiments, chloroform and acetonitrile were used as solvent. The test solution samples were prepared at a fixed content of Npx with gradually augmenting amounts of AA. These series of mixed solutions were scanned on ultraviolet spectrometer with the corresponding concentration of AA solution as the reference, respectively. The UV absorption spectra of Npx with background correction for AA and solvent were shown in Figure 1.

Naproxen is a molecule with the molecular structure of naphthalene in which 2,7-hydrogens are substituted by the 2-propionyloxyl and methoxyl groups, respcetively. Absorption bands in these spectrums are caused by transition of $\pi \rightarrow \pi^*$ on the naphthalene ring and $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$ in the substituting groups. These two groups of UV absorption spectrum for naproxen in the two solvents showed different position and shape of peaks in E and B bands (ethylentic and benzenoid bands), what was due to the solvent effect. When different nature of solvent surrounded the naproxen molecules, they had different impact on the electronic structure and freedom of these elements. So that, the electronic transition energy varied, resulting in the transformation of the absorption band. However, when adding functional monomer (AA), another change in the intensity and position of these peaks took place, regularly. With the addition of the monomer, the absorption peak intensity lowered and the maximum absorption peak position shift, gradually. It indicated that some strong intermolecular forces occurred between Npx and AA. On the basis of molecular structures of Naproxen (Npx with the methoxyl and carbonyl groups) and acrylamide (AA with the amide group), it could be deduced that hydrogen bonding might play a key role between them and a template-functional monomer complex formed (like Fig. 2) through the molecular self-assembly process, in which the electron cloud distribution and density of big pi (π) bond on naphthalene are

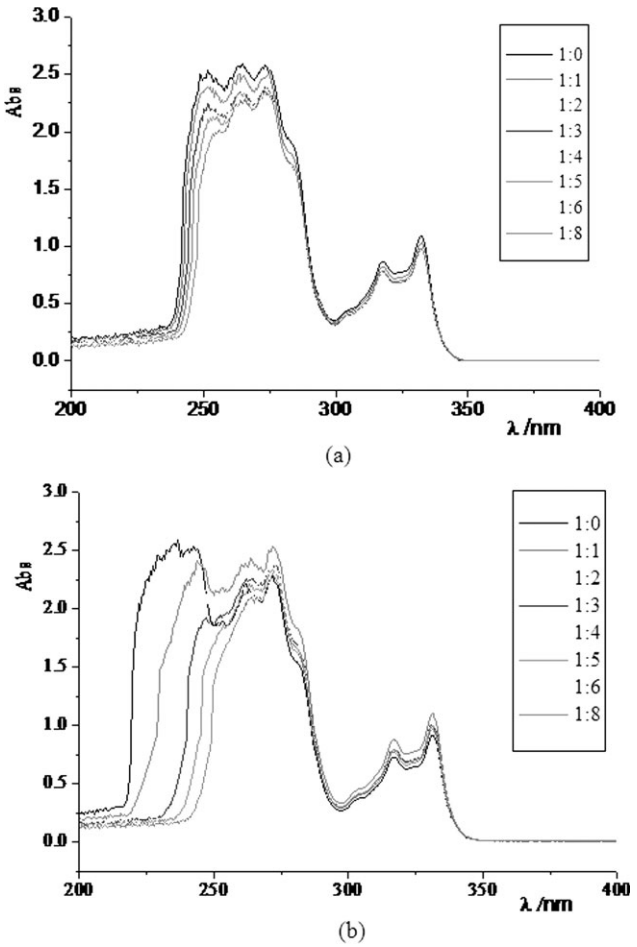


Figure 1 UV-spectrograms of Npx and Npx-AA in different ratios (Npx : AA). (a) With chloroform as solvent. (b) With acetonitrile as solvent.

transformed. This is the result of changes in the ultraviolet spectrum of Npx.

Solutions of chloroform (dielectric constant, $\varepsilon = 5.20$) and acetonitrile ($\varepsilon = 37.50$) used in this study have dissimilar nature, such as solvent polarity, dielectric constant, protonation, complexation and so on. Their effects on the imprintogenicity of naproxen and function of monomer are different. Hence, they impact on the supramolecular self-assembly process in varying degrees. The above-mentioned results showed that noncovalent interaction of functional monomer (AA) with imprinting molecule (Npx) had rivaled the solvent effect of acetonitrile. So that the red shift of peak and less intensity of absorption took place. This result strongly proved that a stable supramolecular complex had formed through self-assembly between Npx and AA.

In addition, the strength of interaction force between the molecular template and functional monomer has a direct effect on the selectivity of MIPs' recognition. The weak force would reduce the stability of the complex, which will lead to poor selective recognition of the molecular imprinted

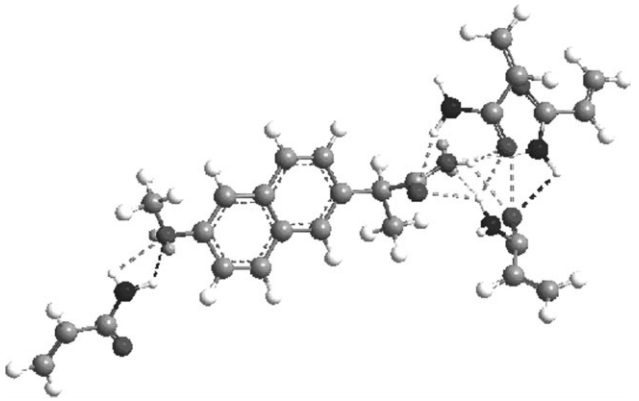


Figure 2 Simulation structure of Npx-AA supramolecular complex.

polymer. Through the UV spectrum analysis, the force strength of two different functional monomers (AA and MAA) to naproxen was detected.

Figure 3 was UV-spectrogram, for MAA as functional monomer, measured in the same way with AA in accordance. Contrast to these two groups of

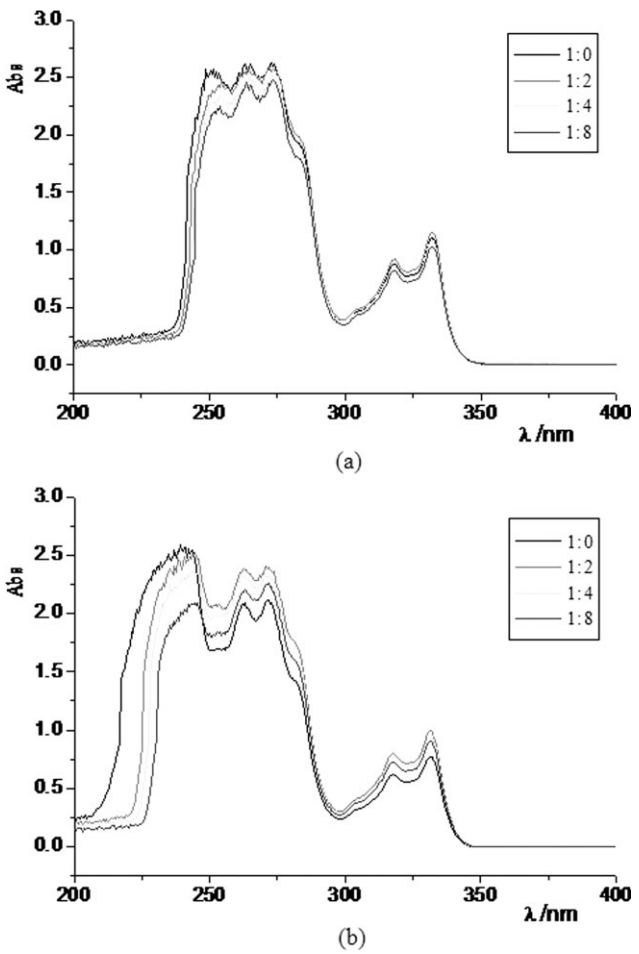
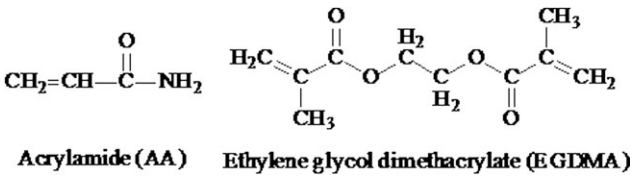


Figure 3 UV-spectrograms of Npx and Npx-MAA of different ratios. (a) With chloroform as solvent. (b) With acetonitrile as solvent.



Scheme 1 The molecular structures of acrylamide and ethylene glycol dimethacrylate.

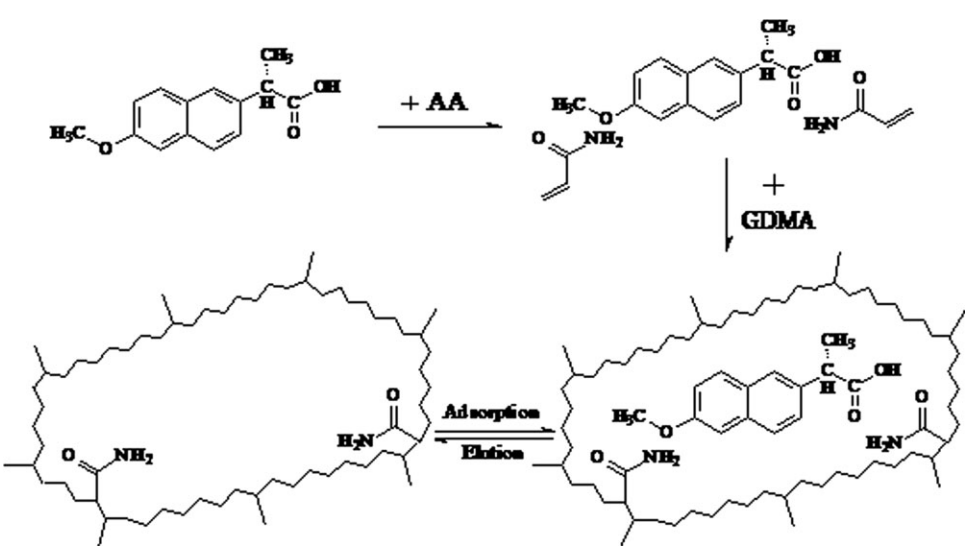
spectrums, Figure 2 showed more conspicuous changes. This indicated that a stronger intermolecular interaction formed by AA and Npx (naproxen), when the same concentration of AA and MAA compounded with Npx. It can be inferred that AA, compared with MAA, is a preferred functional monomer, and it can form better complex with Npx, resulting in better identification of the binding sites.

Structural analysis of polymers by IR spectrum

The information from IR absorption spectra, such as position, shape, and intensity of the absorption peaks, could access to the relevant structure of molecules. In this experiment, the IR analysis was applied to the characterization of the synthetic polymers' structure and the certification of functional groups existing in molecules. The molecular structures of functional monomer (acrylamide, AA) and crosslinking agent (ethylene glycol dimethacrylate, EGDMA), used in this synthesis, are shown in Scheme 1.

The synthesis route in this experiment is shown in Scheme 2. The infrared spectra of products are shown in Figure 4.

There were six spectral lines in Figure 4, where lines marked as N_1, M_2, and M_3 corresponded to NIP, MIP (UV), and MIP (Thermal) before eluted, respectively, and Line N_2 and Line M_4 corresponded to the NIP and MIP after eluted. Besides, Line M_1 was for the spectrum of MIP after adsorbing Npx. These six lines almost had similar absorption peak forms in each district. Among them, the absorption peak at 2955 cm^{-1} of all the polymers arised from the C—H stretching vibration (υ_{C-H}). 1730 cm^{-1} was for the carbonyl groups absorption both in AA and in GDMA ($\upsilon_{C=O}$). And absorption band around 1157 cm^{-1} was attributed to the C—O ester group of GDMA (υ_{C-O}). While the wide absorption band around 3452 cm^{-1} or so corresponded to asymmetric stretching vibration of H—N—H in amide group. The stretching vibration absorption peaks of C—N and the bending or rocking vibrations absorption peaks of N—H in amide group appeared in the regions of 1455–1391 cm^{-1} and 750–880 cm^{-1}, respectively. The existence of molecular structure earlier proved that products of crosslinked copolymer were synthesized by EGDMA and AA in this experimental preparation conditions. And they do have the chemical group, amide group, which can interact with Npx molecule. Besides, in the infrared spectrums of line M_1, M_2, and M_3, there were a few absorption peaks more appearing at 3367, 1609, 666, 475 cm^{-1}, etc. And the original peaks in the 850–960 cm^{-1} region, which seemed to be identical in all the spectra, exhibited a subtle difference at a closer look. This was due to elements of naproxen existing in the system. They were presumably assigned to stretching vibration of O—H in carboxyl group, naphthalene skeleton vibration, bending (rocking or twisting) deformation of C—H in naphthalene ring, and bending modes about C—O—C or the torsional modes about the single bonds C—O, respectively. It suggested that naproxen molecules were successfully introduced into the



Scheme 2 The synthesis route in this experiment.

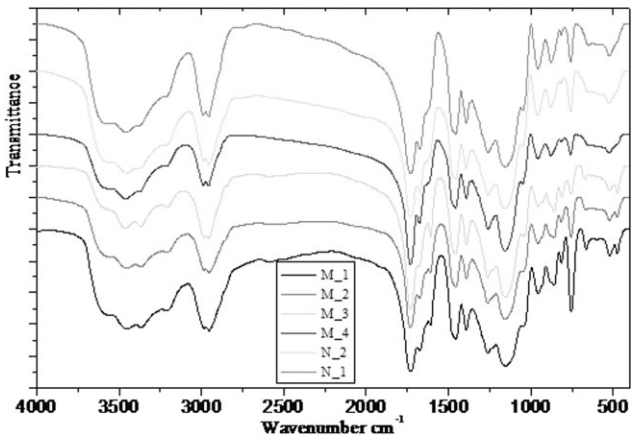


Figure 4 Infrared spectra of polymer products.

AA-EGDMA copolymer through imprinted molecule-functional monomer complexes. And they can also be eluted off and readsorbed on.

The morphology of molecular imprinted polymers characterized by TEM

The properties of material are determined by their microarea composition, microstructure, surface, interface, phase structure, and microscopic texture. These factors materials are commonly impacted with the system composition and environment of synthesis and post-treatment course. Solvent used in molecular imprinting reaction played two important roles in the process of synthesis. First, it dissolved the imprinted molecule and functional monomer achieving to form supramolecular complexes. Second, it occupied a certain volume of space, during the polymerization process, around the reaction substances and resulted in porous structure of the polymers. In this sense, solvent can also be referred as porogen. This porous structure could reduce the amount of imprinting holes embedded in the polymers. So the accessibility of recognition site was enhanced. The dosage of porogen have a significant impact on the porosity, pore distribution, and average pore size of the material. Therefore, the usage of porogen must be optimized in the process of molecular imprinting polymerization. In this experiment, chloroform with a low dielectric constant, and good solubility and volatility was selected as solvent.

The micromorphology and network structure of polymer materials can be directly observed by the transmission electron microscope (TEM). We used TEM to characterize the molecular imprinted polymers and explored the pore-forming function.

Figure 5 shows the three groups of TEM photos taken from P1, P2, and P3 in three magnification times, respectively. Compared with these three groups of photos, it can be seen that MIPs which were synthesized by different dosage of porogen have different morphology. Actually, the amount of porogen determined the concentration of both functional monomer and crosslinker in the solution. The less amount of porogen used, the higher is the concentration of the solution produced. When the polymerization occurred, the viscosity would increase rapidly. The porogen was not enough to enter into the polymer network to form the structure of pore channel. It affected the extent of the template-monomer complex packaged in the matrix. Image a. showed a large bulk of polymer with nonpore structure. It can be inferred the imprinting points had a serious degree of embedding in this material matrix with uneven thickness, which might be detrimental to the process of mass transfer. But when the amount of porogen was too much, concentration of the reaction solution would be too dilute. The radical polymerization reacted in slower rate and the polymerization degree of product might be lower. It was not easy to frame a spatial structure of space net-shape, so the material is loose (seen from Image c). In macroscopic view, the material had disadvantages of low hardness, poor mechanical strength, and easy to crush. Whereas, a moderate amount of porogen seemed to provide an appropriate condition forming an adequate construction on microscopic morphology. In Image b, the polymer presented irregular three-dimensional mesh patterns. Those pore channels running through the material were very useful. On one hand, they can greatly increase the surface area of the material and reduce the number of embedded imprinting points in MIP, so as to enhance the accessibility of binding site. On the other hand, they can provide a passing medium for in-house channels. This is conducive to the achievement of combination and absorption.

Solvent used in the molecular imprinting process is not only to contribute to the uniform composition, which formed before polymerization reaction, but also to impact on the porosity and surface area of the polymers, which determine molecularly imprinted polymer physical properties in the end. It plays an important role of influence on the physical properties of molecularly imprinted polymer.

Meanwhile, MIP_P7 prepared in different synthetic conditions had also been observed by TEM (Fig. 6). Compared them with image b, we can see that MIP synthesized in photoinitiated way has more uniform distribution of mesh than products of thermal-initiated polymerization. The pore shape are homogeneous (diameter of about 100–200 nm). Thus, the material matrix has a greater advantage on the morphology. This was probably due to the temperature and pressure factors, which had a certain impact on the polymerization process and the polymer structure. At present, it is hard, for less research

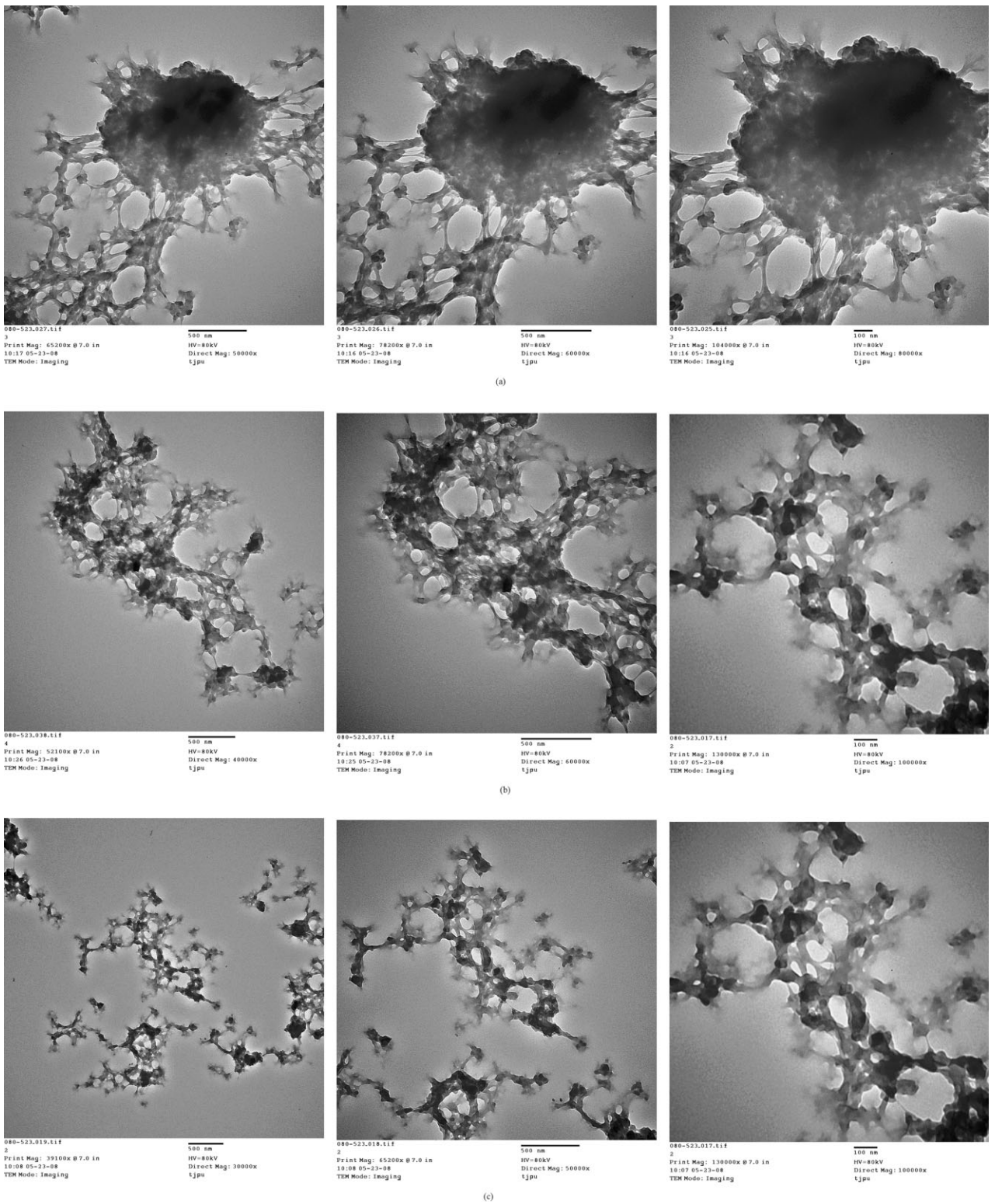


Figure 5 TEM images of MIPs prepared in different amount of solvents. (a) TEM image of MIP_P1. (b) TEM image of MIP_P2. (c)TEM image of MIP_P3.

of synthesis temperature and pressure factors in the field of molecular imprinting technique engaged, to draw firm conclusions on the polymer forming mechanism in different process. So a further study is needed in the future.

In addition, we also observed the MIP which had been used for several times (See Fig. 7).

These photographs show that the materials could maintain the original skeleton structure after the process of elution-adsorption-elution for several

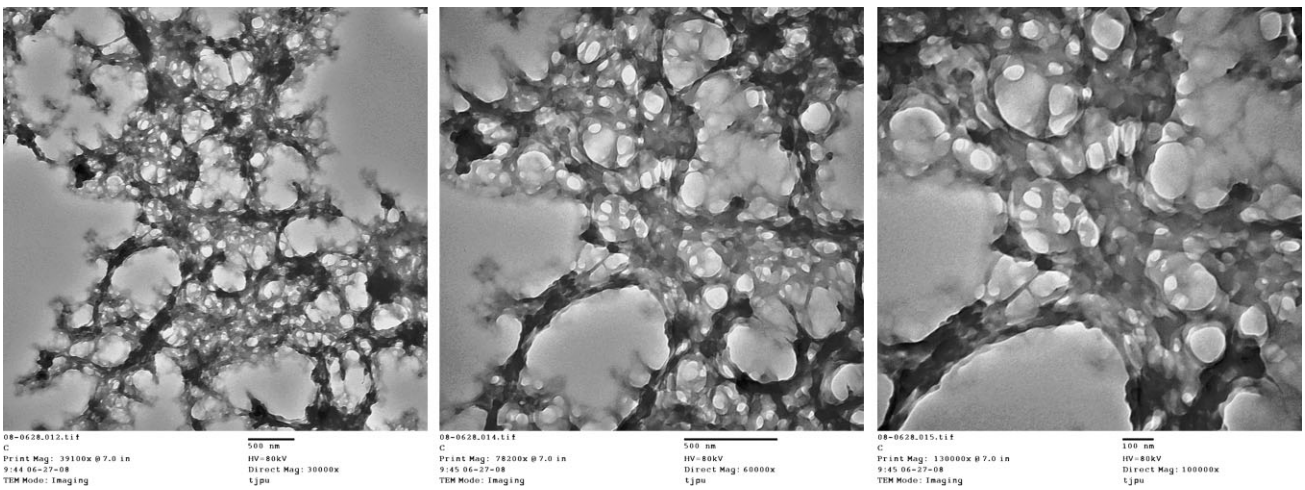


Figure 6 TEM images of MIP_P7.

times. So it can be certified that the synthetic material has good physical properties and solvent resistance.

Research on binding kinetics of adsorption property for MIPs

To study the adsorption kinetics properties of molecular imprinted polymers, the adsorption volume in different time was mensurated, using a fixed quality of MIP (100 mg) and initial concentration of target molecule solution (4 mmol/L). The kinetics curves drawing by adsorption volume Q to adsorption time t is as shown in Figure 8.

Molecular imprinted polymer with selective adsorption is mainly due to the "imprinting hole" or "imprinting cavity," which was produced by template molecule through the process of imprinting and eluting course. These holes have stationary shape and size as well as a fixed array of functional groups matching to the imprinting molecule. When a template molecule re-enters into this "hole" or "cavity," it will bind with the corresponding functional groups again. The adsorption process of the target molecule on MIPs are usually divided into three stages: First of all, the target molecules transfer in the vicinity of MIP; then, MIP physical adsorb the target molecules nearby; the last, MIPs combine the target molecules through the functional group. Usually the physical adsorption rate is very fast and can be completed in an instant.[15]

In this experiment, functional groups in the MIPs combined with naproxen in the main form of noncovalent bond; this combination speed was quite fast. Therefore, the combination rate of MIPs was mainly determined by the mass transmission speed of naproxen through them. By the dynamics of the curve over, we can see that all the MIPs had gone, on the

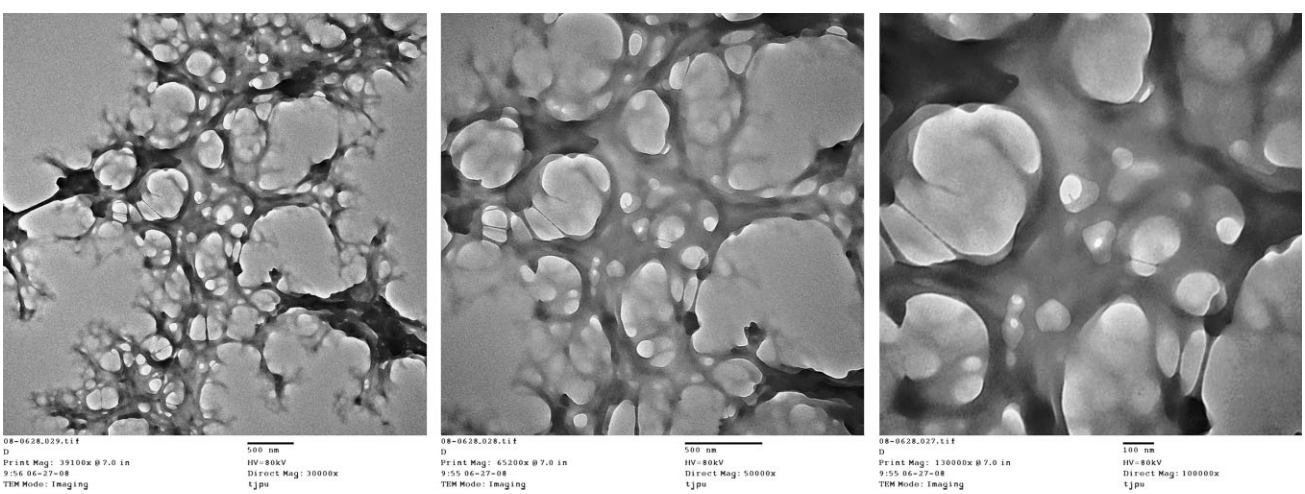


Figure 7 TEM images of MIP_P7 used for several times.

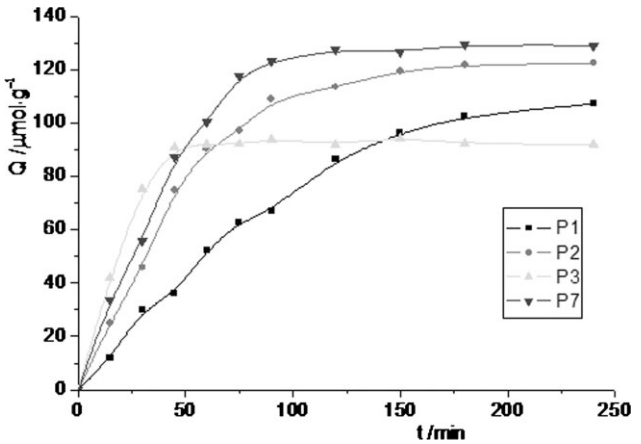


Figure 8 The kinetics curves of MIPs.

adsorption kinetics, through these several stages: adsorption in a uniform speed, deceleration, and balance of saturation. The part of the curve in a straight line reflected the adsorption and combination of the molecular cavity and binding site on the surface of MIPs. As the molecular mass transfer could keep a high speed without change, the combination speed was a constant. With the increase in absorption volume, the number of surface cavity (the molecular holes not combined with elements) reduced. The absorption and combination of MIPs on elements transferred nearby decreased gradually; thus, the rate declined at the beginning. Once the surface molecular holes were filled fully, the ones inside MIPs would lead a major effect in combination. But there were too much resisting forces from interface of material matrix and pore walls for the substrates driving into to the inner body of polymers. So the speed of mass-transfer cut down. Thereupon, the adsorption rate became slower and slower. After period of time, interior binding sites had been basically saturated. The increase in the amount of absorption is no longer clear. At this time, the absorption reached equilibrium.

As the holes are posed by crosslinking agent and functional monomer through copolymerization, their distribution cannot be completely uniform. So the depth of the holes is certainly different. The shallow holes are favorable to the adsorption of Npx on MIPs; it makes the absorption rate, at the beginning of assay, stable and fast. When they are filled up, the deeper transmission of naproxen encountering more resistance goes on. So the rate declines till it reaches the full saturation balance, eventually. In the earlier section of the TEM characterization and analysis, we sw that the dosage of porogen has a crucial influence on the morphology of materials. The structure of three-dimensional network formed by pores would change the surface area and volume distribution of polymer materials, which were bound to affect the extent of imprinting points being embedded. When the small amount of solvent was used, structure of large bulk material, in which a large number of imprinted sites had been embedded, was easily to form. Those deep holes were not conducive to the mass transfer. The kinetic curve (Line P1) indicated that the adsorption increased slowly and was not easy to reach saturation in a short period of time. When the large amount of solvent was used, structure of the material was loose. With a large specific surface area, the polymer's binding sites become easy-to-reach. As the naproxen molecule avoided driving in deep part of molecularly imprinted polymer matrix, a high adsorption speed had been produced (as shown in Line P2). However, because the over dilute concentration of the solution may affect the degree of polymerization, stiffness of the MIP was weaken, and the number of imprinted sites decreased, which reduced the saturated adsorption amount at the same time. Whereas, when the amount of solvent was moderate, the molecular imprinted polymers had the maximum absorption capacity. However, these polymers synthesized in two initiation ways made slightly different kinetic curves of Line P3 and Line P7. MIP of P7 synthesized in a photoinitiated way had a shorter deceleration period of absorption and a larger capacity of saturated adsorption. This was because that MIP material of P7 has relatively homogeneous porous structure on morphology. On this point, it has more excellent performance than the MIP of P3, which was synthesized in thermal-initiated way.

Determination of adsorption isotherms and Scatchard analysis

In the experiment, the equilibrium adsorption capacity Q of imprinted polymers on Npx, in different initial concentrations (range of 0.25–7.00 mmol/L), was determined using an equilibrium binding experiment method. The adsorption isotherm drawn from the experimental data of P7 is shown in Figure 9.

As can be seen from Figure 9, the adsorption of polymers adheres to the rules of monolayer adsorption of Langmuir model.[16] From the comparison of these two binding isotherms, it can be seen that, with the increase of the initial concentration, the absorption capacity of both MIP and NIP to the substrate has increased. However, the combination amount of template molecules on MIPs is always greater than that of NIP's, obviously; it would be saturated in a high concentration range. Thus, it showed more advantageous binding ability. In many of the receptor binding assay,[17] as the curve of nonselective combined volume to the initial concentration increases linearly, it is difficult to reach

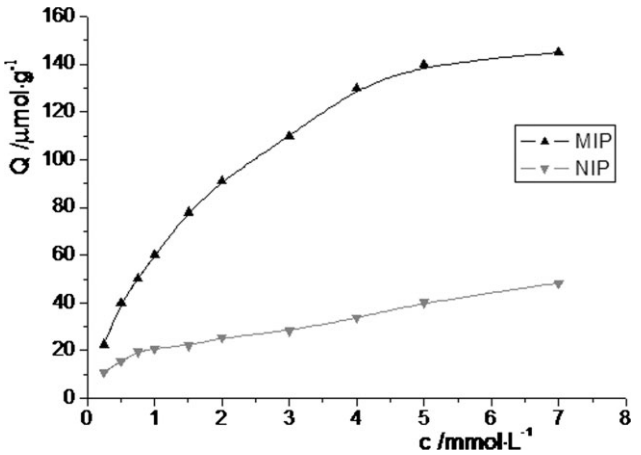


Figure 9 Adsorption isotherm of imprinting molecule on MIP and NIP.

saturation as a rule. This graph inferred that adsorption of NIP on Npx was non-selective, and MIP exhibited a selective absorption. This is because that many specific structure holes with fixed three-dimensional shape and position of functional groups matching to Npx formed during the synthetically process in the presence of imprinted molecule. These imprinted holes with active binding site in them caused a good ability to selectively binding on the MIPs. It also proved that the property of interaction force between imprinted molecule and functional monomer, when they presented as supramolecular complex in the course of synthesis and played an important role in specific identification and strong affinity.

In the study of molecular imprinting technique, the Scatchard model is often used to evaluate the molecular imprinted polymer adsorption characteristics, Scatchard equation is as follows[18,19]:

$$Q/c_{[\mathrm{Npx}]} = (B_{\max} - Q)/K_d \quad (2)$$

where K_d (mmol/L) is defined as the equilibrium dissociation constant of the binding sites, Q (μmol/g) is the amount of naproxen bound to the polymer, $B_{\max}$ is the apparent maximum number of binding sites and $c_{[\mathrm{Npx}]}$ (mmol/L) is equilibrium concentration of naproxen in the solution.

The data of P7 obtained from isothermal adsorption experiment were plotted according to the Scatchard equation.

As shown in Figure 10, there were two distinct sections within the plot, which can be regarded as straight lines. It suggested that there were two classes of heterogeneous binding sites in respect to the affinity for naproxen in this polymer. The linear equations and regression coefficients (correlation coefficients) were as follows, respectively:

$$Y = 157.02811 - 2.79088X \ (R = 0.99498)$$

$$Y = 24.94943 - 0.15944X (R = 0.95614)$$

From the slope ($-1/K_d$) and intercept ($B_{\max}/K_d$) of the straight line, the K_{d1} and the apparent maximum number $B_{\max 1}$ of the higher affinity binding sites can be calculated to be 358.31 μmol/L and 56.26 mmol/g, respectively. Similarly, the K_{d2} and $B_{\max 2}$ values of the lower affinity binding sites are 6.27 mmol/L and 156.48 μmol/g, respectively. The absorption by these two types of binding sites is a common result.

The cause of these two classes of adsorption sites might be: there may be two inequable modalities of complex combined in a variety of interaction between the imprinting molecule and functional monomers in the solution of reaction mixture before and during the polymerization. Both of them were able to enter into the cavity in the polymer matrix in different period of the process. Then, they could be crosslinked by the crosslinking agent and fixed in the copolymer. After the imprinting molecules were eluted, these two different absorption properties of the binding sites formed in the MIP.

Evaluation of the imprinted efficiency for all the MIPs products

According to literatures reported, there are several main parameters for the efficiency evaluation on molecular imprinted polymers. They are listed below:

The specific adsorption, ΔQ, and the imprint factor of the polymer, β, which are defined as[20]:

$$\Delta Q = Q_m - Q_n \quad (3)$$

$$\beta = Q_m/Q_n \quad (4)$$

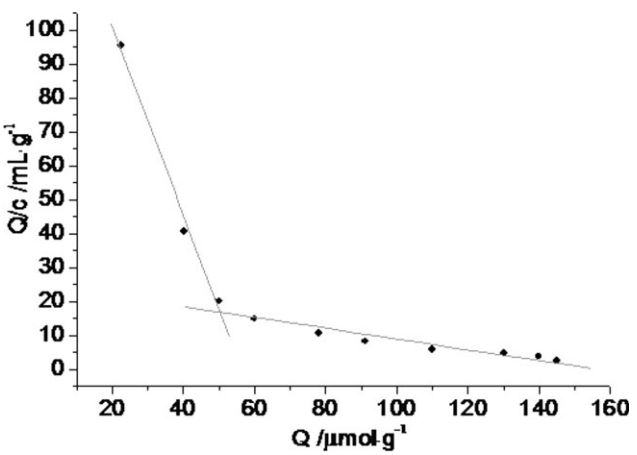


Figure 10 Scatchard plot analysis for the binding of naproxen to imprinted polymer.

TABLE II
The Evaluation of Imprinted Efficiency[a]

Polymer	Q_i (μmol/L)	Q_m (μmol/g)	B_m (μmol/g)	Q_n (μmol/g)	B_n (μmol/g)	$\alpha = \Delta Q$ (μmol/g)	β	E_{ia} (%)	E_i (%)
P1	235.36	107.19	172.34	30.14	71.53	77.05	3.556	73.22	42.83
P2	235.36	122.38	206.80	32.58	90.16	89.80	3.756	87.86	49.56
P3	235.36	91.77	179.01	25.28	85.39	66.49	3.630	76.06	39.78
P4	441.21	232.42	421.32	91.26	209.80	141.16	2.547	95.49	47.94
P5	160.49	80.45	112.10	20.12	21.47	60.33	3.998	69.85	56.47
P6	441.21	248.97	432.52	91.35	219.99	157.62	2.725	98.03	48.17
P7	235.36	129.01	212.75	33.07	88.87	95.94	3.901	90.39	52.63

[a] Q_m and Q_n were measured in solution for the initial concentration of 4 mmol/L. B_m and B_n were calculated from the results of Scatchard analysis.

where Q_m and Q_n are the volumes of template bound on the imprinted and nonimprinted polymers under the same conditions. The parameter of ΔQ can be the visual representation to the specific absorption capacity of MIPs in a given environment. β reflects the selectivity of MIPs on molecular recognition.

In addition, Xue-Jun Wang et al.[21] had put forward the apparent imprinting efficiency, E_{ia}, and the practically efficient imprinting efficiency, E_i, which could be used as evaluations on the efficiency during the imprinting process and the proportion/percentage of the molecules successfully imprinted on MIPs, respectively. Their definition formulas are as follows:

$$E_{ia} = B_m/Q_i \tag{5}$$

$$E_i = (B_m - B_n)/Q_i \tag{6}$$

where Q_i is theoretical maximum number of imprinted sites on MIP, B_m and B_n are the apparent maximum number of binding sites on MIP and NIP, respectively.

The imprinted efficiency of molecular imprinted polymers synthesized in our work was evaluated, after the adsorption performance test, according to the above-mentioned methods. The data of calculation and results are listed in Table II.

Comparing the data in the Table III, it can be found that the effect of solvent to the absorption capacity (α) and selectivity (β) of MIPs was not in law. But the crosslinking degrees had a distinct influence on them. In theory, the shape of the imprinted holes and the location of the binding sites are fixed in the stereo reticular structure, which is formed by a crosslinking agent, such as a "freezing" process.[22] So an adequate rigidity of the crosslinked polymers is needed to keep the spatial structure during the imprinting, removal and binding of template processes. According to the above-mentioned data, with the increase in the amount of crosslinker (P4 < P2 < P5), the selectivity of MIP was improved (β4 < β2 < β5), enhancing the ability of identification. This might be because a greater degree of crosslinking could strengthen the stability of the imprinted polymer as well as the ability to maintain those three-dimensional holes so as to achieve a better recognition effect. But the adsorption capacity was reduced (α4 > α2 > α5) for the less proportion of imprinted sites on a higher crosslinked MIP. Because of the hard network structure of this high crosslinked system and poor swelling behavior of polymer in solvents, the target elements were subject to the steric hindrance of crosslinking network and difficult of diffusion. Moreover, the physical properties of the material were often not satisfactory for practical requirements. When the amount of crosslinking agent had been fewer, although the ratio of binding sites in the polymer increased and the interspaces between the molecular chains became wider, even material had a good absorption capacity and adsorption kinetics, the low degree of crosslinking could not keep the memory on imprinted molecular. The flexible molecular chains were difficult to maintain the structure of the hole. And the grinding process would cause too much damage to the cavity. So the MIP had the lower selectivity, which was adverse for the distinction of structural analogs. Thus considering the molecular recognition properties of polymers, with the capacity and rate of absorption, as well as the physical properties of materials, the polymers of structure and form should be design to make the materials not only had a well accessibility of imprinting point, a high speed of mass transfer and capacity of the exchange on, but also had good selective recognition properties.

Further more, MIPs (P6 and P7) synthesized in photoinitiated polymerization had more excellent performance than the corresponding ones (P1 and P2), in the same ratio of raw materials, prepared on thermal-initiated method, respectively. So the photoinitiated polymerization in a low-temperature environment may be a better way. Among all the MIPs

we synthesized, P7 showed the most outstanding performance. Its imprinting efficiency is $E_{ia} = 90.39\%$ and $E_i = 52.63\%$.

CONCLUSIONS

Molecularly imprinted polymer is porous material with stereo-mesh structure on the morphology. Through the study of TEM observation and absorption kinetics test, we found that an appropriate amount of solvent would make the mesh uniform, which resulted in good adsorption performance for the material. The capability of molecular imprinted polymers for specific recognition and selective absorption depends on stability of the template-functional monomer complex during the imprinting course. In addition, crosslinking degree, after the copolymerization process, is also one of the important factors. To obtain the higher selectivity with the more absorption capacity at the same time, it is necessary for the molecular imprinted polymers to control the dosage of crosslinker. After the efficiency evaluation in this article, we gained the best ratio of raw materials for preparing highly-efficient D-naproxen imprinted polymer, which was: Npx : AA : EGDMA = 1 : 4 : 20 and the best amount of the solvent was 20 mL accordance with their dosage in this experiment.

In addition, MIPs products synthesized, at the same ratio of raw materials, by photoinitiated polymerization were better than by thermal-initiated polymerization. It was inferred that this result might be caused by the temperature and pressure of environment. These factors need to be studied in a further work.

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