Selective Solid-Phase Extraction of Naphazoline Using Imprinted Polymers as Matrix Prepared by Precipitation Polymerization

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ABSTRACT: The molecularly imprinted polymers (MIP) for drug naphazoline (NAZ) have been synthesized by precipitation polymerization. The effect of the dispersive solvents dichloromethane (DCM), acetonitrile (ACN), and Methanol (MeOH) on particle size and morphology of MIP (P1, P2, and P3) was investigated by scanning electron microscopy (SEM). The selectivity of P1, compared with nonimprinted polymer (NIP), C_8 and C_{18} were evaluated via static adsorption using UV spectrophotometer. The result showed that the bond amount of P1 for NAZ was significantly higher than other sorbents. The P1 were applied as a solid-phase extraction (SPE) stationary phase to extract the NAZ from nasal drops and recoveries of more than 89% (relative standard deviations, RSD <5%) were obtained by high performance liquid chromatograph (HPLC) analyses. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 881–886, 2010

Key words: molecular imprinting polymer; precipitation polymerization; solid-phase extraction; HPLC; nasal drops

INTRODUCTION

Molecular imprinting technique is "the construction of ligand selective recognition sites in synthetic polymers where a template is employed to facilitate recognition site formation during the covalent assembly of the bulk phase by a polymerization or polycondensation process, with subsequent removal of some or all of the template being necessary for recognition to occur in the spaces vacated by the templating species," which has attracted great interests due to their high selectivity for a target molecule in terms of size, shape, and functionalities.[1] They have been successfully applied widely in solid-phase extraction (SPE).[2–4] Different polymerization methods can be used to obtain molecularly imprinted polymers (MIP) for SPE applications.[5] The simplest method available in the laboratory is bulk polymerization. For SPE applications, crushing, grinding, and sieving of the monolith is necessary, which is time-consuming, labor intensive and wasteful procedure since only 30–40% of the ground polymer are recovered as useable material.[6] Furthermore, the particles possess heterogeneous binding sites distribution with poor site accessibility and low mass transfer kinetic properties for the target analyte. As a result, the irregular particles generally exhibit low separation efficiency in SPE applications.[7] To streamline the production and performance of MIP particles, alternative synthetic strategies, such as suspension,[8,9] dispersion,[4] and seed polymerization[10,11] have been evolved. However, residual emulsifier or stabilizer may remain on the surfaces of the particles, leading to impair selective rebinding of template molecules to the MIP-sorbent.[12] Precipitation polymerization has emerged as a simple and attractive method without residual emulsifier or stabilizer. Nearly monodisperse and high-quality imprinted spherical particles can be routinely prepared in good yields by precipitation polymerization, meanwhile size and porosity of the particles can be tuned through control of the polymerization conditions.[13–15]

Naphazoline (NAZ, Fig. 1) is a relatively long-lasting vasoconstrictor, which acts on the alpha receptors of smooth vascular muscle. Clinically, patients poisoned with NAZ may exhibit miosis, mydriasis, palpitations, hypertension or hypotension, bradycardia, pallor, cyanosis, diaphoresis, anxiety, insomnia, tremor, agitation, hallucinations, seizures, lethargy, obtundation, and coma.[16] Cases of NAZ poisoning by accidental over dose have also been reported.[17] The patients show severe cardiovascular effects as a result of NAZ intoxicatio.[18] Therefore, it is necessary to develop an effective determination of trace NAZ,

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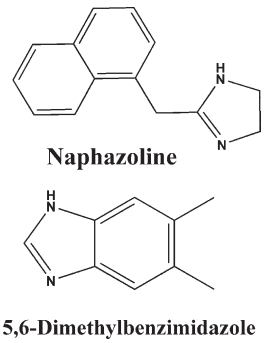

Figure 1 Structures of naphazoline(NAZ) and 5,6-Dimethylbenzimidazole (DBI).

which would be employed for toxicological analysis of NAZ in poisoned patients. To our knowledge, there exists no report on the synthesis, characterization and application of MIP for NAZ.

In this study, NAZ-imprinted microspheres were prepared using precipitation polymerization. The effects of different dispersive solvents on the morphology and particulate size distribution were investigated in detail. The microspheres were further characterized by scanning electron microscopy (SEM), BET physisorption investigation. Then, the property of them was studied by batch tests such as adsorption and selectivity experiments, and compared with commercial C_8 and C_{18}, indicating that the selectivity of prepared MIP was superior to C_8 and C_{18}. At last, the obtained MIP was used as SPE sorbent coupled with high performance liquid chromatograph (HPLC) and applied in real sample.

EXPERIMENT

Materials

NAZ hydrochloride [4,5-dihydro-2-(phenylmethyl)-1H-imidazolehy-drochloride] (99%), methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were purchased from Acros Organics (NJ). MAA and EDMA were purified by general distillation *in vacuo* to remove the polymerization inhibitor. 5,6-Dimethylbenzimidazole (DBI, 99%, Fig. 1) was purchased from Sigma-Aldrich chemical (St. Louis, MO). α,α′-azobis(isobutyronitrile) (AIBN) was purchased from Kelong Chemical Engineering Reagent (Chengdu, Sichuan, China) and recrystallized before use. C_{18} and C_8 were obtained from DaLian Sipore Precision Engineering, LID (40 μm, Dalian, China).Ultra pure water used throughout the experiments was obtained from a Millipore water purification system (MILLI-Q-PLUS, 18.2 MΩ). All solvents used were of HPLC quality and obtained from Kelong Chemical Engineering Reagent (Chengdu, Sichuan, China).

Commercial nasal drops were purchased from local drugstore (Sichuan, China). The glass tubes for samples storage were thoroughly washed with detergents, water, methanol, and doubly deionized water, and dried before use. Samples were adjusted to pH about 7.0 with NaOH to obtain free NAZ and insure the efficient SPE of the analytes by the sorbent. 1 mL of nasal drops was diluted 10, 100, and 1000 times with ethanol and the resulting solutions were applied on the MIP-SPE. The final solution was analyzed by HPLC. Each sample was assayed (P1 extraction and HPLC) three consecutive times. NAZ hydrochloride, as hydrochloride salts were extracted with chloroform from an alkaline aqueous solution and finally isolated as a free base by evaporation of the solvent.[19]

Polymers preparation

The molecular imprinting polymers were synthesized using precipitation polymerization. 1 mmol of template NAZ and 4 mmol of MAA were dissolved in 50 mL of dichloromethane (DCM) and then the mixture was incubated for 30 min. After 4 mmol of EDMA and 1 mmol of AIBN were added, the solution was mixed by ultrasonic for 5 min, and then thermo-polymerization was initiated at 65°C. The polymerization was left for 24 h under nitrogen atmosphere. After that, particles were collected by vacuum filtration. The obtained microspheres were washed by methanol three times, and then the templates were removed by Soxhlet's. Nonimprinted polymers (NIP) was prepared following the same procedure but without the template of NAZ.

Morphological characteristics of the MIP and NIP

Morphological characteristics including pore analysis and SEM analysis of the polymers were investigated. The determinations of specific surface areas were performed using an AUTOSORB-1 Gas Sorption Analyzer (Quantachrome), based on the nitrogen BET. Microscopic analysis of the MIP was carried out in an SEM at 15 Kv.

Determination of binding capacity of polymers (MIP, NIP, C_{18} and C_8)

Alcoholic solutions of NAZ were added to 10 mL vials containing 30 mg of polymers, remained for 3 h at 4°C, subsequently centrifuged for 10 min with 5000 rpm at ambient temperature. After that the supernate were measured by UV spectrophotometer at 279 nm. All tests were conducted in triplicate. Adsorption and recognition studies were also performed with structurally related compound 5,6-Dimethylbenzimidazole (DBI).

TABLE I
Effect of Different Porogenic Solvents on Yield and Average Binding Capacity of MIP

MIP (mmol)	Template (mmol)	MAA (mmol)	EDMA (mmol)	AIBN (50 mL)	Porogenic solvents	Yield (%)	Average binding capacity
P1	1	4	4	1	DCM	78.3	high
P2	1	4	4	1	ACN	75.1	high
P3	1	4	4	1	MeOH	95.4	low

Uptake kinetic of NAZ by P1 and NIP were examined as follows: Thirty milligrams of the sorbent was added to 10 mL of 0.065 mg mL^{-1} of NAZ ethanol solution. The mixture was mechanically shaken for 5, 10, 30, 60, 90, 120, 150, 180, and 210 min at room temperature, respectively, and then separated centrifugally. The supernatants were measured for the NAZ by UV spectrophotometer.

The data of the static absorption experiment were further processed with the Scatchard equation to estimate the binding parameters of NAZ on P1. The Scatchard equation is as follows: Q is the amount of NAZ bound to P1 at equilibrium, Q_{max} is the maximum binding capacity, C_{free} is the equilibrium concentration of NAZ and K_D is the dissociation constant.

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

Information on equilibrium was extracted by Scatchard analysis of the calibration curve, a tool already applied in MIP work.[20]

Procedures for P1 as SPE absorbent for extraction of NAZ

Thirty milligram of MIP was packed in a cartridge (SPE apparatus Waters, Milford, MA) and the upper frit was placed on top. The sample solution was introduced at a flow rate of 1 mL min^{-1}. Different solvents were utilized in the process of washing and eluting. The eluate was collected and dried at room temperature under a stream of N_2. The residues were dissolved in 200-μL mobile phase and an aliquot of 20 μL was analysed by HPLC.

All tests were carried out in a HPLC system including a Waters 515 HPLC pump (Milford, MA), 2487 UV-Vis detector and an Empower Chromatographic workstation. The NAZ was determined at 279 nm through an analytical reversed-phase column (5 μm, 4.6 × 150 mm, Waters Corporation) with a mobile phase containing methanol and water (50 : 50, V/V) at a flow rate of 0.8 mL min^{-1}.

RESULTS AND DISCUSSION

Preparation and characterization of the MIP

Precipitation polymerization is a surfactant-free method that involves polymerization of monomers under diluted conditions (<5% w/v) in a suitable dispersive solvent.[21] As the polymerization proceeds, the growing polymers chains become insoluble in the liquid phase and precipitate. Micro- and nanospheres can be generated when accurate control of the polymerization parameters is achieved (i.e., polymerization temperature, cross-linker and so on).[7] In this work, the microspheres of NAZ–MIP were prepared and the polymerization parameters such as polymerization temperature, rate of stirring, and porogen solvent were studied to control size and porosity of the microspheres. The results indicated that polymerization temperature and rate of stirring presented no obvious influence and porogen solvent generated remarkable effects, shown in Table I and Figure 2. P3 using methanol as porogen solvent gave the highest yield but lower binding capacity, and presented slightly agglomeration compared to P1 (DCM) and P2 [acetonitrile (ACN)]. P1 offer better results in yield and binding capacity. Thereby, DCM was selected as solvent.

The morphology of obtained products was characterized by SEM. From Figure 2, the size distribution of P1, P2, and P3 was all homogeneous relatively, and P1 were about 500 nm. The BET specific surface areas, pore volumes and average pore diameter of P1and NIP were got from nitrogen adsorption experiments (respectively, 149.3 m^2 g^{-1}, 0.2834 mL g^{-1}, 3.591 nm for P1 and 126.9 m^2 g^{-1}, 0.2318 mL g^{-1}, 3.899 nm for NIP). The similar data displayed morphologies of P1 and NIP did not be influenced significantly in presence of template during the precipitation polymerization.

In a process of preparing MIP, porogen solvent is one of the most important factors forming effective molecular recognition. The accuracy of the assembly between the template and the monomer is closely related to the physical and chemical characteristics of the solvent. In this work, MIP was prepared using MAA as functional monomer and EDMA as cross-linker. Nonpolar DMC and moderately polar ACN are both aprotic solvent, while methanol is polar and protic solvent. They were used as porogen in MIP preparation. From Table I, DCM and ACN showed high binding adsorption than methanol, indicting that P1 and P2 possessed higher recognition for the template than P3. As we know, the interactions between the template and functional monomer were contributed to hydrogen bonding and

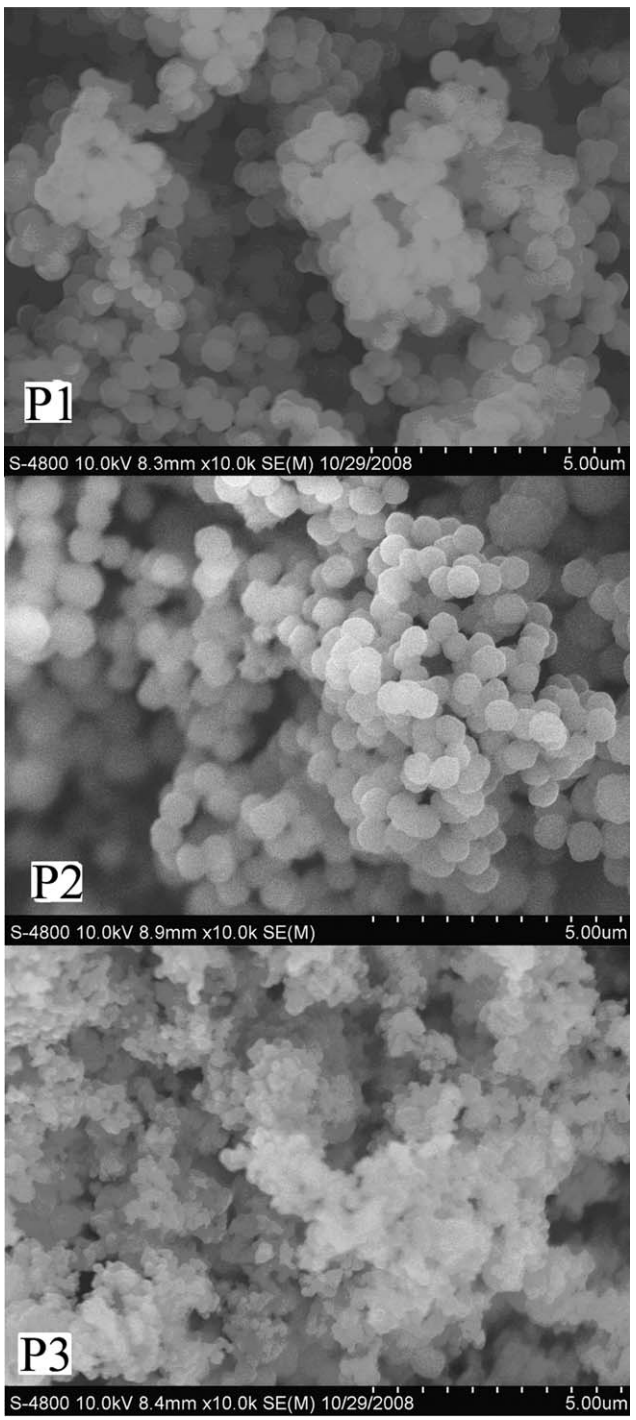

Figure 2 Scanning electron micrographs of naphalazoline-imprinted microspheres using dichloromethane (P1), acetonitrile (P2) and Methanol (P3) as porogenic solvents.

electrostatic interaction.[1] In DCM and ACN media, little interference occurred for the interaction between the template and monomer, however, hydrogen bonding and electrostatic interaction between solvent and the template or monomer can take place in methanol which destroyed the assembly between the template and monomer. Thus, P1 prepared in DCM performed better property.

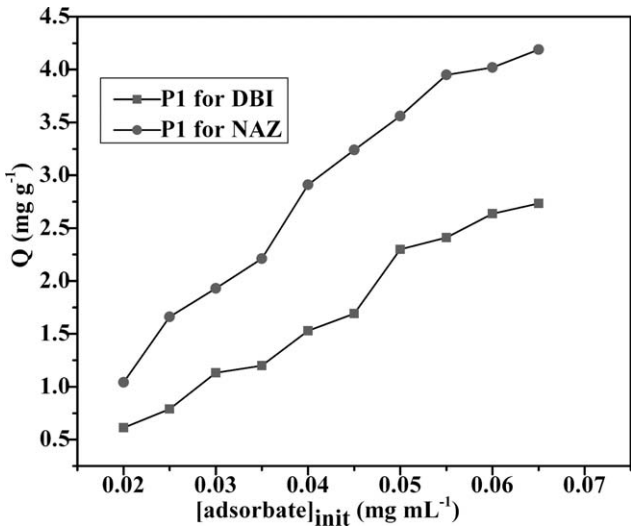

Figure 3 Binding isotherms of P1 for NAZ and DBI $[adsorbate]_{init}$ is the initial concentration of NAZ or DBI.

Selectivity and uptake of P1

To evaluate the selectivity of P1, the structurally similar compound DBI was selected as a competitor in competitive assay. NIP, C_{18}, and C_8 also were involved in the competitive tests. From Figures 3 and 4, the binding capacity of P1 was obviously more than NIP, C_{18} and C_8, the selectivity of P1 was superior to them, and P1 exhibited higher affinity for NAZ than BI, confirming that the synthesis of MIP (P1) was successful. It was may be due to the following factors. As known, hydrophobic interactions contributed to the adsorption of analytes onto C_{18} and C_8. However, hydrogen bonding or electrostatic interactions also contributed to the adsorption onto P1 based on the structure and component of

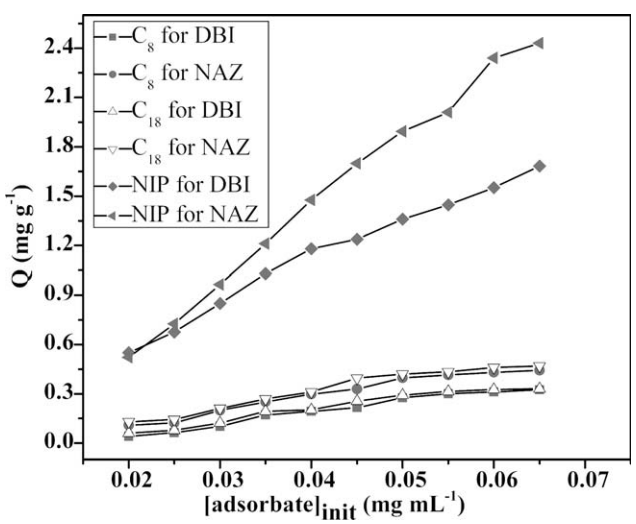

Figure 4 Binding isotherms of NIP, C_8 and C_{18} for NAZ and DBI $[adsorbate]_{init}$ is the initial concentration of NAZ or DBI.

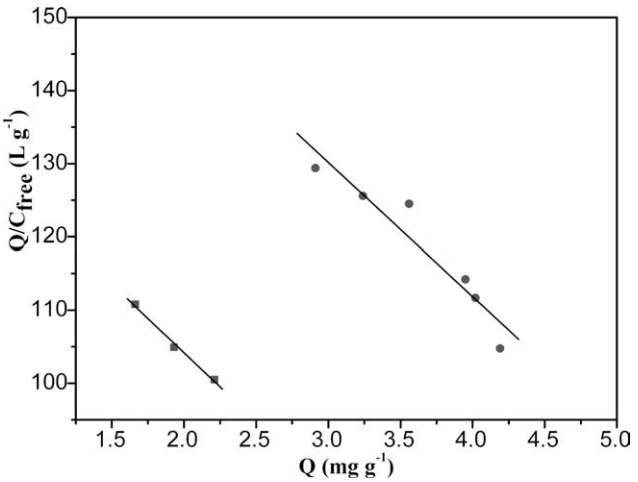

Figure 5 Scatchard plots to estimate the binding nature of P1.

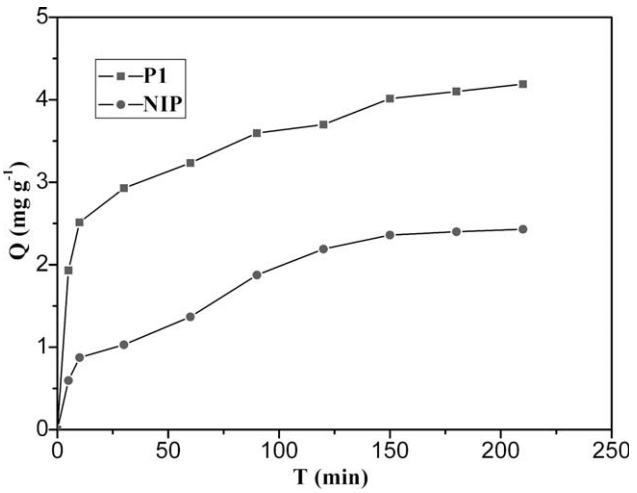

Figure 6 Curves of binding dynamics of P1 and NIP.

NAZ and DBI (Fig. 1). Furthermore, the specific sites formed during synthesis of P1 hold a multidimension spatial structure complementary for NAZ. Thereby, P1 performed excellent selectivity than others and affinity for DBI. In Figure 5, it was showed that the Scatchard plots were two parallel straight lines, implying that there existed two binding sites and they belonged to weak interaction. As mentioned earlier, hydrophobic interactions and hydrogen bonding or electrostatic interactions between N of NAZ and —COOH of P1 existed when the templates were adsorbed by P1.

Uptake kinetics of NAZ by P1 and NIP were also investigated (see Fig. 6). 60% of binding capacity (0.065 mg mL^{-1} NAZ onto 30 mg of P1) was obtained within 10 min while only 36% was obtained by NIP at the same time, indicating that the P1 had faster uptake kinetic than NIP. In addition, P1 hold greater adsorption capacity than NIP. It was due to that NIP possessed only nonspecific adsorption, but P1 owned nonspecific and specific adsorption.

Application of SPE-HPLC using P1 as sorbents to determinate NAZ

To evaluate the performance of P1 as SPE sorbents, the process of loading, washing, and eluting were investigated for extracting NAZ, and NIP and P3 were also studied. All results obtained under same conditions (30 mg of sorbents and 0.85 μg mL^{-1} of NAZ samples) were shown in Table II. After loading of sample, the outflow was determined. Almost the NAZ were retained on three sorbent. It is important for SPE to conduct a wash-step immediately after loading, which ensures the reduction of matrix effect and protects the cartridge and analytical column. In addition, the clean-up step can suppress the nonspecific interactions without disrupting the selective interactions between the MIP and the target molecule. Thereby, nonselective adsorption could be eluted, whereas the template would remain in the polymer. For the purpose, 3 mL methanol was employed as the rinsing solvent. From Table II, 67.3% (NIP) and 54.8% (P3) of NAZ in washing solvent were determined, implying that the major portion of NAZ was not retained by either the NIP or P3. In elution step, a less polar solvent (DCM) was utilized. Recovery of more than 84.3% was achieved for P1. The results confirmed that performance of P1was superior to NIP and P3 which was attributed to specific binding.

The SPE method using P1 as sorbents was applied to real sample. For evaluation of the recovery from real samples, the content of NAZ in nasal drops was analyzed. The results were reproducible and in good

TABLE II
Recovery of Naphazoline During Loading, Washing and Elution Fractions

	Recovery (%)								
	NIP			P1			P3		
Fractions	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Loading	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1
Washing	65.3	67.1	69.6	10.2	11.5	14.8	53.2	56.5	54.7
Elution	19.0	14.1	10.5	89.1	86.9	84.3	29.1	28.6	27.9

TABLE III
Recovery of Naphazoline From Nasal Drops Extracted Using P1 as Sorbents

Sample	Recovery (%)	RSD (%, $n = 3$)
Nasal drops[a]	89.6	4.11
Nasal drops[b]	95.3	4.52
Nasal drops[c]	99.1	4.94

[a] 8.5 μg mL^{-1}.
[b] 0.85 μg mL^{-1}.
[c] 0.085 μg mL^{-1}.

agreement with the recoveries of reference standard solutions. Recovery of more than 89% was obtained for NAZ from nasal drops samples with RSD values of 4.9% ($n = 3$) (Table III).

CONCLUSIONS

In this study, the proposed sorbent of P1 was synthesized by precipitation polymerization. The results of characterization and binding tests confirmed that p1 possessed superior property than other materials. The mechanism of interaction between template and P1 was investigated also. The SPE using P1 as sorbents was coupled to HPLC system, and the developed method was proven to be an efficient tool for the extraction, separation, and determination of NAZ in nasal drops. This work represents the first application of a NAZ imprinted polymer in SPE.

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