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Synthesis of surface nano-molecularly imprinted polymers for sensitive baicalin detection from biological samples

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Abstract

Surface molecularly imprinted polymers (MIP@SBA-15) imprinted on the surface of hybrid nanostructured organic/inorganic materials (SBA-15) were prepared for the selective extraction and detection of baicalin (BA) from biological samples. The surface morphologies and characteristics of the imprinted and non-imprinted polymers were characterized by Fourier transform infrared (FT-IR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), thermo-gravimetric analysis (TGA) and nitrogen adsorption-desorption isotherms. The results indicated that the polymers were successfully grafted on the surface of SBA-15 and possessed a highly ordered mesoporous structure. In binding tests, MIP@SBA-15 reached saturated adsorption within 80 min and exhibited significant specific recognition toward BA with large adsorption capacity. Meanwhile, the prepared MIP@SBA-15 was used as a selective sorbent for solid-phase extraction of BA from biological samples. Recoveries of BA from the liver and spleen ranged from 90.6% to 90.9% with RSD < 3.7%. All these results reveal that this method is simple, rapid and sensitive for effectively extracting and detecting trace BA in biological samples.

Keywords

Surface molecularly imprinted; Baicalin; Mesoporous silica; Recognition; Biological samples

1. Introduction

Radix Scutellariae is the root of *Scutellaria baicalensis* Georgi (SB) that has traditionally been widely used for the prevention and treatment of cardiovascular diseases, hypertension, inflammation, cancer and bacterial and viral infections^{1,2}. Baicalin (BA) is considered as the bioactive constituent in Radix Scutellariae and exhibits multiple biological activities, such as anti-cancer, anti-oxidant³, anti-inflammatory, hepato-protective⁴, anti-bacterial, immunosuppression and spasmolysis functions. In recent years, the strong effect on HIV of

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BA was found⁵. Because of its wide use, significant biological activities, a number of approaches, such as solid-phase extraction, high-speed counter-current chromatography (HSCCC)⁶ and supercritical fluid extraction⁷ have been developed with the intention of extracting or separating BA from complex samples. However, due to the low concentration, large amounts of structure analogues of BA in SB and biological samples, all these methods are tedious and time consuming. Therefore, it is essential to develop a new absorbent material with specific binding properties for target molecules (BA).

Molecular imprinting, which has attracted more and more attention, is a promising man-made technique to obtain a type of synthesized material with specific molecular recognition properties. Molecularly imprinted polymers (MIPs) have specific binding sites to the template molecules and can effectively overcome the interferences of other analyte species with the similar structures⁸. In many cases, MIPs are relatively cheap and easy to prepare, intrinsically stable for multiple uses without losses in the performance, and are workable in the presence of acids or at high temperatures and pressures⁹.

In reported researches, traditional imprinted polymers, which are prepared by bulk polymerization or precipitation polymerization, are convenient to prepare and show selectivity and simplicity. Nevertheless, they exhibit some disadvantages, including incomplete template removal, low rebinding capacity, slow binding kinetics, poor site accessibility to target species and irregular shape^{10,11}. One of the main reasons for these disadvantages is that the template molecules are located in the interior area of the MIPs. To effectively overcome these limitations, many surface molecularly imprinted strategies have been explored, such as imprinted layer-coated at nanostructured materials¹²⁻¹⁵, magnetic particles¹⁶⁻¹⁷ and mesoporous silica¹⁸. Recently, mesoporous silica has attracted considerable attention because of its wide range of applications as adsorbents. Mesoporous silica possesses the following characteristics: well-defined channels, hydrothermal stability¹⁹, controllable pore sizes²⁰, enormous surface area and pore volume²¹, hydrothermal stability and narrow size distribution²². These materials are known for promising applications in sorbents, catalysis and sensors²³. Mesoporous silica SBA-15 was discovered by Zhao et al. in 1998²² and is an attractive candidate for use as adsorbent due to its great advantages. Compared to other mesoporous materials, SBA-15 exhibits larger pore size and thicker pore wall²⁴. However, little attention has been paid to synthesizing MIPs for BA using the surface molecularly imprinted technique.

Therefore, in this study, we used SBA-15 as a substrate for the synthesis of highly selective molecularly imprinted polymers of BA (MIP@SBA-15). The MIP@SBA-15 was characterized by Fourier transform infrared (FT-IR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), thermo-gravimetric analysis (TGA) and nitrogen adsorption-desorption isotherms. The adsorption properties were tested via static adsorption equilibrium experiments, dynamic adsorption equilibrium experiments and selectivity evaluation. The obtained polymers showed well-defined channels, enormous surface area, high adsorption capacity and fast kinetics for the rebinding of BA. Meanwhile, the molecularly imprinted solid-phase extraction (MISPE) procedure coupled with HPLC was successfully developed to analyze trace amounts of BA from biological samples, then

providing new opportunities in the applications involved in the highly selective separation and detection of BA.

2. Experimental

2.1 Materials and chemicals

BA, Baicalein (BAI) and wogonoside were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); Pluronic P123, tetraethoxysilane (TEOS), ethylene glycol dimethacrylate (EGDMA) and 4-vinyl- pyridine (4-VP) was purchased from Sigma–Aldrich (Steinheim, Germany). Acrylamide (AA), methacrylic acid (MAA) and 2,2-azobisisobutyronitrile (AIBN) from Shanghai Chemical Plant (Shanghai, China) were purified through recrystallization prior to use. 3-Methacryloxypropyltrimethoxysilane (MPS) was purchased from aladdin (Shanghai, China). HCl was purchased from Sinopharm (Shanghai, China). Flavone was purchased from Tokyo Chemical Industry CO., LTD (Tokyo, Japan). Methanol of HPLC grade was purchased from Merck (Darmstadt, Germany). All other chemicals used in this study were of analytical reagent grade. SB was donated by the Department of Pharmacognosy, Nanjing Medical University (Nanjing, China).

2.2 Instrumentation and analytical conditions

Infrared (IR) and ultraviolet (UV) spectra were recorded by Fourier transform infrared (FT-IR) spectrophotometer (Bruker, Switzerland) and UV-2450 UV-vis spectrophotometer (Shimadzu, Japan), respectively. A shake culture box (ZHLY-180, China) was applied during the binding experiment. The morphologies and structures of MIP@SBA-15 were observed by a scanning electron microscopy (SEM, FEI Quanta 200, USA) and a transmission electron microscope (TEM, JEM-2100, JEOL, Japan). The N₂ adsorption/desorption analyses were carried out at 77 K using a Micromeritics ASAP 2010 system. Surface areas were determined using the Brunauer-Emmett-Teller (BET) theory. Total pore volumes and pore size distributions were obtained based on the adsorption branch of the isotherm with the Barrett-Joyner-Halenda (BJH) method. Thermal analysis were carried out using a thermogravimetric analyzer (TGA, Pyris 1 DSC, PerKinElmer, USA) under nitrogen purging from the temperature range of 25-700 °C at a heating rate of 20 °C/min to determine the weight loss (wt.%).

The amount of BA was analyzed by a HPLC system (Shimadzu, Japan) equipped with LC-20AT pump, SPD-M20A detector, CTO-20A column oven, and Shimadzu shim-pack C18 column (5 µm, 150×4.6 mm i.d.). The mobile phase consisted of 2% phosphoric acid water (A) and acetonitrile (B) with a flow rate of 1.0 mL/min. The following gradient was performed: 0–10 min, 25% B; 10–13 min, from 25% B to 36% B; 13–26 min, 36% B. The column temperature was maintained at 40 °C. A 20 µL injection volume was used for all analyses. Sample solutions were filtered through a 0.22 µm microporous membrane before injection, and analytes were then monitored with an ultraviolet detector at a wavelength of 280 nm. An Agilent 1200 liquid chromatograph combined with an Agilent 6410B Triple Quad mass spectrometer was used to identify BA in biological samples. The mobile phase

for LC-MS/MS analysis consisted of methanol and 0.5% formic acid water (75:25, v/v) with a flow rate of 0.5 mL/min.

2.3 Synthesis of SBA-15 support and modified SBA-15

The SBA-15 support was prepared under three different grafting conditions: 2.0 g of P123 was dissolved in a solution of 60 g HCl (2 mmol L^{-1}), 2.0 g of acetic acid (HAc) and 15 g of deionized H₂O with vigorous stirring at 40 °C. Then, 4.25 g TEOS was added dropwise into the above solution under vigorous stirring at 40 °C for 24 h and the mixture was transferred into Teflon-lined autoclaves and heated at 100 °C for 48 h. The resulting precipitate was filtered and washed with distilled water, and then dried at room temperature. The organic template was removed by calcining in air for 6 h at a rate of 5 °C min^{-1} up to 500 °C. The same procedure was followed except that neither HAc nor H₂O were added. As listed in Table 1, SBA-15-1, SBA-15-2 and SBA-15-3 were successfully prepared.

Subsequently, SBA-15 was chemically modified with MPS. 0.5 g SBA-15 was dispersed in 50 mL toluene and 10 mL MPS were added into the mixture solution. Then, the mixture was stirred under nitrogen for 24 h. Finally, the synthesized SBA-15-MPS was separated, washed with toluene and methanol and dried in a vacuum at 40 °C for 24 h.

2.4 Preparation of MIP@SBA-15

MIP@SBA-15 was prepared under the conditions listed in Table 2. Prior to polymerization, the prearranged solution was prepared by template and functional monomer dissolved in 30 mL of solvent and stored at room temperature for 4 h. 0.1 g SBA-15-MPS was dispersed in 20 mL of solvent by ultrasonic vibration. The prearranged solution, the cross-linker and initiator were then dissolved into the above solution. The mixing solution was purged with nitrogen for 10 min and sealed. The prepolymerization was carried out at 60 °C in a shake culture box for 24 h. Finally, the synthesized MIP@SBA-15 was separated, cleaned by a mixture solution of methanol/acetic acid (9:1, v/v) to remove the template, then washed by methanol and dried in a vacuum at 40 °C for 24 h.

The non-imprinted polymer (NIP@SBA-15) was also synthesized under the same chemical conditions but without the addition of the BA template.

2.5 Preparation of MIP@SiO₂

To prepare silica nanoparticles, 6 mL of TEOS was firstly dissolved in 86 mL of ethanol. Then, 5 mL of ammonia was added into the above solution under magnetic stirring (500 r min^{-1}) at room temperature and the mixture was allowed to react for 24 h. Finally, the products were separated from the mixed solution by centrifugation and washed with ethanol to remove the residual ammonia¹¹⁻¹³.

The SiO₂-MPS and MIP@SiO₂ were also prepared using an identical procedure as SBA-15-MPS and MIP@SBA-15, but SiO₂ replaced SBA-15.

2.6 Evaluation of the binding ability of MIP@SBA-15

In order to evaluate the recognition properties of MIP@SBA-15 to target molecules, three kinds of adsorption experiments were performed.

Static binding capacity was measured by suspending 20 mg MIP@SBA-15, MIP@SiO₂, NIP@SBA-15 and SBA-15 in 5 mL of ethanol with various BA concentrations (1.0–3.5 mmol L⁻¹), respectively. After incubation in a shaker for 80 min at room temperature, the supernatant was separated and measured by UV-vis spectrophotometer at a wavelength of 278 nm. The amount of BA bound on the polymer was calculated according to Eq. (1),

$$Q_e = \frac{(C_o - C_e) V}{M} \quad (1)$$

Where Q_e is the equilibrium adsorption amount of BA, C_o and C_e are the BA concentration before and after adsorption, V is the volume of solution tested, and M is the amount of the polymer.

The binding kinetics were tested by monitoring the temporal evolution of the BA concentration in the solution. 20 mg of MIP@SBA-15 was added to 5 mL of BA ethanol solution (3.0 mmol L⁻¹). The mixture was shaken at certain time intervals (10–100 min) respectively and then the supernatant was separated and measured by UV-vis spectrophotometer at a wavelength of 278 nm. The amount of BA bound on the polymer was calculated as mentioned above.

Selective recognition studies of MIP@SBA-15 and NIP@SBA-15 were performed with the structure analogues (wogonoside, BAI and flavone). Polymers (20 mg) were added into the 5 mL ethanol solution of each analyte at a concentration of 3.0 mmol L⁻¹. After incubation in a shaker for 80 min, the filtrate solution was analyzed for the amount of unbound analyte as described above. All the tests were conducted in triplicate.

2.7 Application of the polymer to real sample

2.7.1 Preparation of biological samples—The powder of SB (50 g) was extracted with 400 mL of 70 % ethanol at a temperature of 80 °C for 3 h. The extraction solution was collected after extraction, filtered and concentrated under vacuuming until the volume of the solution was 10 mL, and then diluted to 50 mL with physiological saline. The rats were starved overnight prior to drug administration. Then, they received the extract solution via gastric gavage with a dose equivalent to 9 g kg⁻¹ of SB.

Thirty minutes after dosing, tissues (liver and spleen) were isolated from mice and then washed with physiological saline and then were immediately frozen in liquid nitrogen and kept at 80 °C until analysis²⁵. The blank tissues were also obtained under the identical conditions, except for the drug administration. The tissues were weighed and homogenized by adding cold ethanol (1:2, w/v) in a polypropylene centrifuge tube. Homogenates were vortexed for 30 s and centrifuged at 12000 r min⁻¹ for 15 min. The supernatant was collected as the analysis samples.

2.7.2 Enrichment of BA from biological samples by MISPE—MIP@SBA-15 and NIP@SBA-15, each weighting ~0.5 g, were packed in an empty SPE cartridge. Each cartridge was rinsed with methanol/acetic acid (9:1, v/v) until no template was detected in the obtained solution by HPLC. After activation by 4 mL ethanol to remove any air bubbles that may affect solvent flow, 4 mL of each analysis samples was loaded, and washed with 4 mL of ethanol to remove the interfering substances. The SPE cartridge needed to be dried thoroughly by vacuum application after washing. Then, an elution was performed with 4 mL of methanol/acetic acid (9:1, v/v). All the collected solutions were evaporated to dryness and redissolved in 1.0 mL methanol for HPLC analysis.

Results and discussion

3.1 Optimization of conditions for SBA-15

Hexagonal porous structure of SBA-15 provides a sheltered environment for the association of a template molecule and functional monomers. It is necessary to successfully prepare the SBA-15 before it is functionalized with the silane coupling agent MPS. Nitrogen adsorption/desorption analysis was used to evaluate SBA-15, which is shown in Table 1. The surface area, pore volume and pore size of SBA-15-3 are $631.6 \text{ m}^2 \text{ g}^{-1}$, $1.129 \text{ cm}^3 \text{ g}^{-1}$ and $67.79 \text{ \AA}$, respectively. It can be seen that SBA-15-3 exhibits a large number of mesopores with average pore diameter. For this reason, we prepared SBA-15 without using HAc.

3.2 Optimization of conditions for surface-imprinting synthesis

3.2.1 Functional monomers—The stability of the complex formed by BA and functional monomers can influence the selectivity and absorption of the imprinted polymers. In this study, AA, MAA and 4-VP were used as functional monomers to prepare the polymers. In Table 2, when using AA as functional monomer, the selectivity of MIPs was better than those of MAA and 4-VP, so AA was chosen as the functional monomer in the experiment. The ratio of template-functional monomer can also influence the selectivity of the imprinted polymers. In this study, ratios from 1 : 7 to 1 : 9 were investigated to prepare the MIPs and NIPs. As shown in Table 2, largest difference was obtained while the ratio was 1 : 8. Therefore, the ratio of BA/AA of 1 : 8 was chosen to prepare the MIPs.

3.2.2 Solvents—Solvents have a large impact on the self-assembly of template-functional monomers, and the size of mesopores and macropores in the particles. N,N-Dimethyl formamide (DMF) and ethanol were investigated to prepare the imprinted polymers. The results are shown in Table 2, when using DMF as the solvent, the selectivity of the MIPs was not obvious, and as a result ethanol was selected as the solvent.

3.2.3 Cross-linker—Cross-linker is one of the parameters that can affect the degree of cross-linking of the polymeric network. To determine the suitable ratio of functional monomers/cross-linker is crucial in the study of MIPs. Ratios from 1 : 4 to 1 : 6 were investigated to prepare the MIPs and NIPs. Table 2 showed that 1 : 5 is more suitable for the preparation of the MIPs.

3.3 Characterization of MIP@SBA-15

To confirm the successful modification of SBA-15 and preparation of MIP@SBA-15, FT-IR spectra were obtained for SBA-15, MPS, SBA-15-MPS, BA, NIP@SBA-15 and MIP@SBA-15 before elution. Fig. 1A shows the FT-IR spectra of SBA-15, MPS and SBA-15-MPS. The absorptions at 3462 and 1634 cm^{-1} resulted from O-H vibration, while peaks at 1087 and 818 cm^{-1} were attributed the Si-O-Si stretching vibration. The observed peak at 1700 cm^{-1} indicates that C=O stretching vibration on the surface of modified SBA-15 and the relatively strong bands at the range of 2800–3000 cm^{-1} corresponded to the stretching vibration of C-H bonds from the methyl (or methylene) groups of MPS²². Fig. 1B shows the FT-IR spectra of BA, NIP@SBA-15 and MIP@SBA-15 before elution. The MIP@SBA-15 before elution displayed clearly the characteristic peaks of BA, including the peaks at 1068 and 851 cm^{-1} corresponding to the glycosyl, while the peaks were not shown in spectrum of NIP@SBA-15. It confirmed the existence of BAI in MIP@SBA-15 before elution. Based on the above results, it can be known that the modification of SBA-15 and preparation of MIP@SBA-15 have been successfully performed.

Fig. 2 shows the SEM and TEM images of SBA-15 and MIP@SBA-15, respectively. As shown in Fig. 2a and Fig. 2b, SBA-15 appears as hexagonal rods while MIP@SBA-15 has a honeycomb-like porous structure. As shown in Fig. 2c, SBA-15 possesses a well-defined channel structure. Fig. 2d is a TEM image of the MIP@SBA-15; it shows the dark layers or dots inside the pores of the SBA-15 attributed to the grafting of MPS. In addition, the image indicates the highly ordered channel structures of the SBA-15 are still preserved.

Fig. 3 shows the thermal decomposition pattern of SBA-15, SBA-15-MPS and MIP@SBA-15. SBA-15 had only one weight loss stage around 100 °C, corresponding to the release of solvent residues and physically adsorbed water. SBA-15-MPS had two weight loss stages: the first stage around 100 °C, corresponding to the release of solvent residues and physically adsorbed water. The second stage around 100–700 °C, corresponded to the degradation of the MPS silane coupling agent. The curve of MIP@SBA-15 is similar to SBA-15-MPS, but the 2nd stage could be assigned to the degradation of MIP@SBA-15 and the loss weight (89%) was much more than that of SBA-15-MPS (14%), which indicates that MIP had grown on the surface of SBA-15.

Nitrogen adsorption/desorption analysis was used to evaluate the surface area, pore size and volume of the polymer, which are shown in Table 3. The results show that the surface area, pore volume and pore size of SBA-15-MPS decreased slightly compared with SBA-15. However, the surface area, pore volume and pore size of MIP@SBA-15 decreased drastically. These results also indicate that the polymerization of the MIPs occurred both on the whole surface of SBA-15 and on the inside of pores. Furthermore, these results show that the mesoporous structure of the modified SBA-15 did not change.

3.4 Evaluation of the adsorption characteristic of MIP@SBA-15

The binding capacity of MIP@SBA-15 was evaluated by comparing with MIP@SiO₂, NIP@SBA-15 and SBA-15. As shown in Fig. 4A, the binding capacity of MIP@SBA-15 increased with the increase of BA concentration until it reached an equilibrium state. The

maximum binding capacities of MIP@SBA-15, MIP@SiO₂, NIP@SBA-15 and SBA-15 were 68.29, 40.84, 33.22, 20.07 $\mu\text{mol g}^{-1}$, respectively. Thus, the binding capacity of MIP@SBA-15 is about 1.67, 2.06 and 3.40 fold that of MIP@SiO₂, NIP@SBA-15 and SBA-15, respectively. From the above results, it is easier to conclude that the MIP@SBA-15 and MIP@SiO₂ show more significant selectivity for BA than NIP@SBA-15 and SBA-15. This may be ascribed to the specific molecular recognition sites on the surface of MIP@SBA-15 and MIP@SiO₂, and the adsorptivity of NIP@SBA-15 and SBA-15 are mainly physical adsorption. For further comparison, MIP@SBA-15 had a greater adsorption for BA than MIP@SiO₂. This could be due to the higher surface area of SBA-15 compared with silica micro-particles²³.

The binding kinetics were investigated with 2.5 mmol L⁻¹ BA at different time intervals. It can be seen from Fig. 4B that the adsorption capacity increased sharply within 80 min and reached equilibrium after that. The fast rate was attributed to the most recognition sites at the surface of MIP@SBA-15 and this favors the use of MIP@SBA-15 as an adsorbent.

The selectivity of MIP@SBA-15 was investigated with wogonoside, BAI and flavone as the structural analogues of the BA template. The results are shown in Fig. 5, and the binding capacity of MIP@SBA-15 to BA is about 1.29, 1.69 and 2.07 fold that of wogonoside, BAI and flavone, respectively. It shows that the more similar structure of compound is, the higher the binding capacity is. A possible explanation for this phenomenon was that MIP@SBA-15 provides an imprinted polymer layer directly at the surface and this layer has functional groups and selective cavities that specifically interact with the BA. However, NIP@SBA-15 does not exhibit an obvious difference in the binding capacities and adsorbed less BA than MIP@SBA-15.

3.5 Method validation

Under the optimal conditions, liner regression calibration curves are shown in Table 4. The results indicated good linearity was achieved for the liver in the range of 1.0–15.0 $\mu\text{g mL}^{-1}$ with correlation coefficients (R^2) of 0.9997 and for spleen in the the range of 1.0–7.0 $\mu\text{g mL}^{-1}$ with correlation coefficients (R^2) of 0.9993. The limits of detection (LODs) and limits of quantification (LOQs) of this proposed method ranged from 0.6 $\mu\text{g mL}^{-1}$ to 0.7 $\mu\text{g mL}^{-1}$ and from 2.3 $\mu\text{g mL}^{-1}$ to 2.4 $\mu\text{g mL}^{-1}$, respectively. The intra- and inter-day precisions were determined by measuring relative standard deviations (RSDs) at concentrations 7.0 and 4.0 $\mu\text{g mL}^{-1}$ for BA. The RSDs were within the range of 1.1–2.6% and 1.8–2.0%, respectively. The results demonstrated that the precision of this method was acceptable.

The amounts of BA that were detected from the analysis samples (liver and spleen) were 7.90 and 3.54 $\mu\text{g mL}^{-1}$, respectively, and the accuracy of the MISPE method was estimated by determining the tissue samples spiked with BA at the concentration of 8.00 and 3.50 $\mu\text{g mL}^{-1}$. As can be seen in Table 5, the average recoveries of two tissue samples on the MISPE sorbent were 90.9% and 90.6% with RSDs of 2.6% and 1.5%, respectively. However, the average recoveries of the NISPE sorbent were 28.5% and 28.8% with RSDs of 0.8% and 3.7% under the same conditions.

Table 6 shows a comparison between the characteristics of recovery values obtained in some reported literatures and this study. Compared with the other sample pretreatment and detection methods, this developed method is simple and reliable due to its high sensitivity and selectivity to the target analyte (BA).

From these results, it can be concluded that MIP@SBA-15 had good recovery and reproducibility and could satisfy the determination of BA in the two different kinds of tissues.

3.6 Applications to biological samples

The proposed method was successfully applied to the detection of BA in rat tissues.

Fig. 6A shows the chromatograms of BA in analysis samples, which were obtained with direct injection, MISPE and NISPE (non-imprinted solid-phase extraction), respectively. The target analyte in the elution solution with MISPE has been identified by LC-MS/MS (Fig. 6B).

Curves a, c and e were the chromatograms of BA in the liver and curves b, d and f were in the spleen. From curves a and b, we found the obvious target peaks and some other interfering peaks, such as wogonoside and BAI, which arose from the complex biological matrices. In contrast to the chromatogram of curves a and c, the interfering peaks, such as wogonoside and BAI, were suppressed while the peaks in curve d were also not found, indicating that the remarkable enrichment ability of the MISPE sorbent to BA. As shown in curves e and f, the peak area of BA by NISPE was much lower than that by MISPE, which indicated that there was no such selectivity with NIP@SBA-15 as a SPE material. These results proved that MIP@SBA-15 as a SPE materials has good applicability to the selective extraction of trace BA in biological samples.

4 Conclusions

In this study, an efficient surface molecularly imprinted polymer (MIP@SBA-15) was synthesized using AA as the functional monomer and ethanol as the polymerization solvent, as well as 1:8 molar ratio of BA to AA and 1:5 molar ratio of functional monomers to cross-linker. The polymer exhibited the ordered mesoporous structure, good selectivity and reproducibility, high absorption capacity and fast kinetics for the binding of BA. MIP@SBA-15 as a MISPE material was used to selectively enrich and determine BA in rat tissues. Therefore, this simple and reliable strategy is proposed for the development of molecularly imprinted polymers for the highly selective separation and fast enrichment of BA from biological samples.

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Fig. 1.

(A) The FT-IR spectra of (a) SBA-15, (b) SBA-15-MPS, (c) MPS. (B) The FT-IR spectra of (a) BA, (b) NIP@SBA-15, (c) MIP@SBA-15 before eluting templates.

Fig. 2.
SEM images of (a) SBA-15 and (b) MIP@SBA-15; TEM images of (c) SBA-15 and (d) MIP@SBA-15.

Fig. 3. Thermogravimetric weight loss curves of (a) SBA-15, (b) SBA-15-MPS and (c) MIP@SBA-15.

Fig. 4.

(A) Adsorption isotherms of (a) MIP@SBA-15, (b) MIP@SiO₂, (c) NIP@SBA-15, and (d) SBA-15 for BA and (B) the adsorption kinetic curve of MIP@SBA-15.

Fig. 5.

Binding capacities of BA, wogonoside, BAI and flavone with MIP@SBA-15 and NIP@SBA-15 at the 2.5 mmol L^{-1} level.

Fig. 6. (A) Chromatograms of BA in the tissue samples (a) and (b) by direct injection without SPE, (c) and (d) with MISPE, (e) and (f) with NISPE: (a), (c) and (e) the liver; (b), (d) and (f) the spleen. (B) Mass spectrum and total ion chromatography (TIC) of BA in samples by LC–MS/MS.

Table 1

Porosities of SBA-15 determined by nitrogen adsorption/desorption analysis.

Samples	P123 (g)	TEOS (g)	HCl (g)	H ₂ O (g)	HAc (g)	S _{BET} (m ² g ⁻¹)	V _T (cm ³ /g)	D _P (Å)
SBA-15-1	2.0	4.25	60	15	2.0	598.9	1.138	69.54
SBA-15-2	2.0	4.25	60	—	—	623.0	1.128	68.31
SBA-15-3	2.0	4.25	60	15	—	631.6	1.129	67.79

Table 2

The preparation of MIP@SBA-15 under different conditions.

Polymer ^a	Functional monomer (mmol)	Cross-linker (mmol)	Solvent	Q ^b (μmol g ⁻¹)	IF ^c
SBA-15@MIP-1	2.4 (AA)	12 (EGDMA)	Ethanol	71.62	2.30
SBA-15@MIP-2	2.4 (4-VP)	12 (EGDMA)	Ethanol	49.40	1.19
SBA-15@MIP-3	2.4 (MAA)	12 (EGDMA)	Ethanol	35.55	1.53
SBA-15@MIP-4	2.1 (AA)	10.5 (EGDMA)	Ethanol	61.95	1.47
SBA-15@MIP-5	2.7 (AA)	13.5 (EGDMA)	Ethanol	46.72	1.06
SBA-15@MIP-6	2.4 (AA)	12 (EGDMA)	DMF	50.97	1.26
SBA-15@MIP-7	2.4 (AA)	9.6 (EGDMA)	Ethanol	57.25	1.38
SBA-15@MIP-8	2.4 (AA)	14.4 (EGDMA)	Ethanol	40.78	1.66

^aThe polymer was prepared using BA (0.3 mmol) as template and AIBN as initiator.

^bThe binding properties were determined by adding 5 mL of BA ethanol solution (3.0 mmol L⁻¹) in 20 mg polymer for 80 min (n=3).

^cIF=QMIP/QNIP, where QMIP and QNIP were the binding properties of BA on MIP@SBA-15 and NIP@SBA-15, respectively.

Table 3

Porosities of polymers determined by nitrogen adsorption/desorption analysis.

Sample	S_{BET} (m ² /g)	V_t (cm ³ /g)	D_p (Å)
SBA-15	631.6	1.070	71.46
SBA-15-MPS	436.9	0.7433	63.06
SBA-15@MIP	47.85	0.04100	34.28

Table 4

The performance characteristics of the proposed method (n = 3).

Tissue	Linearity range ($\mu\text{g mL}^{-1}$)	R^2	RSD(%)		LOD _s ($\mu\text{g mL}^{-1}$)	LOQ _s ($\mu\text{g mL}^{-1}$)
			Intra-day	Inter-day		
Liver	1.0-15.0	0.997	2.6	1.8	0.6	2.4
Spleen	1.0-7.0	0.993	1.1	2.0	0.7	2.3

Table 5

Determination of BA in tissue samples (n =3).

Tissue	Cartridge	Determined level ($\mu\text{g mL}^{-1}$)	Spiked level ($\mu\text{g mL}^{-1}$)	Recovery (%)	RSD (%)
Liver	MISPE	7.90	8.00	90.9	2.6
Liver	NISPE	7.90	8.00	28.5	0.8
Spleen	MISPE	3.54	3.50	90.6	1.5
Spleen	NISPE	3.54	3.50	28.8	3.7

Table 6

Comparison with other proposed methods and other techniques for the determination of BA.

Pretreatment	Analytical method	Sample type	Recovery (%)	Reference
C18-SPE	HPLC-DAD	Rat plasma	88.7	[26]
Oasis® HLB-SPE	LC-MS/MS	Rat brain	52.0-66.0	[27]
LLE ^a	LC-MS/MS	Rat plasma	68.2-79.5	[28]
MISPE	HPLC-UV	Rat tissue	90.6-90.9	This article

^aLLE liquid-liquid extraction