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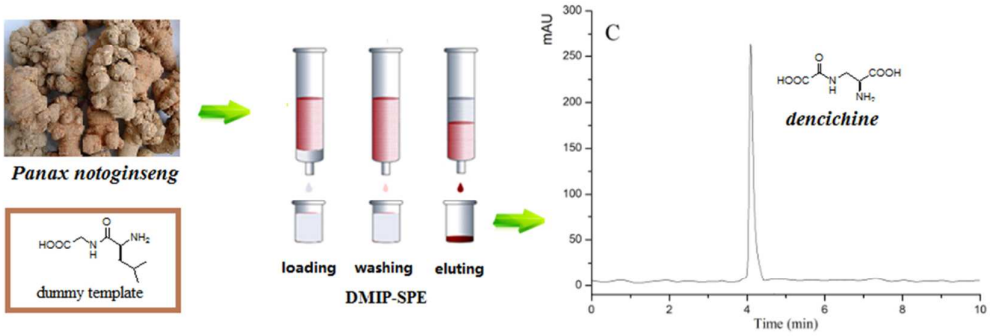

Surface molecularly imprinted polymers with dummy templates for the targeted separation of dencichine from *Panax notoginseng.*

Surface molecularly imprinted polymers with dummy templates for the separation of dencichine from *Panax notoginseng* †

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Abstract:

In this work, surface molecularly imprinted polymers with dummy templates were developed as the selective sorbents for preparation of dencichine from the extract of *Panax notoginseng* for the first time. The polymers were characterized by scanning electron microscopy and Fourier transform infrared spectroscopy. The performances of molecularly imprinted and non-imprinted polymers were evaluated, which included selective recognition, adsorption isotherms and adsorption kinetics. Optimization of various parameters affecting molecularly imprinted solid phase extraction, such as sample loading pH and flow rate, the composition and volume of the eluting solvent and the composition and volume of the washing solvent were investigated. Compared with NISPE, MISPE displayed improved specific adsorption performance. Dencichine with a purity of 98.7% was obtained from the aqueous extract of *Panax notoginseng* with the average recovery of 83.7% (n = 3).

1. Introduction

Molecularly imprinted polymers (MIPs) are man-made porous materials with specificity and selectivity towards the template and analogous molecules [1-4]. Due to their capability of specific molecular recognition, MIPs have been used in many fields such as chemical separation, molecular sensing, catalysis and protein crystallization, and so on [5-18]. The specific disadvantages of MIPs prepared by precipitation polymerization or bulk polymerization include: (1) difficulty of removing target molecules from interior binding sites; (2) the rebinding capacity is limited by the

small number of binding sites on/near the surface; and (3) target molecules are easily hindered from accessing binding sites deep in the interior of the particles [19]. In order to overcome these drawbacks effectively, the surface molecular imprinting technique has been developed. Surface molecularly imprinted polymers (SMIPs) have attracted much attention for their some advantages over the traditional MIPs, including more accessible binding sites, adequate selectivity, fast mass transfer rate and binding kinetics. These properties have made SMIPs extremely attractive for extraction of bioactive constituents from complicated mixture, such as artemisine [20], resveratrol [21] and tanshinone [22], and so on. However, no attention has been paid to synthesize SMIPs for water-soluble bioactive constituents.

Dencichine (β-N-oxalyl-L-α, β-diaminopropionic acid, β-ODAP), isolated from the roots of *Panax notoginseng*, is the bioactive component responsible for main hemostatic and platelet count improving properties [23]. To our knowledge, several methods such as colorimetry [24], high-performance liquid chromatography (HPLC) [25-27], gas chromatography - mass spectrometry [28] and liquid chromatographic - tandem mass spectrometric [29] have been developed for the determination of dencichine in *Panax notoginseng*. Unfortunately, there are few reports about the preparation of dencichine. It is urgent to develop an efficient method for the preparation of dencichine for pharmacological studies and clinical trials aiming for use dencichine as a hemostatic agent. Nevertheless, the imprinting of dencichine is difficult. Dencichine is difficult to produce and is currently sold commercially at over $10 for 1.0 mg. In the studies of phytochemical extraction, it is unadvisable to use the target compound

as the template due to their high cost. Therefore, to find the appropriate analogue instead of the target compound is a formidable challenge in molecular imprinting.

In our initial studies, we have evaluated with other dummy templates for the separation of some fat-soluble natural products, such as ginkgolic acids [17], capsaicinoids [18] and gingerols [30]. In this study, the dummy MIPs for dencichine were firstly synthesized by using D-leucine-glycyl (LG) as the analogue. The dummy template possesses the similar spatial configuration and the possible interaction sites of dencichine, such as amidogen (-NH_2), carboxylic acid group (-COOH) and amide group (-NHCO). Moreover, to overcome the low adsorption capacity of MIPs, MIPs were prepared using the surface molecular imprinting technique. The adsorption performances and selectivity of MIPs for dencichine were systematically evaluated. Solid phase extraction on dummy molecularly imprinted polymers (MISPE) was optimized and applied to the selective extraction of dencichine from the aqueous extract of *Panax notoginseng*. Comparing with the reports for separation of natural compounds [20-22], the appropriate analogue instead of the target compound has a broad marketable prospect.

2. Experimental

2.1 Reagents and chemicals

The roots of *Panax notoginseng* were obtained from Wenshan, Yunnan, China. Dencichine (≥98%) was supplied by Zelang Medical Technology Co. Ltd. (Nanjing, China) 2,2-azoisobutyronitrile (AIBN, initiator), acrylamide (AM, functional monomer), ethylene glycol dimethacrylate (EGDMA, cross linker),

(3-aminopropyl)triethoxysilane (APTES), methacryloyl chloride, D-leucine-glycyl (LG, dummy template), glycyl-DL-leucine (GL), phenylpyruvic acid (PHA), DL-tyrosine (TYS), glycyl-L-phenylalanine (GP) and phenethyl alcohol (PA) were purchased from Aladdin chemistry Co. Ltd (Shanghai, China). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The molecular structures of chemicals are shown in Fig. 1.

2.2 Instruments and operation parameters

Scanning electron microscopy images (SEM) of the surface morphology of imprinted and non-imprinted polymers were recorded on a SWPRATM55 microscope (Carl Zeiss, AG, Aalen, Germany).

Fourier transform infrared spectroscopy (FT-IR) was recorded using a PE Spectrum One FT-IR spectrometer from Perkin-Elmer (Foster City, CA, USA).

The HPLC analysis was performed on an YMC-Pack ODS-A (4.6×250 mm, i.d. 5 μm) analytical column. The samples were analyzed by an Agilent Series 1120 (Agilent Technologies, USA) system, controlled by Chemstation B0403 Chromatographic Software. The mobile phase was acetonitrile: 20 mM NH_4Ac (65 : 35, *v/v*) with the flow rate of 0.5 mL min^{-1} at 30 ^{o}C. Spectra were monitored at 213 nm. The injection volume was 10 μL.

2.3 Chemical modification of silica particles.

Aminopropyl modification of silica was carried out with APTES, as described by Daming Gao [31]. Typically, 1.0 g of silica and 20 mL of APTES were added into 200 mL of anhydrous toluene. The mixture was refluxed for 12 hours under dry nitrogen.

The resulting APTES-silica particles were separated by centrifugation and washed with toluene.

The amino end groups of APTES monolayer were further acryloylated with methacryloyl chloride. Typically, 200 mL of APTES-silica toluene solution was mixed with 10 mL of acryloyl chloride and anhydrous potassium carbonate added into this reaction system as a catalyst. The mixture was vigorously stirred for 12 hours at room temperature under dry nitrogen. The product was separated by centrifugation and washed with toluene, water, and ethanol, in that order. Finally, the AA-APTES-silica particles were obtained.

2.4 Synthesis of dummy molecularly imprinted polymers

Surface molecularly imprinted polymers: AA-APTES-silica particles (200 mg) were dispersed in 200 mL of acetonitrile by ultrasonic vibration. Acrylamide (170 mg, 2.4 mmol), EGDMA (1.8 g, 9.2 mmol), LG (350 mg) and AIBN (20 mg) were then dissolved into the above solution. This mixing solution was purged with nitrogen for 10 min while cooled in ice bath. The polymerization reaction was conducted with vigorous stirring. Pre-polymerization was first performed at 60 °C for 6 hours and the final polymerization completed at 70 °C for 24 hours. The resultant SiO_2@LG-MIP particles were then separated from the mixed solution by centrifugation, and washed with acetonitrile and ethanol. Original templates in the imprinted particles were extracted with a mixing CH_3OH/HAc solvent (9:1, *v/v*) in a Soxhlet extractor.

The corresponding SiO_2@LG-NIP particles were synthesized in the same manner with omission of dummy templates.

Bulk polymerization: Acrylamide (170 mg, 2.4 mmol), EGDMA, (1.8 g, 9.2 mmol), LG (350 mg) and AIBN (20 mg) were then dissolved into 2 mL of acetonitrile. This mixing solution was purged with nitrogen for 10 minutes with cooling in ice bath. The polymerization reaction was carried out under a nitrogen atmosphere for 24 hours at 60 ^{o}C. The resultant bulk rigid polymers were crushed, ground into powder and sieved through a 35~45 μm stainless steel filter. The sieved particles were washed in a mixture of methanol/acetic acid (9:1, *v/v*) using soxhlet apparatus until no templates were detected by HPLC in the extraction, and then washed with methanol until neutral. Fine particles were removed by suspension in acetone. The obtained polymer particles (LG-MIP) were dried under vacuum for 12 hours at 60 ^{o}C.

For the preparation of non-imprinted polymers (NIPs), the similar manner was adopted with omission of dummy templates.

2.5 Binding experiments of SiO_2@LG-MIP and SiO_2@LG-NIP

To investigate the steady-state binding ability of MIPs for dencichine, 5 mg of MIPs and NIPs sorbents were equilibrated with 5.0 mL various concentrations of dencichine (8.0-90.0 μg mL^{-1}). The sorbents were isolated by centrifugation after shaken for 180 min at 25^{o}C, and then the solutions were analyzed by HPLC. The adsorption capacity (Q_e, mg g^{-1}) was calculated following the equation [32].

$$Q_e = (C_i - C_e)\, v / m \quad (1)$$

where Q_e (mg g^{-1}) is the adsorption capacity. C_i (μg mL^{-1}) and C_e (μg mL^{-1}) are the initial and final concentrations of dencichine. *v* (mL) and m (mg) are the volume of solution and the mass of sorbents, individually.

The equilibrium dissociation constants (K_d, μg mL^{-1}) of MIPs and NIPs were further calculated according to the *Scatchard* equation [32].

Scatchard equation: $Q_e / C_e = (Q_{max} - Q_e) / K_d$ (2)

The kinetic study was performed with 5 mg of MIPs or NIPs and 5.0 mL standard solutions of dencichine at a concentration of 52.8 μg mL^{-1}. The mixture was shaken at 25 °C for different periods of time (0-180 min) and the adsorption amount was determined by HPLC.

The Lagergren's pseudo first order and pseudo second order models were used to describe the adsorption kinetic mechanism of MIPs. Both the first and second order rate equations were commonly employed in parallel, and one was often claimed to be better than another according to a marginal difference in correlation coefficient [32].

$$\ln(Q_e - Q_t) = -k_1 t + \ln Q_e \quad (3)$$

$$t / Q_t = t / Q_e + 1 / k_2 Q_e^2 \quad (4)$$

2.6 Selectivity study

The selective recognition capacity was performed with dencichine, four analogues including GL, PHA, TYS and GP, a reference compound PA. The SiO_2@LG-MIP or SiO_2@LG-NIP (5 mg) sorbents were added to 5.0 mL of the standard solution at a concentration of 0.3 mmol L^{-1} and mechanically shaken for 240 minutes at 25 °C. After the solution was centrifuged, the concentrations of five analytes were determined by HPLC. The partition coefficient (K_D) is calculated as:

$$K_D = Q_e / C_e \quad (5)$$

For comparison of the selectivity of polymers, the selectivity coefficient k^{sel} and

relative selectivity coefficient k^{rel} values were calculated according to the following formulas:

$$\text{selectivity coefficient: } k^{sel} = K_{D,\ \text{dencichine}} / K_{D,\ \text{analogues}} \quad (6)$$

$$\text{relative selectivity coefficient: } k^{rel} = k^{sel}_{MIPs} / k^{sel}_{NIPs} \quad (7)$$

2.7 Solid phase extraction on dummy molecularly imprinted polymers (MISPE)

0.1 g of *Panax notoginseng* roots powder was added to 50 mL water in a 100 mL extraction flask. The mixture was extracted under 105 ^{o}C for 2 h. Then, the supernatant was filtrated through a 0.45 μm PTFE membrane.

500 mg polymers were packed into a SPE column (5 mL). Next, the extraction solution was adjusted with KH_2PO_4/NaOH buffered solution to pH at 7.0 and passed through the MISPE column at flow rate of 3.0 mL min^{-1}. Finally, the column was eluted with 5 mL 10% hydrochloric acid. The eluted solution was analyzed by HPLC and the recovery of dencichine was calculated.

3. Results and discussion

3.1. Optimization of SiO2@LG-MIP preparation conditions

Dencichine is a non-protein amino acid with a short carbon chain. The carboxylic acid group (-COOH), amidogen (-NH_2) and carbonyl group (C=O) possess possible interaction sites. Based on the structural features and the spatial configurations of dencichine, LG was chosen as the dummy template, which contains the groups of amidogen (-NH_2), carboxylic acid (-COOH) and amide groups (-NHCO).

The imprinting was subsequently assessed by comparing the adsorption of the

dencichine (52.8 μg mL^{-1}) in water on SiO2@LG-MIP and LG-MIP with corresponding SiO2@LG-NIP and NIP. The equilibrium adsorption capacity (Q_e, mg g^{-1}) and the imprinting factor ($\alpha = Q_{e\ SiO2@LG\text{-}MIP} / Q_{e\ SiO2@LG\text{-}NIP}$) were applied to evaluate the affinity of polymers to dencichine. As shown in **Fig. 2**, Q_e and α of dencichine were higher on polymers prepared using dummy templates LG compared with others. The dummy template LG both contains the possible interaction sites of dencichine and possesses the similar spatial configuration. SiO2@LG-MIP prepared by the surface molecular imprinting technique showed excellent adsorption capacity compared with LG-MIP obtained by bulk polymerization ($Q_{e\ SiO2@LG\text{-}MIP} > 30$ mg g^{-1} and $Q_{e\ LG\text{-}MIP} < 10$ mg g^{-1}).

3.2 Morphological analysis

Scanning electron microscopy (SEM) was used to characterize the morphologies of MIPs and NIPs. The results shown in **Fig. 3** indicate that the SEM micrographs of SiO_2@LG-MIP and LG-MIP are morphologically different. The uniform sphere morphology of SiO_2@LG-MIP indicated that surface molecularly imprinted polymers were obtained.

The FT-IR diffuse reflectance spectra of pure silica, APTES-silica, AA-APTES-silica and SiO_2@LG-MIP are shown in **Fig. S1**†. Compared with the infrared data of pure silica, the APTS-silica particles displayed characteristic peaks of amino groups at the range of 1384-1491 cm^{-1} and the AA-APTES- silica particles displayed the relatively strong band of carbonylic groups at 1789 cm^{-1}s. Simultaneously, the existence of the bands of SiO_2@LG-MIP at 1730 cm^{-1} and weak

bands at 1639 cm^{-1} indicated that the surface molecularly imprinted polymer, which was prepared using AA as the functional monomer and EGDMA as the cross-linking agent, had formed.

The physical characteristics of SiO_2@LG-MIP and SiO_2@LG-NIP were also investigated by the evaluation of BET N_2 adsorption isotherms. The surface areas of SiO_2@LG-MIP and SiO_2@LG-NIP were 282 and 132 m^2 g^{-1}, and the pore volumes are 0.45 and 0.19 m^3 g^{-1}, respectively. The surface area and the pore volumes of SiO_2@LG-MIP were about 2.1 and 2.4 times of these of SiO_2@LG-NIP, which indicates that the surface area and pore volume of SiO_2@LG-MIP were increased by the imprinted cavity. The porosity of SiO_2@LG-MIP was beneficial to the adsorption of analytes from complex matrices.

3.3 Selectivity study of the sorbents

The selectivity study of SiO2@LG-MIP was evaluated by using dencichine, four analogues including PHA, TYS, GL and GP, and a reference compound PA.

Fig. 4 illustrated the data obtained from the selectivity experiment for both SiO2@LG-MIP and SiO2@LG-NIP, concerning the adsorption amounts and the ratios between $Q_{e,\ MIP}$ and $Q_{e,\ NIP}$. The SiO2@LG-MIP exhibited obviously higher adsorption capacity than SiO2@LG-NIP for dencichine due to the presence of the specific binding sites and the similar spatial configuration. The adsorption capacity for dencichine on SiO2@LG-MIP sorbents was above 30 mg g^{-1}, which was significantly higher than those for the four analogues, indicating that the binding cavities in SiO2@LG-MIP sorbents had no specificity for four analogues. Moreover,

the low adsorption capability of SiO2@LG-MIP for PA was observed due to the different structures in comparison with dencichine. This result indicated that SiO2@LG-MIP had no specific interaction site to the compounds with significantly different structures.

Distribution ratio (K_D), selectivity coefficient (k^{sel}) and relative selectively coefficient (k^{rel}) values of SiO2@LG-MIP and SiO2@LG-NIP for analytes were listed in **Table 1**. The selectivity coefficient (k^{sel}) indicated the cross-selectivity between analogues and dencichine. It can be seen from **Table 1** that the significantly high k^{sel} value of SiO2@LG-MIP had been achieved indicating a high discrimination property of SiO2@LG-MIP between dencichine and analogues. In addition, the relative selectivity coefficients (k^{rel}) were all more than 4 which showed the higher selectivity of SiO2@LG-MIP than SiO2@LG-NIP.

3.4 Adsorption isotherms

The experimental equilibrium isotherms for the adsorption of dencichine onto the SiO2@LG-MIP and SiO2@LG-NIP with different initial concentrations were investigated. As it can be seen in **Fig. 5A**, the amount of dencichine binding to the polymers increased along with its initial concentration. Moreover, SiO2@LG-MIP had a higher affinity for dencichine than SiO2@LG-NIP. The stronger adsorption properties of MIPs may be attributed to MIPs possessing a large number of specific binding sites whilst NIPs did not.

To obtain insight into the binding affinity of sorbents and the theoretical number of binding sites for the template, Scatchard experiments were used to analyze the data

of the static adsorption experiment. As shown in **Fig. 5B**, the Scatchard plot for MIPs shows two different straight lines, corresponding to the low and high affinity binding sites. This also suggested that the binding sites in the MIPs were heterogeneous. The linear regression equations for two curves were $Q_e/C_e = -0.086\ Q_e + 2.403$ and $Q_e/C_e = -0.013\ Q_e + 1.347$, respectively. The K_d and Q_{max} values were calculated as 76.92 μg mL^{-1} and 103.62 mg g^{-1} for the low-affinity binding sites, and 11.63 μg mL^{-1} and 27.94 mg g^{-1} for the high-affinity binding sites. The NIPs curve indicated a linear slope and the linear regression equation was $Q_e/C_e = -0.043\ Q_e + 0.955$. It revealed homogeneous binding sites with K_d and Q_{max} values of 23.26 μg mL^{-1} and 22.21 mg g^{-1}, respectively. When the initial concentrations of dencichine were more than 0.15 mmol L^{-1}, the lower K_d and higher Q_{max} values indicated that MIPs were more suitable for SPE as sorbents than NIPs.

3.5 Kinetic adsorption characteristics

In order to determine the binding rate of dencichine on SiO2@LG-MIP, kinetic adsorptions studies were carried out. From **Fig. 6** it can be seen that adsorption equilibrium can be achieved within 10 minutes, whilst equilibrium cannot be reached in more than 100 minutes for SiO2@LG-NIP. The higher adsorption rate of SiO2@LG-MIP may have resulted from the preferential and rapid adsorption of the template onto the recognition sites. The results indicated that it was suitable in the practical application of the sorbents for the SPE procedures.

To determine the rate controlling and mass transfer mechanisms, kinetic data were correlated to linear forms of the first-order equation and the second-order

equation. The results of kinetic parameters and correlation coefficients (R^2) were shown in **Table 2** and kinetic models for SiO2@LG-MIP and SiO2@LG-NIP were presented in **Fig. S2**† and **Fig. S3**†. The correlation coefficient (R^2) of the first-order model exhibited a lower value than that of the second-order adsorption model. In addition, the calculated equilibrium adsorption capacity, $Q_{e,cal}$, from the second-order model fitted well with the experimental data, $Q_{e,exp}$. This indicated that the second-order kinetic equation fitted the kinetic adsorption data better than the first-order kinetics equation.

3.6 Optimization of MISPE

The factors for optimizing the MISPE procedure include sample loading pH and flow rate, the composition and volume of the eluting solvent, and the composition and volume of the washing solvent. For all the steps, SPE columns packed with 1.0 g SiO2@LG-MIP or SiO2@LG-NIP were used.

3.6.1 Effect of sample loading pH and flow rate

In the SPE method, the solution pH can affect the adsorption capacity. Therefore, 10 mL of loading solutions (0.3 mmol L^{-1} for dencichine) with a range of pH from 4.0 to 10.0 (pH: 4.6, 5.4, 6.4, 7.0, 8.5, 9.2 and 10.1, adjusted with 0.1% formic acid or 10% ammonia) were investigated. As shown in **Fig. S4**†, the retention rates of dencichine onto the MISPE increased with the pH from 4.6 to 7.0 and then remained almost constant at pH 8.5-10.1. In the adsorption process, it is critical for the carbonyl groups of dencichine to form hydrogen bonds with the amines in the polymers. The lower retention rates of dencichine onto the MISPE at pH 4.6-6.4 might be attributed

to the protonation of the carbonyl groups of dencichine, which could not form hydrogen bonds with the residual amines in the polymers, therefore dencichine could not be retained on the cartridge effectively.

The effect of sample loading flow rate (0.1 mL min^{-1} to 5.0 mL min^{-1}) on dencichine recoveries was studied (**Fig. 7**). When the flow rate increased (0.1 mL min^{-1} to 3.0 mL min^{-1}), the retention rates of dencichine were almost 98%. However, when the flow rate further increased (3.0 mL min^{-1} to 5.0 mL min^{-1}), the retention rates decreased in the MISPE cartridge. Thus, the flow rate of 3.0 mL min^{-1} was selected as an optimum compromise between the flow rate and retention rate.

3.6.2 The composition and volume of the washing solvent

The washing step was a crucial procedure both to maximize the specific interactions and to produce non-specific interactions between the target analytes and MISPE. After loading 10.0 mL of spiked samples onto the cartridge, 5.0 mL of methanol, methanol-H_2O (10:1, *v/v*), acetone, acetone-H_2O (10:1, *v/v*), tetrahydrofuran, acetonitrile as the washing solvent were investigated. After washing with 5.0 mL of acetone-H_2O (10:1, *v/v*), the retention rate of dencichine onto MISPE was still near 90%, whereas the retention rate from NISPE decreased to less than 30%, as shown in **Fig. S5**†. Acetone was sufficient to remove dencichine from NISPE, but most of the dencichine was e also eluted simultaneously. For choosing an optimal volume of washing solution, various volumes of acetone-H_2O (10:1, *v/v*) from 2.0 to 8.0 mL were investigated (**Fig. 8**). The retention rates of dencichine kept almost constant with the volume from 2.0 to 5.0 mL, and then decreased with the increasing

volume from 5.0 to 8.0 mL. With comprehensive consideration of the recoveries and purification effects, 5 mL of mixture of acetone-H_2O (10:1, *v/v*) was chosen as the washing solution in further experiments.

3.6.3. Elution solvent selection

The final elution of dencichine was conducted by using 5 mL of 10% hydrochloric acid and 5 mL TFA-H_2O (1:5, *v/v*). Both elution solvents achieved dencichine recoveries close to 100%. Considering the cost, 5 mL of 10% hydrochloric acid was selected as the optimum elution solvent.

3.6.4 Accuracy of the methods

In order to assess the accuracy of the optimization experiments, the pure media spiked with three different levels of dencichine (10.0, 50.0, 100.0 μg mL^{-1} for dencichine) were subjected to extraction by the MISPE and NISPE cartridge under the optimized conditions and then analyzed by HPLC. The recoveries were 87.9%, 86.1% and 86.6% with the RSD% values 4.1%, 3.7% and 4.7% (n = 5), respectively. This result demonstrated that the proposed method was a suitable method for the determination of dencichine in samples.

3.7 Application of MISPE to samples

The optimized MISPE methods have been used for preparation of dencichine from *Panax notoginseng*. After adjusted with KH_2PO_4/NaOH buffered solution to pH at 7.0, the solution was passed through the MISPE column. The column was washed with acetone-H_2O (10:1, *v/v*) and eluted with 10% hydrochloric acid. The final eluent from the MIPs and NIPs cartridge were analyzed by HPLC.

352 The chromatograms were shown in **Fig. 9**. After the enrichment of dencichine
353 with MISPE cartridge, and eluted by 10% hydrochloric acid, the peak of dencichine
354 appeared distinctly (**Fig. 9C**). As shown in **Fig. 9B** and **Fig. 9C**, the extraction
355 efficiency and selectivity of the MISPE column were much higher than those of the
356 NISPE column. **Fig. 9B** demonstrated a large impurity peak for dencichine in case of
357 the NIP indicating there is still considerable undesired non-specific interaction.

358 **3.8 The stability of polymers**

359 3.8.1 The reproducibility of the MIP synthesis

360 In order to evaluate the repeatability and reproducibility of the preparation of the
361 polymers, we did three independent syntheses experiments for SiO_2@LG-MIP. The
362 separate batches of polymers exhibited excellent adsorption capacity to dencichine,
363 including high affinity, capacity, selectivity and specificity. The recoveries of
364 extraction obtained using three MIPs resulting from three independent syntheses after
365 applying the optimized procedure of extraction were 83.5%, 84.9% and 82.6%
366 respectively, with the average recovery of 83.7% (n = 3). These results showed that
367 the SiO_2@LG-MIP as a selective sorption material could prepare the dencichine from
368 *Panax notoginseng* and had a broad marketable prospect.

369 3.8.2 Thermal and chemical stability and lifetime of polymers

370 The lifetime of polymers is important for practical application (decline of
371 efficiency with the recovery of analysis). The results showed that the specific
372 recognition ability had no obvious decline after polymers were damaged mainly by
373 high temperature, acid solution and basic solution (**Table S1**†). The high thermal and

chemical stability is due to the strong chemical binding formed into polymers. **Table S1**† also showed the change of selective enrichment efficiency of polymers after being used for 5, 10, 15, 20 and 30 times. The recovery did not evidently decrease after being used for 30 times. The results indicate that selective enrichment efficiency had no obvious decline. Polymers were still stable and reusable.

4. Conclusions

In this study, surface molecularly imprinted polymers with dummy template for the separation of the water-soluble natural product, dencichine, were prepared and characterized for the first time. Because of the simultaneous possession of high affinity, capacity, selectivity and specificity, MISPE method based on SiO_2@LG-MIP was established under optimized conditions. The approach provided a novel method for targeted extraction of dencichine from natural products. These results indicate that the presented dummy molecular imprinting technique is an efficient method for the separation of bioactive components.

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Table 1. Distribution ratio (K_D), selectivity coefficient (k^{sel}) and relative selectively coefficient (k^{rel}) values of SiO2@LG-MIP and SiO2@LG-NIP for different analytes.

Analytes	$K_{D, NIP}$ (L/g)	k^{sel}_{NIP}	$K_{D, MIP}$ (L/g)	k^{sel}_{MIP}	k^{rel}_{MIP}
dencichine	0.33	-	2.11	-	-
PHA	0.58	0.57	0.64	3.30	5.79
TYS	0.35	0.94	0.44	4.79	5.09
GL	0.30	1.10	0.40	5.27	4.79
GP	0.23	1.43	0.30	7.03	4.92
PA	0.17	1.94	0.19	11.10	5.72

Table 2 Comparison of pseudo-first-order and pseudo-second-order rate constants and experimental Q_e values.

	Q_e[a](exp) (mg g^{-1})	Pseudo-first-order kinetics			Pseudo-second -order kinetics		
		k_1[b] (min^{-1})	Q_e[a](cal) (mg g^{-1})	R^2	k_2[c] (g mg^{-1} min^{-1})	Q_e[a](cal) (mg g^{-1})	R^2
MIP	32.62	0.047	3.32	0.802	0.021	33.33	0.999
NIP	13.41	0.021	4.74	0.943	0.002	15.63	0.987

[a] Q_e is the amounts of template adsorbed at equilibrium; [b] k_1 is the rate of pseudo first-order. [c] k_2 is the rate of pseudo second-order.

Captions to the figures

Fig. 1. Chemical structures of investigated compounds.

Fig. 2. The imprinting factor of surface and bulk polymers with different dummy templates for dencichine (n = 3, RSD < 5%).

Fig. 3. SEM images of (A) SiO_2@LG-MIP (20.00K). (B) SiO_2@LG-MIP (50.00K). (C) LG-MIP and (D) LG-NIP.

Fig. 4. Adsorption amounts of SiO_2@LG-MIP and SiO_2@LG-NIP and ratios between Q_{MIP} and Q_{NIP} for six analytes.

Fig. 5. (A) The adsorption isotherms of dencichine on SiO_2@LG-MIP and SiO_2@LG-NIP. (B) Scatchard plots of the DMIPs and NIPs isotherms.

Fig. 6. Kinetic adsorptions isotherms of SiO_2@LG-MIP and SiO_2@LG-NIP for dencichine.

Fig. 7. Effect of the flow rate on the retention rate of dencichine.

Fig. 8. Effect of the washing solvents volume on the retention rate of dencichine.

Fig. 9. (A) Chromatogram of crude extract of *Panax notoginseng* before percolating through SPE column. (B) Chromatogram of eluting solutions from NISPE column. (C) Chromatogram of eluting solutions from MISPE column.

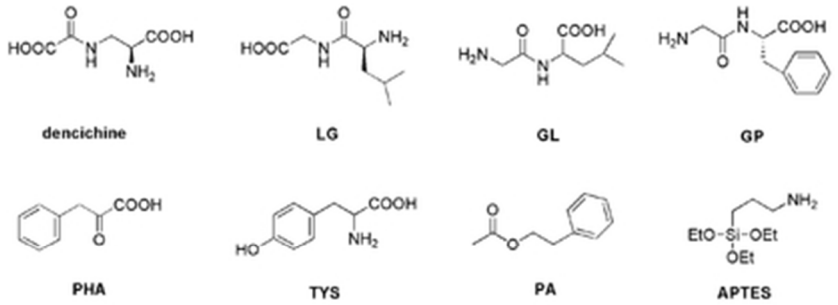

Figure 1

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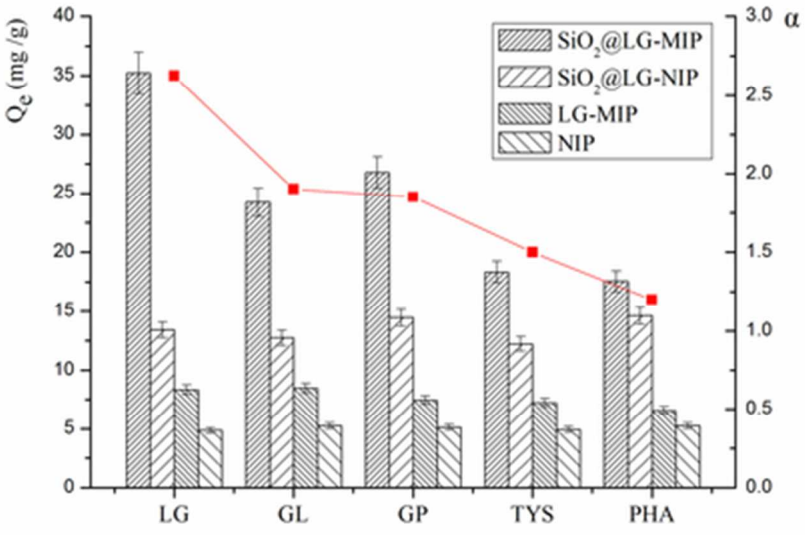

Fig.2

39x27mm (300 x 300 DPI)

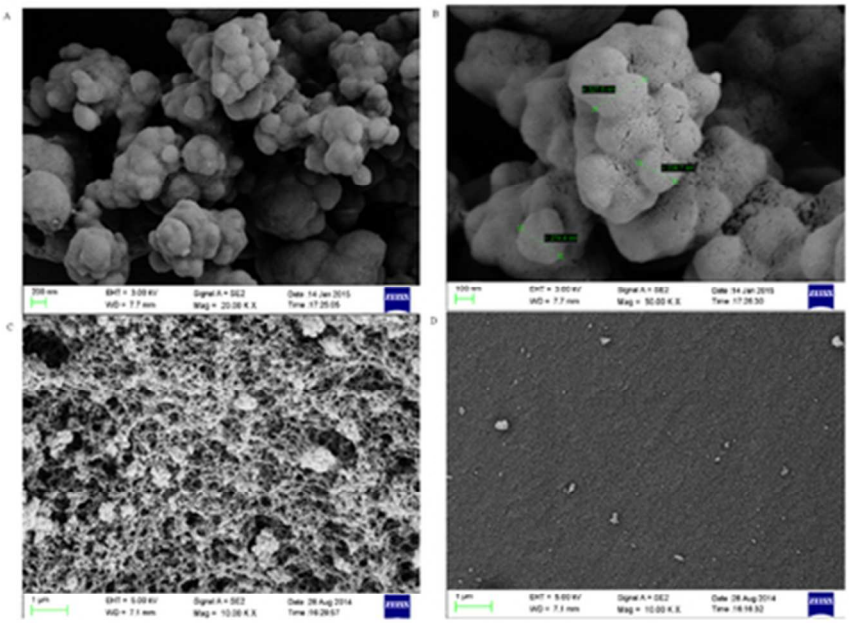

Fig. 3

39x30mm (300 x 300 DPI)

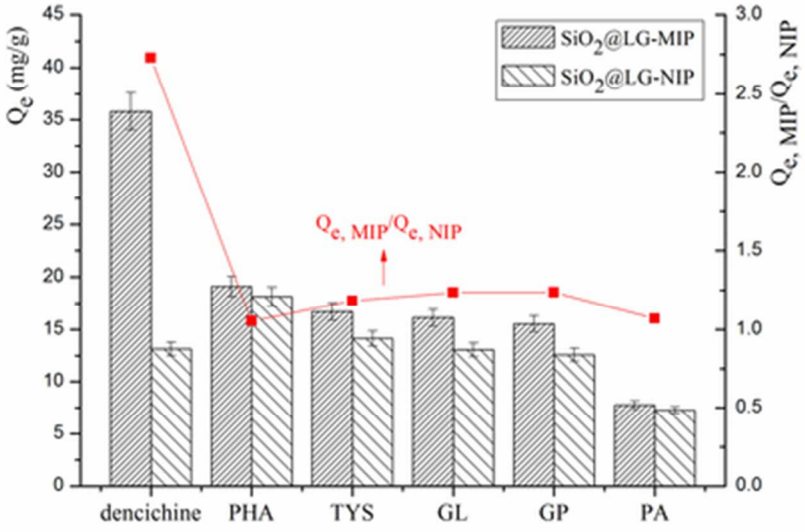

Fig.4

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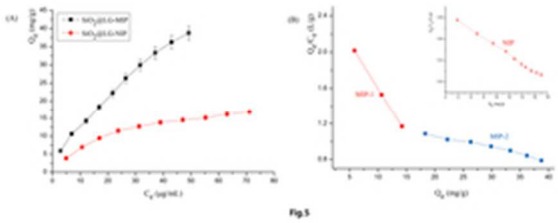

Fig.5

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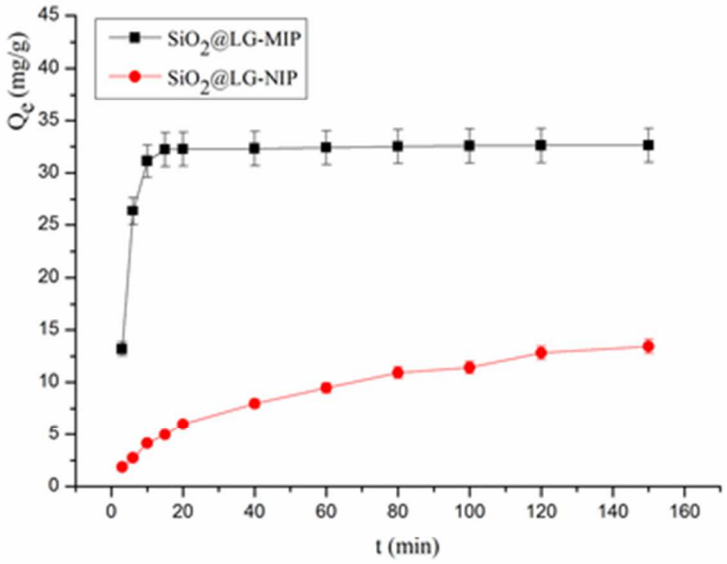

Fig.6

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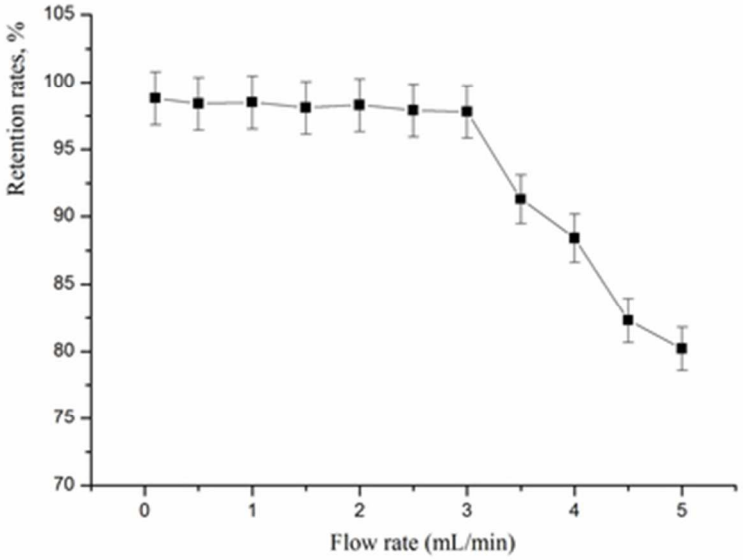

Fig. 7

39x27mm (300 x 300 DPI)

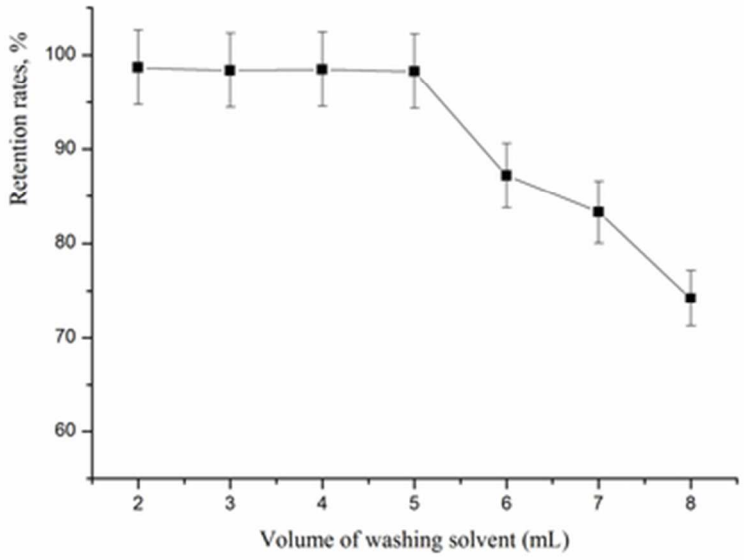

Fig. 8

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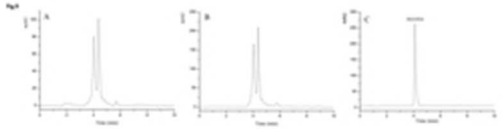

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