JOURNAL OF Applied Polymer SCIENCE

Highly Selective Extraction of Baicalin in Natural Herbs and Medicinal Preparations by Molecularly Imprinted Polymer

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ABSTRACT: Selective extraction of bioactive compounds in natural products and traditional medicines is difficult but necessary for drug analysis and new drug development. In this study, baicalin-imprinted polymer particles were prepared for selective recognition and extraction of baicalin from herbs and traditional Chinese medicines. The molecularly imprinted polymer (MIP) was synthesized by co-polymerization of 4-vinylpridine and ethylene glycol dimethacrylate in the presence of baicalin with a ratio of 8 : 40 : 1, where *N,N*-dimethyl formamide/acetonitrile served as porogen. The prepared MIP particles showed good selectivity by comparing with non-imprinted polymer and possessed an imprinted factor of 2.17; selectivity to other molecules was also tested by extracting an analogue, naringin. The performances of the MIP particles were systematically investigated, including adsorption kinetics, isotherm experiment, and Scatchard analysis. The imprinted particles were also applied to extract baicalin from natural herb *Plantago asiatica L*, and traditional Chinese medicine *Longdanxiegan bolus*. The results showed that baicalin in these complex matrixes could be selectively extracted and well isolated with other components. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 1873–1878, 2013

KEYWORDS: adsorption; applications; biomedical applications

Received 19 November 2012; accepted 15 April 2013; Published online 7 May 2013

DOI: 10.1002/app.39410

INTRODUCTION

Scutellaria baicalensis Georgi is a kind of plant which belongs to the family of labiates. *Scutellaria baicalensis Georgi* has been used as traditional Chinese medicine for tens of century for the treatment of dysentery, hot eyes, carbuncles and furuncles, and damp-heat jaundice.[1] Researches have revealed that baicalin (Figure 1) is one of most important and active compounds attributed to the pharmaceutical application of *Scutellaria baicalensis Georgi*. Besides *Scutellaria baicalensis Georgi*, baicalin is also found in *Plantago asiatica L*, Lobular loquat, etc. Baicalin possesses extensive bioactivities including antibacterial,[2] antiviral,[3] anti-inflammatory,[4] anti-oxidant,[5] and so on. Baicalin also serves as the main active component of dozens of compound medicinal preparations, such as *Longdanxiegan bolus*, Shuanghuanglian tablets, Niuhuangjiedu tablets, Sanhuang tablets, and Qingkailing capsules.

Generally, rude extracts of *Scutellaria baicalensis Georgi* are directly used for manufacturing these preparations. For the quality control, quantification of baicalin in the herbs, crude extracts, and the commercial preparations is necessary. And, in order to further explore its pharmaceutical activities and develop new drugs based on baicalin, it is also significant to purify baicalin in crude extracts of herbs. To date, several extraction methods have been developed to purify and remove the interferences co-existed with baicalin. Soxhlet extraction and maceration extraction are often effective but time consuming and labor intensive, moreover, their capability of clean-up is also quite limited.[6] Solid-phase extraction column with C-18 as sorbents has also been used,[7–9] however, the elimination of interferences with similar polarity with baicalin could not be realized neither.

Molecularly imprinted polymers (MIPs) are tailor-made materials with high selectivity to template molecules. MIPs can be synthesized conveniently by co-polymerizing functional monomers and cross-linkers in the presence of template molecule. After polymerization, template molecules are removed by solvents and polyporous polymer with selectively functional binding sites and complementary cavities for template molecules are obtained. For the advantages such as high stability, ease of preparation, and high sensitivity, MIPs have been used widely in different application, including chromatographic separation, solid phase extraction (SPE), catalysis, and sensing.[10–15] MIPs-based SPE is one of the most successful and useful application. MIPs-

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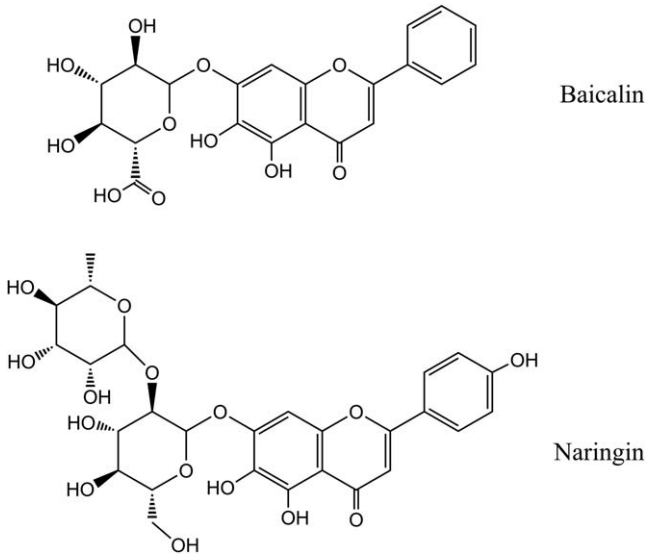

Figure 1. Chemical structure of baicalin and naringin.

based SPE combines both the advantages of MIPs and SPE, and exhibits good extraction efficiency, stability, and selectivity to different kinds of analytes,[16] which is promising to selectively and effectively extract active compounds in complex matrix of natural products and medicinal preparations.

In this study, we aim to synthesize baicalin-MIP particles for selective screening of baicalin in different matrix. Different kinds of functional monomers and porogens were investigated to obtain MIP with good selectivity towards baicalin molecules and the performances of the MIP particles were systematically studied, including adsorption kinetics, isotherm experiment, and Scatchard analysis. The selectivity of the prepared MIP was also demonstrated for recognizing and extracting baicalin in commonly used herb and compound medicinal preparation samples.

EXPERIMENTAL

Materials and Chemicals

Baicalin was purchased from Nanjing ZeLang Medicinal Technology Co. (Nanjing, China). Methacrylic acid (MAA), 4-vinylpridine (4-VP), ethylene glycol dimethacrylate (EGDMA), and naringin were purchased from Sigma-Aldrich (Shanghai, China). Azo-*N,N*-9-diisobutyronitrile (AIBN), acrylamide (AA), and the other reagents were purchased from National Medicinal Company (Shanghai, China). *Plantago asiatica L* and *Longdanxiegan bolus* were purchase from the local drug stores. Acetonitrile and methanol were of HPLC grade and the other chemicals were of analytical grade. And water was purified by a Milli-Q system (MA).

Before use, EGDMA was dried by bitter salt after being extracted with 10% sodium hydroxide and water. AIBN was recrystallized with methanol.

Instrumentation

A Shimadzu HPLC system (Tokyo, Japan) consisted of two 20AD pumps, a degasser, a UV detector, and a thermostat controlled column compartment were used for HPLC analysis. Data collection was performed in a Shimadzu LC Solution software. The chromatographic separation was performed by a Sepax ODS C18 column (4.6 mm × 250 mm, 5 μm), the mobile phase was methanol/0.2% phosphoric acid–water (70/30, v/v, 1.0 mL/min), and detection wavelength as set at 280 nm.

Preparation of the MIP Particles

About 0.1 mmol baicalin, 0.8 mmol 4-VP (or AA, MAA) were dissolved in 1.5 mL DMF : AcN (1/1, v/v) in a borosilicate bottle. The mixture was sonicated and then stirred in an ice bath (0°C) for 4 h. Subsequently, 4 mmol EGDMA, 66 mg AIBN were successively added to the mixture. After sonicating for homogeneity, the mixture was sparged with nitrogen for 15 min to remove dissolved oxygen. The polymerization was carried out in water bath at 60°C for 24 h. The produced polymer was grounded and sieved by a 48-μm sieve; fine particles were further removed by repeated sedimentation in acetone. The uniform MIP particles were washed with methanol–acetic acid (9 : 1, v/v) in a Soxhlet extraction apparatus until no baicalin was detected by HPLC; finally, the particles were washed with methanol and dried in an oven (60°C) for further use. The control, non-imprinted polymer (NIP) particles were prepared by same procedures but in the absence of baicalin.

Loading and Eluting Experiments of MIP

MIP particles (10 mg) and baicalin solution were mixed and stirred for a period of time (0.5, 1.5, 4, 5, 18, and 21 h). Then, the loading solution was filtrated and the amount of baicalin was determined by HPLC. The adsorption amount of baicalin (Q_1, μmol/mg) was calculated by *eq.* (1), in which c_t for the concentration of baicalin in solution, c_0 for the initial concentration of baicalin, V for the volume of sample solution, and W for the weight of the polymer.

$$Q_1 = \frac{(c_0 - c_t)V}{W} \quad (1)$$

The MIP particles adsorbed with samples were desorbed with eluting solvents and determined by HPLC. The eluting rate of baicalin was calculated by *eq.* (2), in which Q_2 stands for the eluting rate, c_0 for the loading concentration of baicalin, A for the peak area of solution being eluted, V_e for the volume of eluting solvent, A_0 for the peak area of control baicalin, W for the amount of polymer, and Q_1 for the ratio of adsorbed baicalin by polymer.

$$Q_2\% = \frac{Ac_0V_e}{A_0WQ_1} \times 100\% \quad (2)$$

Evaluation of MIP

Adsorption Kinetics and Isotherm Experiments. Adsorption kinetics and isotherm experiments were carried out in order to evaluate the recognition performance of the polymer towards the target molecules. The adsorption kinetic experiment was carried out in the "Loading and Eluting Experiments of MIP" section; MIP particles were added into standard baicalin solution with a constant concentration and extraction was performed. The rate of adsorption [calculated by *eq.* (1)] was determined at different times. The adsorption isotherm experiment was performed similar to the adsorption kinetic

Table I. UV Absorbency (A) of Baicalin and Baicalin-Functional Monomers

Functional monomers	A (baicalin)	A (baicalin-monomers)	ΔA
AA		276 nm : 0.88	0.62
		242 nm : 1.63	1.15
MAA	276 nm : 1.50	276 nm : 0.99	0.51
	236 nm : 2.78	238 nm : 2.56	0.22
4-VP		280 nm : 0.80	0.72
		238 nm : 0.97	1.89

experiment; MIP particles were added into standard baicalin solution with different concentration, and the rate of adsorption [calculated by *eq.* (1)] was determined. Both of the experiments were carried out at room temperature.

Scatchard Analysis of the Polymer. The experiment was carried out in the "Loading and Eluting Experiments of MIP" section and the curve can be plotted by *eq.* (3),[17,18] in which Q_1 (μmol/mg) stands for the amount of baicalin adsorbed by the polymer, c_e (μmol/mL) for the concentration of baicalin in solution, Q_{max} (μmol/mg) for the theoretical largest adsorption amount of the polymer, and K_d for the breaking dissociation constant.

$$\frac{Q_1}{c_e} = -\frac{Q}{K_d} + \frac{Q_{max}}{K_d} \quad (3)$$

Selectivity of MIP Towards Analogues. The selectivity of baicalin–MIP towards other molecules was also studied. A structure analogue baicalin, naringin was loaded onto the baicalin–MIP particles and the extraction efficiency was evaluated. The experiment was carried out in the "Loading and Eluting Experiments of MIP" section and the adsorption rate of naringin was calculated according to *eq.* (1).

Application of the Polymer to Real Samples

Plantago depressa Willd. The herb of 70 mg (powder) was added with 8 mL water, after sonication for 15 min, the sample was filtered and diluted to 100 mL by water. About 25 mL of the solution was taken and 10 mg MIP particles were added and evaluated as described in the "Loading and Eluting Experiments of MIP" section.

Longdanxiegan bolus. After wiping off the coating outside, the preparation (1.3 mg) was grounded and extracted with 13 mL water under sonication for 15 min. After filter, the solution was diluted to 25 mL, then 10 mg MIP particles were added and evaluated as described in the "Loading and Eluting Experiments of MIP" section.

RESULTS AND DISCUSSION

Effects of Functional Monomers

The stability of the complex formed by baicalin and functional monomers can influence the selectivity of the imprinted polymer particles. Generally, if the hydrogen bond between baicalin and functional monomer is formed, red shifts in the UV spectra would be found and the absorbency of baicalin solution would decrease as a result.[19] To select proper functional monomers, UV spectra of baicalin with different kind of functional monomers were investigated. In Table I, the typical UV wavelength of baicalin was 276 and 236 nm. The UV spectra of baicalin changed little when using MAA as functional monomer, while obvious changes at 236 nm were observed when using AA and 4-VP as functional monomers. Moreover, in the situation of baicalin–4-VP, obvious red shifts could be found at 276 nm, the change of absorbency value was the largest. The results showed that 4-VP can firm stable hydrogen bonds with baicalin molecules. To further convince the choice of the functional monomers, AA, MAA, and 4-VP were used as functional monomers, respectively, and the selectivity of synthesized polymers were compared. In Figure 2(a), when using AA as functional monomer, the selectivity of MIP could not be seen. While using 4-VP, the selectivity of MIP was better than MAA–MIP and could elute more baicalin. Therefore, 4-VP was chosen as the functional monomer and the result was the same to UV analysis.

The ratio of template/functional monomer can influence the selectivity of MIP, as well as the density of the polymer based on our previous experiment.[20] To certain extent, larger amount of functional monomer is beneficial to hydrogen bond formation between template and functional monomer, but the non-specific adsorption should also be considered. The most used ratio of template to functional monomer is 1 : 4, in this study; ratios from 1 : 4 to 1 : 8 were investigated to prepare MIPs and NIPs. The results are shown in Figure 2(b). Largest difference was obtained when the ratio of template/functional monomer was 1 : 8, and good extraction efficiency was also obtained by the soft polymer, therefore, the ratio of baicalin/4-VP of 1 : 8 was chosen to prepare the MIP.

Effects of Porogens

Porogens served as the solvents for the polymerization, which could influence the self-assembly of template-functional

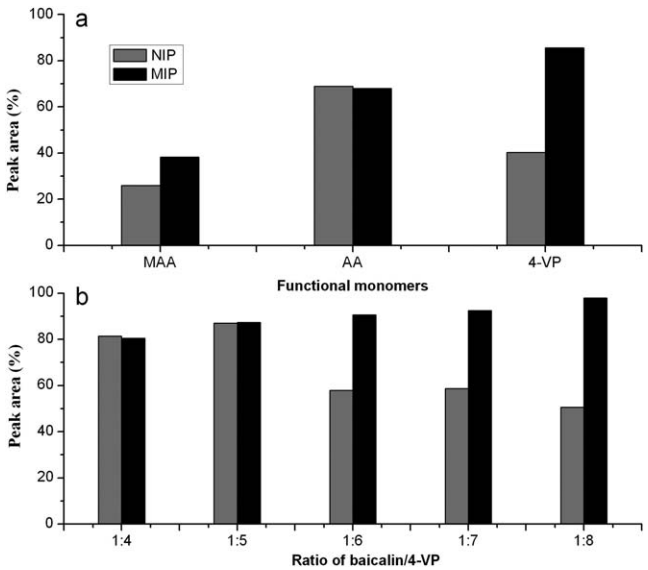

Figure 2. The effects of (a) different functional monomers and (b) different ratios of template/functional monomer.

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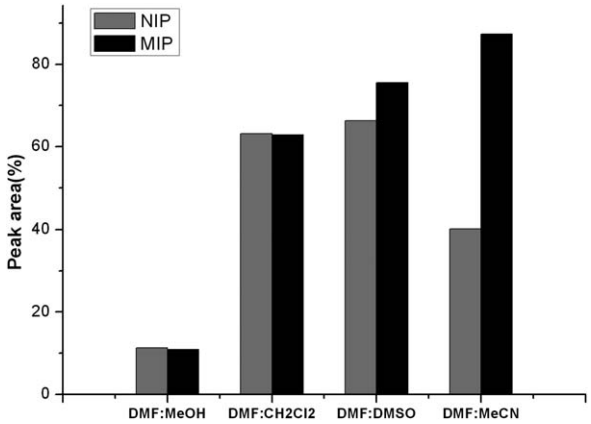

Figure 3. The effect of different porogens.

monomers, and affect the size of mesopores and macropores in the MIP particles.[21] In the system of baicalin-4-VP, the main non-covalent interaction was the hydrogen bond; therefore, the polarity of porogen should be weak enough to avoid disturbing the stability of complex formed by baicalin and 4-VP. *N,N*-Dimethyl formamide (DMF) was a good solvent to dissolve baicalin and DMF/MeOH, DMF/CH_2Cl_2, DMF/AcN, and DMF/DMSO at a ratio of 1/1 (v/v) were investigated to prepare MIPs. The results were shown in Figure 3. When using DMF/MeOH as porogen, the extraction efficiency of the polymers was relatively low, the reason is possibly that MeOH was a kind of polar solvent, which could disturb the hydrogen bond between baicalin and 4-VP. When using DMF/CH_2Cl_2 and DMF/DMSO as porogens, the selectivity of MIPs was not obvious. DMF/AcN was selected as the porogen considering the selectivity and extraction efficiency of MIP particles.

Evaluation of the Performance of MIP Particles

The Adsorption Kinetic Study. The adsorption kinetics of the synthesized polymer particles was studied. The amount (Q) of baicalin that was adsorbed by polymer particles at different times was determined. In Figure 4, the amount of baicalin adsorbed onto MIP and NIP particles increased sharply within 5 h and reached equilibrium after 5 h. Several hours are often required for the equilibrium of particles stirring in a solution.[22–24] The amount of baicalin adsorbed by MIP was more than that by NIP and the result also testified the selectivity of synthesized baicalin–MIP particles.

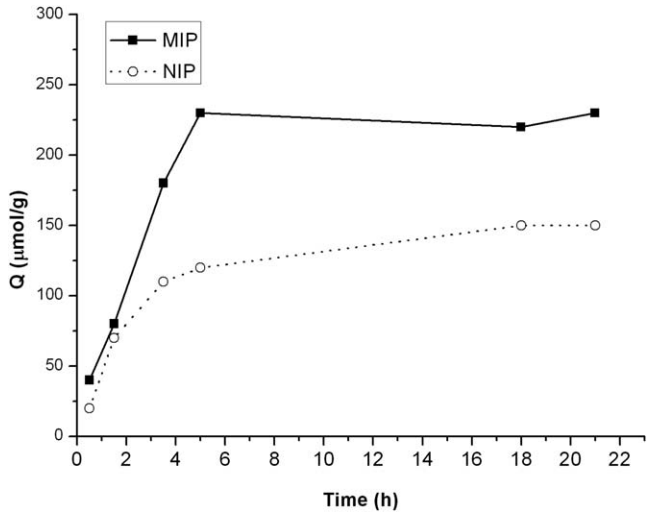

Figure 4. The curves of adsorption kinetics of MIP and NIP particles. Conditions: Polymer, 10 mg; sample volume, 25 mL; baicalin concentration, 0.01 μmol/mL.

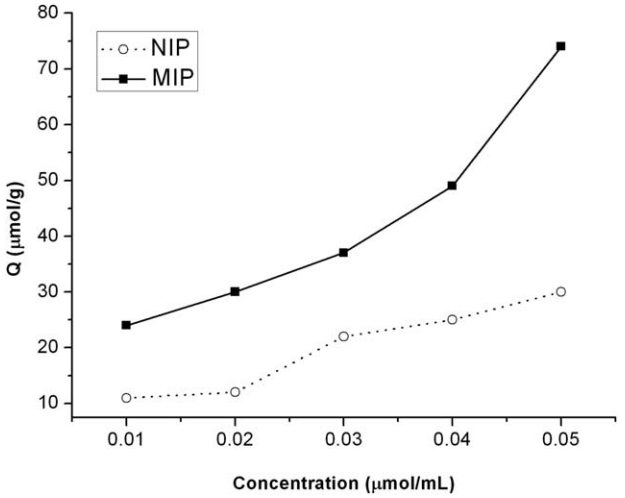

Figure 5. The adsorption isotherm of MIP and NIP particles. Conditions: Polymer, 10 mg; sample volume, 25 mL; stirring time, 12 h.

The Adsorption Isotherm Analysis. The adsorption isotherm of the synthesized polymer particles was also studied. The amount (Q) of baicalin adsorbed by polymer particles was determined with baicalin solutions at different concentrations. In Figure 5, the amount of baicalin adsorbed by the polymers increased with the concentration of baicalin loaded until saturation was obtained [22,23] (not observed in the investigated range). Furthermore, the amount of baicalin adsorbed by MIP was more than that by NIP and showed that MIP particles had larger adsorption capacity and better extraction efficiency than NIP particles.

The Scatchard Analysis of the Polymers. The Scatchard analysis was carried out to investigate the adsorption mechanism of the polymer particles. The Scatchard curves of MIP and NIP are shown in Figure 6. Data sheets in Scatchard plot of MIP [Figure 6(a)] could be separated into two lines, demonstrating that there were two binding sites of MIP: non-specific binding sites and specific binding sites. The specific binding sites of MIP particles probably came from the imprinted cavities which were formed in the process of polymerization. The dissociation constants (K_d) were 0.053 μmol/mL (low energy binding site) and 0.008 μmol/mL (high energy binding site) and the theoretic largest adsorption amount of MIP (Q_{max}) were 0.700 and 0.222 μmol/mg, respectively. For NIP [Figure 6(b)], only one line was observed, indicating that only non-specific binding sites existed; the K_d was 0.012 μmol/mL and Q_{max} was 0.055 μmol/mg. The results also showed that the adsorption ability of MIP was higher than NIP.

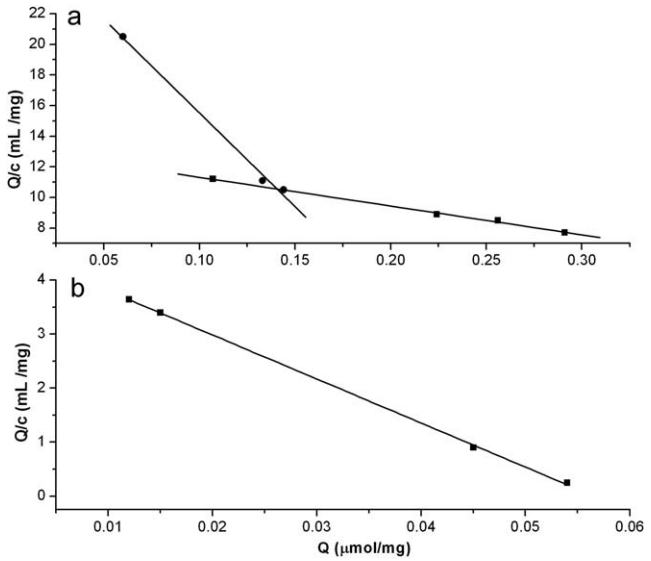

Figure 6. The Scatchard curves of MIP (a) and NIP (b) particles. Conditions: Polymer, 10 mg; sample volume, 25 mL; baicalin concentration, 0.01 μmol/L; stirring time, 12 h.

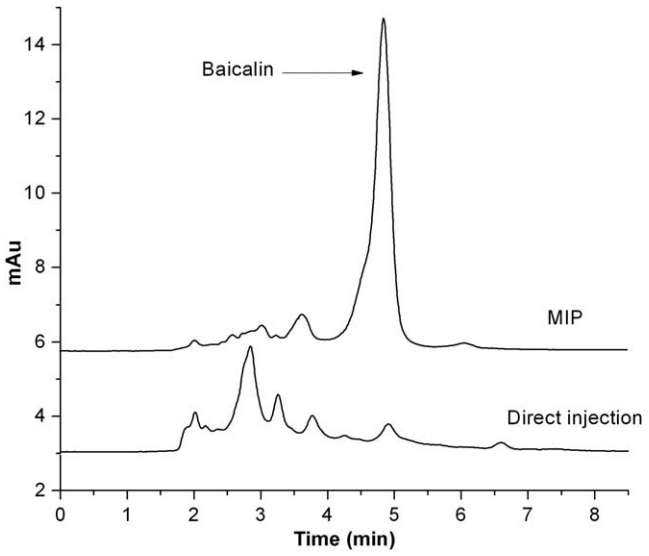

Figure 7. Chromatograms of *Plantago asiatica L* sample with MIP-SPE and without extraction.

Selectivity of Baicalin–MIP Towards Baicalin and Structure Analogues. The selectivity of baicalin–MIP was further investigated by extraction of a structure analogue of baicalin, namely, naringin (Figure 1). The enrichment capability of MIP towards baicalin and naringin were calculated by the ratio of peaks for these two compounds with extraction and without extraction. In Table II, MIP particles obviously possessed better extraction toward baicalin, while only a small portion of naringin can be extracted by the MIP particles. Although both of baicalin and naringin were of flavone family, the locality of hydroxyl group was different, which resulting in their different interaction with functional monomer. Furthermore, the Indian group of naringin was not the same with that of baicalin, leading to mismatch of the shape of naringin with cavities on the baicalin–MIP particles. Difference in extraction capability of baicalin–MIP particles towards baicalin and naringin was a result of specific recognition of MIP.

Application in Real Herb and Medicinal Preparation Samples. Baicalin is the main active compounds of some herbs and a lot of medicinal preparations. Generally, the matrices of these samples are complex and some compounds with similar physical and chemical properties still co-existed with baicalin. It is difficult to extract baicalin molecules from the described complex environment by commonly used extraction methods. MIP based solid-phase extraction is a promising methods. Therefore, the synthesized baicalin–MIP particles were finally applied to selectively extract baicalin in herbs and medicinal preparations. An herb containing baicalin, namely *Plantago asiatica L* and a commonly used baicalin compound preparation, namely *Longdanxiegan bolus* was selected to evaluate the extraction efficiency of the MIP particles. Figures 7 and 8 show the chromatograms of real samples with and without MIP-SPE. Baicalin was extracted and enriched effectively; in *Plantago asiatica L* sample (Figure 7), most interference was removed after MIP-SPE, while in *Longdanxiegan bolus* sample (Figure 8), sample peaks were also observed during 6–7 min, which were possibly ascribed to the more complexity of medicinal preparations, and some compounds with extremely similar structure such as baicalin derivants co-existed in the sample. The results demonstrated the high selectivity of the MIP particles to baicalin and the good clean-up efficiency of the MIP-SPE method.

Table II. Enrichment Folds (EF) of MIP and NIP to Baicalin and Naringin, and Corresponding Imprinted Factors (IF = EF_{MIP}/EF_{NIP})

Compounds	EF_{MIP}	EF_{NIP}	IF
Baicalin	9.1	4.2	2.17
Naringin	0.78	0.75	1.04

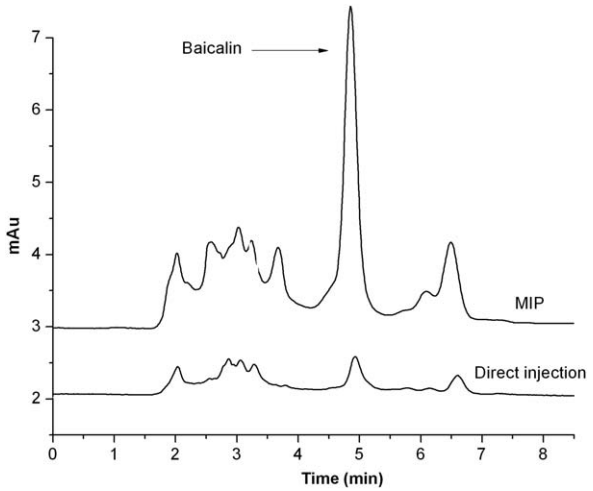

Figure 8. Chromatograms of *Longdanxiegan bolus* sample with MIP-SPE and without extraction.

CONCLUSIONS

In this study, baicalin–MIP was synthesized. The polymer was prepared by bulk polymerization method using baicalin as template, 4-VP as functional monomer, and EGDMA as cross-linker. The synthesized MIP showed good selectivity by comparing with NIP, with an imprinted factor of 2.17; and also showed selectivity to structure analogue of baicalin. The performances of the MIP particles were systematically investigated, and the results showed the good recognition and extraction capability of the synthesized MIP particles. Finally, MIP particles were applied to selectively recognize and extract baicalin in herbs and medicinal preparations. Baicalin was extracted and enriched effectively and most of interference from the samples matrix was removed after MIP-SPE process. The results demonstrated that baicalin–MIP particles are promising to be use for selective recognition and extraction of baicalin in natural products and complex medicinal preparations.

ACKNOWLEDGMENTS

This study was supported by the National Scientific Foundation of China (Grant Nos: 90817103, 30973672, 20775055), Doctoral Fund of Ministry of Education of China (No.: 20110141110024), Hubei Provincial Scientific Foundation (No.: 2011CDB475), and the Fundamental Research Funds for the Central Universities.

REFERENCES

1. Nanjing University of Traditional Chinese medicine. Dictionary of Chinese Medicine; Shanghai Science and Technology Press, Shanghai; **2005**.
2. Cushnie, T. P. T.; Hamilton, V. E. S.; Lamb, A. *J. Microbiol. Res.* **2003**, *158*, 281.
3. Chen, F.; Chan, K. H.; Jiang, Y.; Kao, R.; Lu, H. T.; Fan, K. W.; Cheng, V.; Tsui, W.; Hung, I.; Lee, T.; Guan, Y.; Peiris, J.; Yuen, K.Y. *J. Clin. Virol.* **2004**, *31*, 69.
4. Lu, J.; Wang, J. S.; Kong, L. Y. *J. Ethnopharmacol.* **2011**, *134*, 911.
5. Ng, T. B.; Liu, F.; Wang, Z. T. *Life Sci.* **2000**, *66*, 709.
6. You, J.; Gao, S.; Jin, H.; Li, W.; Zhang, H.; Yu, A. *J. Chromatogr. A* **2010**, *1217*, 1875.
7. Huang, P.; Gao, J. W.; Shi, Z.; Zou, J. L.; Lu, Y. S.; Yuan, Y. M.; Yao, M. C. *Bioanalysis* **2012**, *4*, 1205.
8. Zeng, H. J.; Yang, R.; Guo, C.; Wang, Q. W.; Qu, L. B.; Li, J. J. *J. Pharm. Biomed.* **2011**, *56*, 815.
9. Zgorka, G.; Hajnos, A. *Chromatographia* **2003**, *57*, s77.
10. Zheng, C.; Huang, Y. P.; Liu, Z. S. *J. Sep. Sci.* **2011**, *34*, 1988.
11. Vasapollo, G.; Del Sole, R.; Mergola, L.; Lazzoi, M. R.; Scardino, A.; Scorrano, S.; Mele, G. *Int. J. Mol. Sci.* **2011**, *12*, 5908.
12. Suryanarayanan, V.; Wu, C. T.; Ho, K. C. *Electroanalysis* **2010**, *22*, 1795.
13. Rezaei, B.; Jafari, M. T.; Khademi, R. *Talanta* **2009**, *79*, 669.
14. Liu, J. Z.; Wang, W. Z.; Xie, Y. F.; Huang, Y. Y.; Liu, Y. L.; Liu, X. J.; Zhao, R.; Liu, G. Q.; Chen, Y. *J. Mater. Chem.* **2011**, *21*, 9232.
15. Li, J. P.; Li, Y. P.; Zhang, Y.; Wei, G. *Anal. Chem.* **2012**, *84*, 1888.
16. Augusto, F.; Carasek, E.; Silva, R. G. C.; Rivellino S. R.; Batista, A. D.; Martendal, E. *J. Chromatogr. A* **2010**, *1217*, 2533.
17. Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *22*, 1722.
18. Heny, A. F. *Anal. Biochem.* **1972**, *48*, 317.
19. Chen, L. N.; Jia, X. J.; Lu, Q.; Peng, Y.; Du, S. H.; Chen, Q. *Sep. Purif. Technol.* **2010**, *71*, 324.
20. Zhang, W.; Chen, Z. *Talanta* **2013**, *103*, 103.
21. Zheng, M. M.; Gong, R.; Zhao, X.; Feng, Y. Q. *J. Chromatogr. A* **2010**, *1217*, 2075.
22. Xu, L.; Lv, R.; Wang, Y.; Gao, J. *J. Appl. Polym. Sci.* **2010**, *118*, 881.
23. Rao, R. N.; Maurya, P. K.; Khalid, S. *Talanta* **2011**, *85*, 950.
24. Zhang, W.; Du, J.; Su, C.; Zhu, L.; Chen, Z. *Anal. Lett.* **2013**, *46*, 900.