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Molecularly Imprinted Solid Phase Extraction using Bismethacryloyl-β-cyclodextrin and Methacrylic Acid as Double Functional Monomers for Selective Analysis of Glycyrrhizic Acid in Aqueous Media

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Abstract

In this work, a new molecularly imprinted solid phase extraction protocol was developed for the selective extraction and purification of glycyrrhizic acid from liquorice roots in aqueous media. The molecularly imprinted polymers (MIPs) for glycyrrhizic acid were prepared by using bismethacryloyl-β-cyclodextrin and methacrylic acid as double functional monomers and characterized by Fourier transform infrared spectroscopy, scanning electron microscope, thermo gravimetric analysis, nitrogen adsorption and elemental analysis. In aqueous media, the adsorption properties of MIPs including adsorption kinetics, adsorption isotherms and selectivity adsorption were investigated. The characterization of imprinted polymers indicated that the prepared MIPs had good stability and many cavity structures. The results of adsorption experiments illustrated the MIPs had high adsorption capacity of glycyrrhizic acid (69.3 mg g^{-1}) with the imprinting factor 3.77, and it took ~5 min to get adsorption equilibrium. The MIPs could be used as an solid phase extraction sorbent absorbent for enrichment and purification of glycyrrhizic acid from the crude extraction of licorice roots, and the results showed promising practical value.

Introduction

Glycyrrhiza uralensis Fisch, also known as Chinese liquorice (Gan-Cao), widely distributed in North China, has been used as a traditional medicinal herb in China for over 2000 years (1). Glycyrrhizic acid, a primary active ingredient of liquorice roots, has been widely used for antitussive, anti-inflammatory, antiviral emollient and gastro-protective properties (2). It is reported that glycyrrhizic acid has been used to treat patients with chronic active hepatitis (3), fight against the human immunodeficiency virus (4) and Severe Acute Respiratory Syndromes coronavirus (5). Recently, it has been reported that glycyrrhizic acid can ameliorate the intracerebral hemorrhage-induced injury (6, 7) and liver injury (8) induced by ischemia-reperfusion in rats. Glycyrrhizic acid can also be used to inhibit side effects of contraceptive formulations, such as alterations in blood coagulation and thrombosis (9).

Based on these effects, the enrichment and purification of glycyrrhizic acid from liquorice roots have gained great interest. Many papers have reported several methods for the selective extraction of glycyrrhizic acid with different techniques such as ultrafiltration (10), crystallization (11), ion exchange resin method (12) and macroporous resin adsorption (13). In these techniques, the organic solvent was frequently used, the operational procedures were very complicated and the specificity was poor.

Recently, molecularly imprinted polymers (MIPs) have attracted attention for their high affinity and pre-determined selectivity for target analytes (14). Among the extraction techniques, molecularly imprinted solid phase extraction (MISPE) is an efficient approach for purification of analytes from complex matrices (15) and it has been used in environmental, food (16) and life science fields (17) because of the easy preparation and excellent recognition properties of MIPs for analytes. Hydrogen bonding, the main effect between MIPs and template molecules, was easily destroyed in aqueous media because aqueous solvents can compete with the template for the functional monomers (18). And a paper has reported that preparation of glycyrrhizic acid MIPs using Methacrylic acid (MAA) as functional monomers could not avoid the influence of aqueous solvents (19). Recently, β-cyclodextrin (β-CD) and its derivatives have been chosen as functional monomers for making MIPs due to the internal hydrophobic external hydrophilic properties (20). It is reported that the synthesized MIPs were successfully used to separate some biological compounds, such as cholesterol (21, 22), antibiotics (23), peptides (24) and other compounds in aqueous solution (25).

This study aims to develop a method for the development of a combination of β-CD with MAA as double functional monomers to synthesize MIPs, which can be used to selectively extract glycyrrhizic acid in aqueous media. To the best of our knowledge, this is the first time that MIPs designed for recognizing glycyrrhizic acid in aqueous media have been developed. The recognition abilities and binding characteristics of the synthesized polymers were evaluated by equilibrium binding experiments and selective binding experiments. Finally, the MIPs were applied to SPE sorbent to enrich and purify glycyrrhizic acid from the crude extract of liquorice roots.

Materials and methods

Reagents and materials

Glycyrrhizic acid was purchased from Shaanxi Fu Jie Medicine Co. (Shaanxi, China). β-CD was purchased from Ze Yuan Trading Co. (Guangzhou, China). MAA was purchased from Tianjin Chemical Reagent Plant (Tianjin, China), and was distilled under vacuum to remove inhibitors prior to use. Ethylene glycol dimethacrylate (EDMA) was obtained from Sigma-Aldrich (New Jersey, USA). 2, 2′-Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent Factory (Shanghai, China) and recrystallized from methanol before use. 4-dimethylaminopyridine (DMAP) was purchased from Wilk Chemical Co., Ltd. Kunshan (Jiangsu, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) was purchased from Shanghai Red Swiss Chemical Technology Co., Ltd. (Shanghai,China). Methanol of HPLC grade was purchased from Kemite Co. (Tianjin, China). Water was purified with Molement 1805b (Shanghai, China). All other chemicals of analytical grade were obtained from local suppliers. Empty SPE cartridges (5.0 mL) were purchased from Shanghai Zhiyu Medical Co. (Shanghai, China). Liquorice was obtained from a local drug store in Xi'an city, China.

Instrument and analytical conditions

Glycyrrhizic acid determination was performed on a LC-2010 AHT HPLC system (SHIMADZU, Japan, Kyoto). The column was Kromasil C_{18} (ODS) (250*4.6 mm, 5 μm) and the thermostat was set at 25°C. The mobile phase was PBS (0.2620 g KH_2PO_4 and 0.7018 g K_2HPO_4 were dissolved in 1000 mL water, pH 7.0)—acetonitrile (77:23, v/v), and the flow rate was set at 1.0 mL min^{-1}. The detection wavelength for glycyrrhizic acid and its analogues were 254 nm and 280 nm, respectively. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Thermo Nicolet Nexus 330 FT-IR spectrometer (Madison, USA) using KBr pellets in the range of 400–4,000 cm^{-1}. The morphologies of MIPs and non-imprinted polymers (NIPs) were observed by a TM-1000 Scanning Microscope (Hitachi, Japan). The thermal analysis was carried out on a SDT Q600 thermal gravimetric analysis instrument from TA Company (New Castle, USA). The elementary analysis was carried out on a Vario EL III elemental analysis instrument from Elementar Co. (Germany). The nitrogen adsorption–desorption experiments was carried out on an Autochemii2920 (Quantachrome, USA) with a bath temperature of 77 K.

Synthesis of BMA-β-CD

The synthesis procedure of bismethacryloyl-β-cyclodextrin (BMA-β-CD) was as follows: β-CD (recrystallized) was dissolved in pyridine and the solution was cooled to 0°C. Then, an ice-chilled solution of MAA and DMAP was added to this solution with stirring magnetically under nitrogen. And EDCI was added 15 min later. The reactive mixture was slowly warmed to room temperature and stirred over night. After that, the solvent was evaporated in a rotary evaporator. The residue was washed with ethanol for several times and a white product (BMA-β-CD) was obtained.

Preparation of imprinted polymers

The MIPs stationary phase was prepared by bulk polymerization. Briefly, 0.1 mmol of the template glycyrrhizic acid and 0.1 mmol of BMA-β-CD were dissolved in 2.5 ml of anhydrous DMSO in a 10 mL conical flask. After being magnetically stirred at room temperature for 2.0 h, 1 mmol of MAA, 4 mmol of EDMA and 0.0419 g of AIBN were added. The flask was sonicated for 10 min under nitrogen and thermo-polymerized for 24 h at 60°C. The resultant bulk rigid polymers were crushed, grounded into powder and sieved through a 200 nm stainless steel sieve. The obtained particles were Soxhlet extracted with a mixture of methanol-aceticacid (4:1, v/v) until glycyrrhizic acid in the elution could no longer be detected by a spectrophotometer. Then, the particles were washed with acetonitrile-water (20:80, v/v) to neutral to remove residual acetic acid and dried under vacuum at 60°C. The corresponding NIPs were prepared similarly in the absence of glycyrrhizic acid. Besides, polymers with only MAA or BMA-β-CD as single functional monomers were synthesized to compare with the polymers having both of the functional monomers.

Adsorption experiment

The adsorption experiments were carried out in a thermostatic shaker at 25°C. To evaluate the adsorption capacities of MIPs and NIPs for glycyrrhizic acid, 20 mg of MIPs or NIPs were added into 10 mL of glycyrrhizic acid stock solution in a conical flask, with various concentrations ranging from 50 to 500 μg·mL^{-1}. After being shaken for 2 h, the mixture was filtered through a 0.45 μm filter, the

concentration of the filtrate was determined by HPLC. The adsorption isotherm was plotted by the related data. To investigate the adsorption kinetics, the adsorption was performed using 20 mg of MIPs or NIPs and 10 mL of glycyrrhizic acid water solution (300 $\mu g \cdot mL^{-1}$). The solution was collected at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 60, 90 and 120 min after incubation and detected by HPLC to calculate the adsorbed amount of glycyrrhizic acid onto the particles. The amount of the substrate bound to the particles (Q) was calculated according to Equation (1) and the molecular imprinting factor (IF) which was used to evaluate the imprinting effect was calculated according to Equation (2) (26):

$$Q = (C_0 - C_e)V/m \quad (1)$$

$$IF = \left(Q_{MIPs}/Ce_{MIPs}\right)/\left(Q_{NIPs}/Ce_{NIPs}\right) \quad (2)$$

where V, C_0, C_e and m represent the volume of the solution (mL), initial solution concentration, the final solution concentration (the concentration after adsorption) ($\mu g \cdot mL^{-1}$) and the weight of the particles (mg), respectively.

Each measurement was carried out in triplicate and the data expressed as means ± RSD%.

Selectivity experiment

To investigate the selectivity of MIPs, 10 mL of the solutions containing different substances (glycyrrhizic acid, hesperidin and methyl hesperidin) were added into 20 mg of MIPs for the adsorption, respectively. Hesperidin and methyl hesperidin are composed of polycyclic aglycone and disaccharide, whose structures are similar to glycyrrhizic acid. The selectivity of MIPs was examined between glycyrrhizic acid and its analogs. The concentrations of all the solution were 300 $\mu g\,mL^{-1}$. The adsorbed amount of the substances onto the MIPs were eluted and determined by HPLC analysis.

MISPE conditions

The MISPE column was prepared by packing the dry MIPs (300 mg) in an empty SPE cartridge. The syringe tube was thoroughly cleaned and dried, and attached with two sieve plates at the bottom end and the top end, respectively. The polymers were rinsed with 5 mL of methanol and 5 mL of deionized water, and then with 1 mL of pure water which was the loading solvent, at the of flow rate 3 $mL \cdot min^{-1}$. And 1 mL of glycyrrhizic acid aqueous solution was loaded onto the MISPE column. Then, the column was washed with 1 mL of ethanol/water (9:1, v/v), and eluted with 5 mL of water. The eluates were evaporated to dryness under a nitrogen stream and the residues were dissolved in 500 µL of water for HPLC analysis.

Preparation of samples

A liquorice sample was ground to a fine powder and transferred to a 50 mL round bottom flask together 20 mL of ethanol: water (5/5, v/v). The mixture was maintained by thermostat at 60°C for 25 min with stirring and then centrifuged for 10 min at 3,000 rpm. The supernatant was filtered through a paper filter and an aliquot of the filtrate was subjected to HPLC (27).

Method validation

The method validation was performed for linearity, range, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision. All the following data were obtained after the treatment of samples by using the optimized MISPE–HPLC condition. The calibration was established by preparing different concentrations of the standard solution (0.1–300 $\mu g \cdot mL^{-1}$) before the MISPE–HPLC procedure. LOD and LOQ, defined as the concentration corresponding to a signal equal to 3 and 10 times the standard deviation of the blank. Accuracy was evaluated by means of recovery assays carried out. The precision was evaluated by measuring relative standard deviations (RSD%) of intra- and inter-day tests.

Results

Optimization of polymerization conditions

In this study, the molecules proportion of functional monomers, template and cross-linking agent were optimized. As shown in Table I, the maximum IF was obtained when the molar ratio of BMA-β-CD, glycyrrhizic acid, MAA and EDMA was 1:1:10:40. To investigate the contribution of the used functional monomers, the other two imprinted polymers with one kind of functional monomer were prepared and their corresponding NIPs were also prepared. And the results indicated that the IF of MIPs prepared with double functional monomers was much higher than MIPs prepared with single functional monomers. Hence, double functional monomers were used for the polymerization and the molar ratio of BMA-β-CD, glycyrrhizic acid, MAA and EDMA was 1:1:10:40.

Physical and morphological characterization

FT-IR analysis of imprinted polymers

To confirm the successful synthesis of BMA-β-CD and preparation of MIPs and NIPs, FT-IR spectra were obtained for β-CD, BMA-β-CD, MIPs and NIPs and the results were shown in Figure 1.

Morphology of imprinted polymers

The morphology of the MIPs and NIPs were observed on scanning electron microscope (SEM) as shown in Figure 2. It can be seen that MIPs and NIPs showed appreciable porous structures in morphology, indicating that the obtained MIPs could be available for the adsorption and dissociation of template molecules, and there is no an obvious difference between MIPs and NIPs in morphology.

Elemental analysis

The BMA-β-CD, MIPs and NIPs were characterized by elemental analysis and the results are shown in Table II. As for BMA-β-CD,

Table I. Optimization Results of Preparation Conditions of MIPs for Glycyrrhizic Acid

BMA-β-CD:GL:MAA: EDMA	Q_{MIPs} ($mg \cdot g^{-1}$)	Q_{NIPs} ($mg \cdot g^{-1}$)	IF
1:1:4:40	56.1	42.4	1.73
1:1:6:40	75.1	62.7	1.80
1:1:8:40	72.1	48.9	2.68
1:1:10:40	69.3	37.4	3.77
1:1:12:40	58.7	49.7	1.44
2:1:10:40	101.7	94.6	1.35
0.5:1:10:40	113.6	104.8	1.22
1:1:10:30	92.2	81.2	1.34
1:1:10:50	112.7	86.2	2.2
1:1:0:40	75.4	71.3	1.24
0:1:10:40	69.1	61.3	1.41

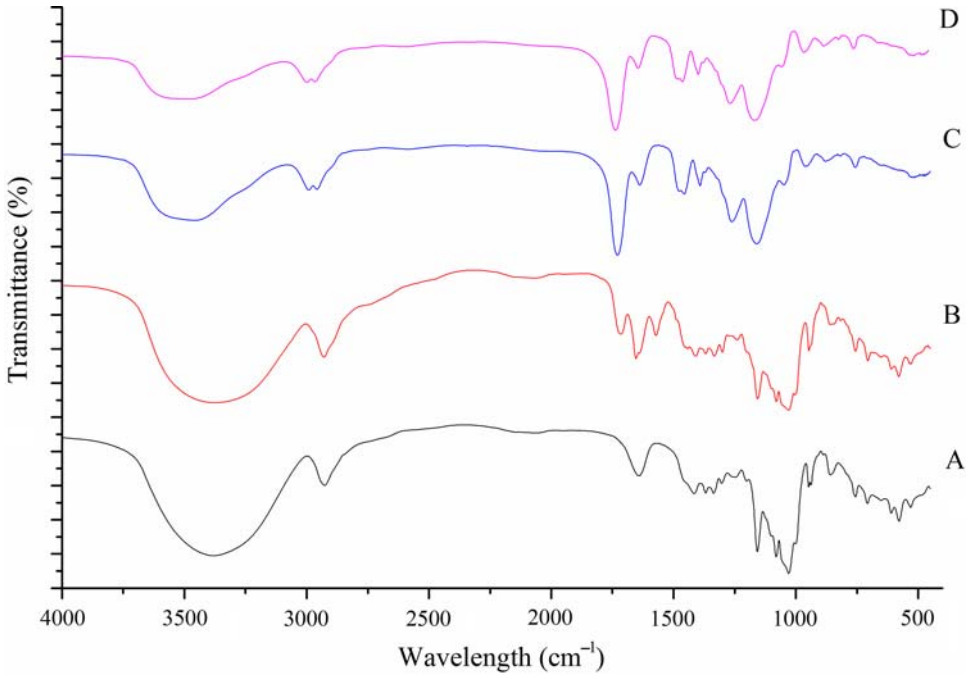

Figure 1 FT-IR adsorption spectra of β-CD (A), BMA-β-CD (B), MIPs (C) and NIPs (D).

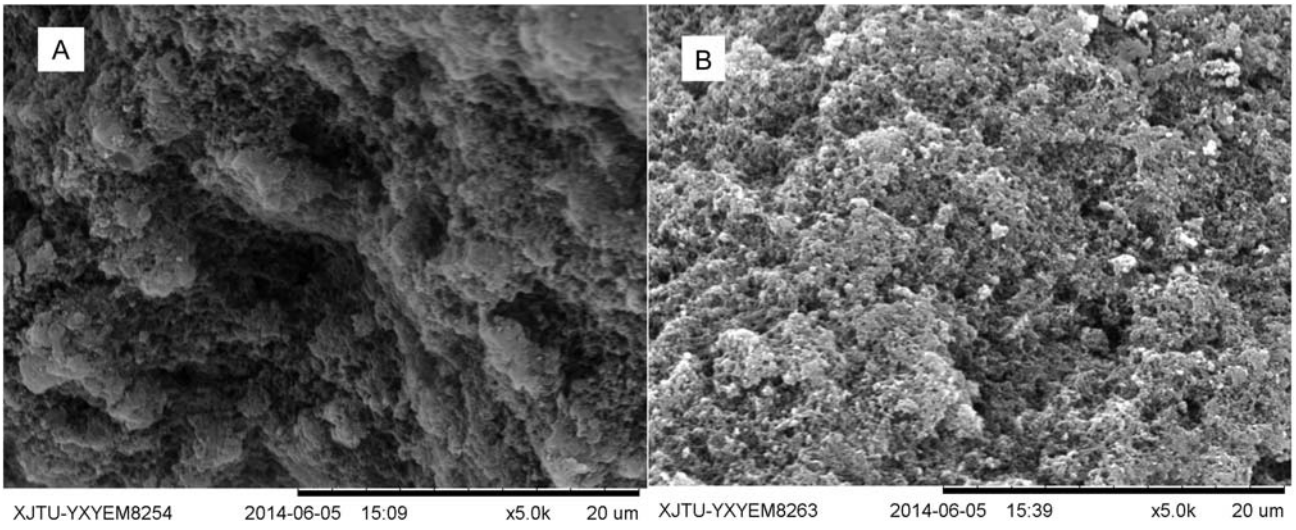

Figure 2 SEM images of MIPs (A) and NIPs (B).

Table II. Elemental Analysis Results of Polymers

Polymers	*N* (%)	*C* (%)	*H* (%)
BMA-β-CD	—	46.51	6.14
MIPs	0.12	56.87	7.72
NIPs	0.11	57.01	7.83

carbon (46.51%) and hydrogen (6.14%) were found, which determined 2 methacryloyl substitutions per β-CD (theoretical value for BMA-β-CD: C: 46.09%, H: 5.99%). After polymerization, nitrogen can be found in MIPs and NIPs owing to AIBN, and the percentages of carbon and hydrogen changed compared with BMA-β-CD. These results suggest that MIPs and NIPs were prepared successfully.

Nitrogen adsorption–desorption experiments

The nitrogen adsorption–desorption experimental results for MIPs and NIPs are shown in Table III according to measurement, the surface area, pore volume and pore size of MIPs were all bigger than NIPs, which would benefit the adsorption of analytes form complex matrices. The results are consistent with the description of the SEM images.

Table III. Nitrogen Adsorption–Desorption Experimental Results of MIPs and NIPs

Polymerization	Surface area ($m^2 \cdot g^{-1}$)	Pore volume ($cm^3 \cdot g^{-1}$)	Pore size (nm)
MIPs	237.9	0.71	6.16
NIPs	179.0	0.52	5.56

Thermal gravimetric analysis

In order to investigate the thermal stability of the polymers, the thermal gravimetric analysis was conducted and the results are shown in Figure 3.

As can be seen in Figure 3, each substance lost ~5% weight below 100°C and loss ~10% weight below 200°C, where the weight loss may be owing to the water loss. Each substance was stable below 200°C. This suggests that the imprinted polymers had good thermal stability and were appropriate for practical applications.

Adsorption properties

The adsorption isotherms of MIPs and NIPs for glycyrrhizic acid are illustrated in Figure 4. Adsorption studies were also carried out at different time intervals. As shown in Figure 5, it took ~5 min for MIPs and NIPs to get the adsorption equilibrium.

The selective sorption properties were carried out with the same concentration of glycyrrhizic acid, hesperidin and methyl hesperidin, which were all polycyclic saponins. As shown in Table IV, the polymers exhibited the best selectivity for glycyrrhizic acid, and the IF is 3.77. On the other hand, the selectivity for hesperidins and methyl hesperidin were lower than the template.

Optimization results of MISPE

In this study, 1 mL of the glycyrrhizic acid was loaded on the column which was almost all retained. According to the polarity of the solvent, different washing and eluting solvents were investigated. In the washing step, the column was washed with 1–3 mL of ethanol, ethanol/water (9:1, v/v), ethanol/water (5:5, v/v), methanol, methanol/water (9:1, v/v), water, isopropyl alcohol, acetone, acetonitrile and methylene chloride, respectively, and the flow rate was 2 $mL \cdot min^{-1}$. When ethanol/water (9:1, v/v) was used, the loss rate of glycyrrhizic acid was the lowest and the leaching rate of impurities was the highest. So ethanol/water (9:1, v/v) was chosen as washing solution. When the volume of washing solution was increased, the loss of glycyrrhizic acid was gradually increased. So the choice of volume was 1 mL. In order to get the best eluting condition, the column was eluted with 1–6 mL of water, ethanol/water (5:5, v/v), methanol/water (9:1, v/v), methanol/glacial acetic acid (8:2, v/v) and 0.1% methanol HCl (9:1, v/v), respectively, and the flow rate was 3 $mL \cdot min^{-1}$. When using water as eluent, the recovery of glycyrrhizic acid was the highest. So water was chosen as eluent. It was found that the recovery of glycyrrhizic acid increased gradually before 4 mL of eluting solution. And there was no significant different for recovery between 5 and 6 mL, so 5 mL was chosen as the eluent volume.

MISPE of liquorice roots

The previous SPE methods have been applied to a liquorice extract for the selective extraction of glycyrrhizic acid and the results are shown in Figure 6 and Table V.

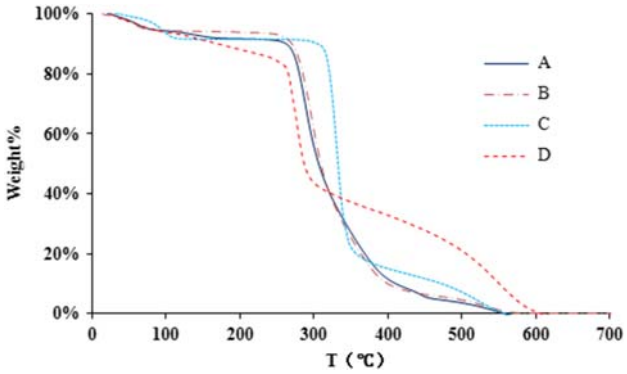

Figure 3 TGA curves of MIPs (A), NIPs (B), β-CD (C) and BMA-β-CD (D).

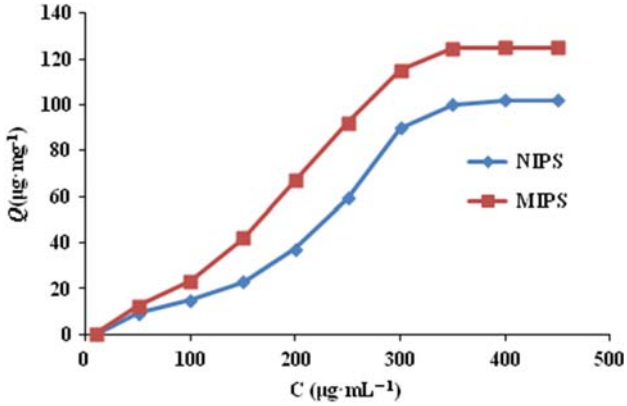

Figure 4 Adsorption isotherm curves of MIPs and NIPs for glycyrrhizic acid.

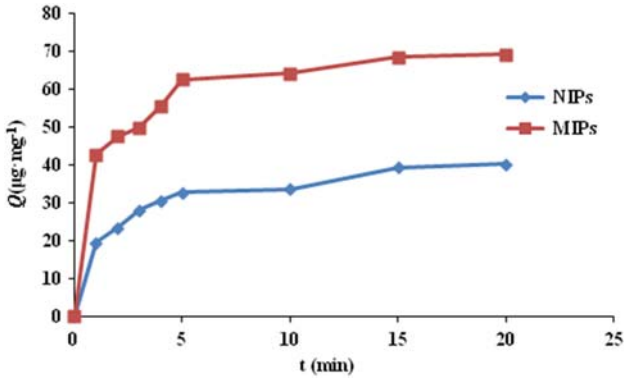

Figure 5 Adsorption kinetic curve MIPs and NIPs for glycyrrhizic acid.

Table IV. The Selectivity of MIPs and NIPs for Glycyrrhizic Acid and its Analogues

Target	Glycyrrhizic acid	Hesperidin	Methylhesperidin
IF	3.77	2.38	1.28

Method validation

Linearity, LOD and LOQ

Under the optimized HPLC conditions, the linearity regression analysis was $y = 8622.5x + 21656$ with a correlation of 0.9989. The LOD was 0.015 μg·mL^{-1}, and the LOQ was 0.045 μg·mL^{-1}.

Precision and accuracy

The repeatability (intra-day) and intermediate (inter-day) precision of this method were assessed using glycyrrhizic acid solution at three concentrations (5, 50 and 250 μg mL^{-1}). The repeatability and intermediate precision were conducted with five replicates for each concentration level on the same day and on three consecutive days, respectively. The result is shown in Table VI.

As shown in Table VI, the absolute recoveries at high, medium and low concentrations were 74.4%, 71.5% and 77.5%, respectively, and the relative recovery was ~100%. The RSD% of intra precision was <3.5% and the inter-day precision RSD% was <2.77%.

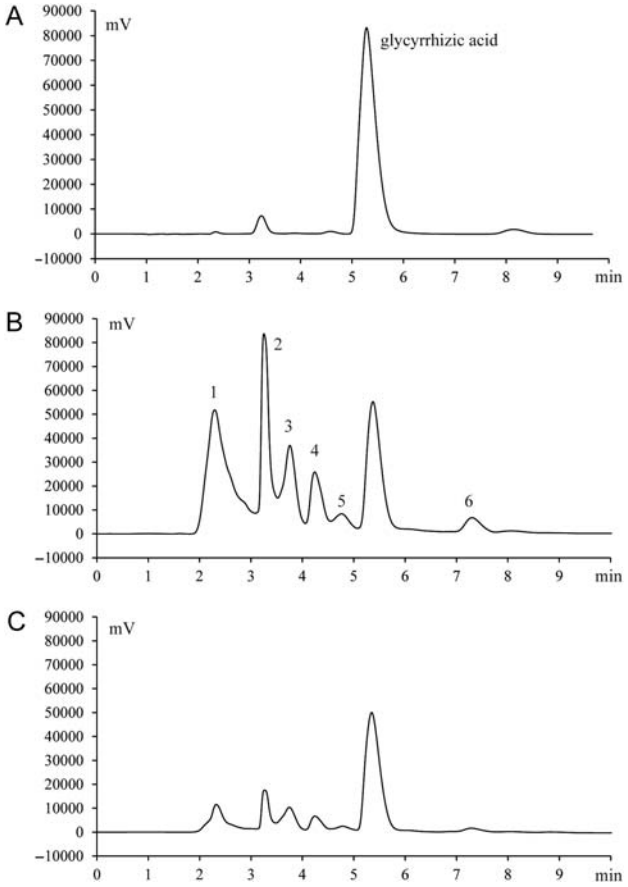

Figure 6 MISPE chromatograms of glycyrrhizic acid (A) Crude extractions of liquorice root with MISPE pretreatment, (B) Crude extractions of liquorice root without MISPE pretreatment, (C) Bulk drug solution of glycyrrhizic acid. The chromatographic conditions are described under Materials and Methods. Column: Kromasil C18 (ODS) (250*4.6 mm,5 μm) and the thermostat was set at 25°C. The mobile phase was PBS (0.2620 g KH2PO4 and 0.7018 g K2HPO4 were dissolved in 1,000 mL water, pH 7.0)–acetonitrile (77:23, v/v), and the flow rate was set at 1.0 mL•min-1. The detection wavelength for glycyrrhizic acid and its analogues were 254 nm.

Discussion

Optimization of polymerization conditions

β-CD molecule is a torus-shaped cyclicoligo saccharide consisting of 1,4-linked D-glucopyranose units with an internal hydrophobic cavity. This structure enables it to form inclusion compounds with many compounds in aqueous media or organic solvents with high polarity through hydrophobic interactions (28). In the pre-polymerization mixture for synthesis MIPs, when BMA-β-CD was mixed with glycyrrhizic acid in DMSO, the aromatic rings of glycyrrhizic acid entrapped inside the hydrophobic core of BMA-β-CD and the more polar carboxyl group (-COOH) of glycyrrhizic acid left outside the cavity. When MAA was added, the carboxyl group can form hydrogen interactions with MAA. Thus, the BMA-β-CD molecule and MAA molecule were regularly placed. The positions and mutual conformations of BMA-β-CD molecule and MAA molecule were fixed by cross-linking procedure.

FT-IR analysis of imprinted polymers

In Figure 1, for BMA-β-CD, the appearance of C=O vibration at 1,715 cm^{-1} and 1,695 cm^{-1} and C=C vibration at 1,575 cm^{-1} compared with β-CD, demonstrated that the CH_2=C (CH_3) COO-group had been grafted onto β-CD. Compared with BMA-β-CD, the FT-IR spectra of MIPs and NIPs displayed characteristic peaks such as: 1,731 cm^{-1} (stretching vibration of -O-C=O bonds), 1,639 cm^{-1} (stretching vibration of C=O bonds), 2,989 cm^{-1}and 2,958 cm^{-1} (stretching vibration of C-H bonds of -CH_2-), and red shift at 3,509 cm^{-1}, attributed to the polymerization of MAA and EDMA, respectively, indicating that the MIPs and NIPs were successfully synthesized by the polymerization of MAA and EDMA.

Adsorption properties

As for the curve of MIPs, the adsorption capacity for glycyrrhizic acid rapidly increased with the increase of the concentrations of

Table VI. The Recoveries of Glycyrrhizic Acid ($n = 5$)

Concentrations (μg·mL^{-1})	Absolute recovery (%)	Relative recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)
5	74.4	104.8	3.50	2.77
50	71.5	102.5	0.47	0.33
250	77.5	102.2	2.61	2.70

Table V. The Content of Each Substance With and Without MISPE Pretreatment

Content (%)	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Glycyrrhizic acid	Peak 6
Before MISPE	46.06	14.63	9.15	7.45	3.56	15.04	4.11
After MISPE	14.73	8.21	11.55	7.71	2.03	53.63	2.14

glycyrrhizic acid in the initial stage, followed by a slow increase till the adsorption equilibrium. While under the same conditions, the adsorbed amount of NIPs increased slowly and the amount of glycyrrhizic acid bound to MIPs was much higher than that of NIPs. It should be noted that the maximum adsorption capacity was 69.3 $mg \cdot g^{-1}$, which was much higher than the reported method (7). Adsorption studies indicated that the binding sites of the MIPs prepared were at the surface or in the proximity of the surface. So it is an easy diffusion for target to access the recognition sites. This merit is especially favorable for SPE. The results showed that the polymers had better absorption performance for glycyrrhizic acid, which illustrated the good specificity of MIPs.

MISPE of liquorice roots

As can be seen in Figure 6, the content of glycyrrhizic acid was highly improved after the MISPE procedure and the content of impurity substances was significantly reduced. To quantify the content of glycyrrhizic acid and impurity substances, Table V shows that after the extraction by the MIPs, the content of glycyrrhizic acid increased to 53.63%, while the content is only 15.03% before SPE, which indicates that the glycyrrhizic acid MISPE has good enrichment and purification properties for glycyrrhizic acid.

Conclusion

In this study, MIPs were prepared for glycyrrhizic acid by using MAA and BMA-β-CD as double functional monomers, which had good recognition characteristics in aqueous media. The prepared MIPs showed large adsorption capacity and fast binding kinetics for glycyrrhizic acid. MIPs as solid-phase extraction sorbent were successfully coupled with HPLC to enrich glycyrrhizic acid from liquorice roots in aqueous media.

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