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A Molecular Imprinted Polymers with β-Cyclodextrin for Adsorption of Paeoniflorin

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β-Cyclodextrin and epichlorohydrin were respectively used as functional monomer and cross-linker to synthesize molecularly imprinted polymers microspheres (MIPs) by suspension polymerization, for adsorption of paeoniflorin (PF), named PF-MIPs. The experiment indicated the PF-MIPs have specific selectivity on paeoniflorin. The paeoniflorin binding site was found inside of PF-MIPs by Scatchard analysis, with K_d and Q_{max} of 0.46 mg/mL and 47.22 mg/g, respectively. The adsorption process was endothermic process, the adsorption of paeoniflorin on MIPs followed Freundlich adsorption model. The PF-MIPs can be re-used to isolation paeoniflorin at least 10 times.

Keywords: Molecularly imprinted polymers, Paeoniflorin, Adsorption, β-Cyclodextrin.

INTRODUCTION

Molecular imprinting technique is a rapidly developing technique to prepare polymers possessing high recognition properties[1,2]. The polymers were prepared by this technique, named molecular imprinted polymers (MIPs). Molecular imprinted polymers is a kind of functional polymer, with several imprint cavities of a template molecule. Considering its superior mechanical strength, good stability, strongly specificity and high efficient, molecularly imprinted technique were widely applied in many areas, such as biochemical separation[3], biosensors[4], mimic enzyme catalysts[5] and clinical analysis[6]. With the development and update of molecular imprinted technique, MIPs has a bright application prospect in various high technology areas.

Paeoniflorin[7] (Fig. 1), a monoterpene glycoside, was isolated by Shibata and Nakahara[8] as the major physiologically active principle from Paeoniae Radix, the root of *Paeonia albifora Pallas*. Paeoniflorin is moisture absorption amorphous powder. Its melting point is 196 ºC. Paeoniflorin, derived from the crowfoot plants, purple peony root, dilate blood vessels and analgesic sedative, anti-inflammatory antioxidants ulcer, antipyretic spasmolysis, diuretic effect.

In this study, the molecularly imprinted polymers for isolation of paeoniflorin (PF-MIPs) microspheres were prepared by suspension polymerization. Paeoniflorin was used as template molecule, β-cyclodextrin as functional monomer, epichlorohydrin as cross-linker. High performance liquid chromatography (HPLC) method was used in this experiment to analysis

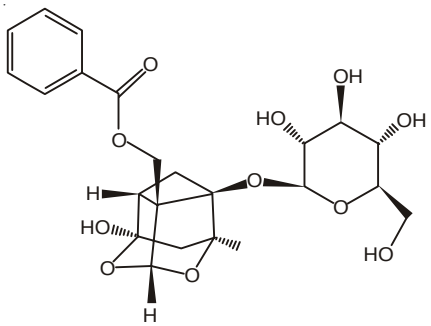


Fig. 1. Chemical structure of paeoniflorin

MIPs' adsorption property and evaluated its specificity adsorption capacity and regeneration capacity. Results of imprinting experiment revealed that PF-MIPs have specific selectivity on paeoniflorin, which can be reused many times without decreasing its adsorption capacities significantly. A highly efficient method to harvest paeoniflorin from herbal extracts by molecular imprinting was provided.

EXPERIMENTAL

β-Cyclodextrin, epichlorohydrin, Tween 20, Span 80 were purchased from National Medicine Group Chemical Reagent Co., Ltd. NaOH, Methanol, Acetone, HAC were purchased from Beijing Chemicals Co., Ltd. Paeoniflorin (≥ 98 %) and albiflorin (≥ 98 %) were provided with Center of Fine Separation.

HPLC-grade methanol was produced by Fisher Scientific CO. (USA). Other agents of analytical grade were purchased from Beijing Beihua Fine Chemicals Co., Ltd. (Beijing, China).

Chromatographic analysis of paeoniflorin and albiflorin: Offline analysis of paeoniflorin and albiflorin by HPLC on a Agilent 2100 (USA) with the UV detector. Sample analysis was performed on a Agilent C_{18} column (150 L × 4.6 mm, I.D., 5 μm) at a column temperature of 25 °C. The mobile phase was methanol-water-acetic acid (30:70:0.1, v/v/v) at a flow rate of 1 mL/min; the analysis wavelength was 230 nm.

The morphology of the polymers was characterized by the scanning electron microscope (model Quanta-200, FEI); and the supernatant was obtained by centrifugation (model 3K-15, Sigma).

Preparation of PF-MIPs microspheres: In order to prepare the MIPs, the template molecules paeoniflorin and the functional monomer β-cyclodextrin were dissolved in deionized water in a round-bottom flask and the water bath temperature lower than 60 °C, stirring by a blender for 10 min. Then 4 g NaOH and 3 g β-cyclodextrin were added in the round-bottom flask, respectively. After being dissolved thoroughly, the cross-linker agent epichlorohydrin (7 mL) was added slowly at 30 °C, with the blender stirring at 350 rpm for 2 h and pre-polymerization was performed. Next, solvent oil 200#, emulsifier Tween 20 and Span 80 were added, followed by rising temperature gradually, until the temperature was up to 80 °C. The synthesis time is not less than 6 h. The MIPs microspheres waer acidified with diluted hydrochloric acid. Polymers were washed with ethanol, deionized water and acetone to remove the template molecule and the un-synthesis β-cyclodextrin. The NIPs microspheres were prepared under identical conditions in the absence of paeoniflorin during the polymerization process.

Analysis of adsorption equilibrium time of PF-MIPs: The adsorption equilibrium time experiment with paeoniflorin was calculated by mixing various 100 mg PF-MIPs with 0.8 g/L of paeoniflorin solution (5 mL). The mixtures were incubated weith continuous stirring at 30 °C and were determined every 10 min, until the paeoniflorin solution concentration was not changed.

Specific selectivity evaluation on PF-MIPs: Paeoniflorin and albiflorin are both the main component of *Paeonia albifora Pallas* and have a similar structure. To gain better results on the specific selectivity of PF-MIPs, the specific selcectivity of PF-MIPs with paeoniflorin was calculated by mixing various 100 mg PF-MIPs with 1 g/L of paeoniflorin and 1 g/L of albiflorin mixed solution (5 mL) and the mixture supernatant were determined every 10 min, until the paeoniflorin solution concentration was not changed.

Scatchard analysis of PF-MIPs: The binding parameters of PF-MIPs were mainly studied by a specific binding model-scatchard analysis model[9], which was a common fitting method. The equation can be described as follows:

$$Q = \frac{Q_{max}[C^*]}{K_d + [C^*]} \quad (1)$$

where Q is the template molecule absorbed to the imprinted polymers, Q_{max} is the theoretical maximum absorption capacity of binding sites, K_d is the desorption constant of binding site and $[C^*]$ is the concentration of the template in adsorption equilibrium solution. Eqn. 1 could be transformed to:

$$\frac{Q}{[C^*]} = \frac{Q_{max}}{K_d} - \frac{Q}{K_d} \quad (2)$$

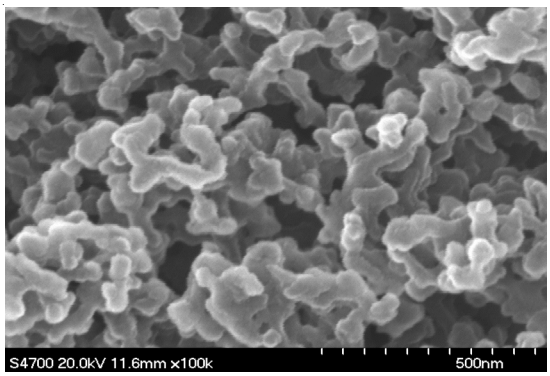
According to the eqn. 2, if Q and $Q/[C^*]$ is a straight line, the slope and intercept are $-1/K_d$ and Q_{max}/K_d, respectively; this shows that there exists a class of equivalent recognition sites in MIPs.

Next, the studies analyzed the adsorption isotherm of paeoniflorin on PF-MIPs. Preparated five different concentration of paeoniflorin solution (5 mL) and added 100 mg PF-MIPs at the temperature of 298, 303 and 308K. Then calculated the adsorption capacity correspondingly and fitted the adsorption isotherm of paeoniflorin on PF-MIPs[10,11].

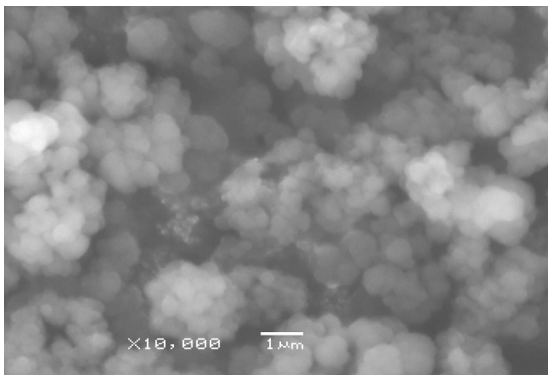
Reusability evaluation on PF-MIPs: In order to harvest paeoniflorin from PF-MIPs and also reuse the MIPs, the reusability of MIPs was studied. After eluted paeoniflorin from PF-MIPs, with the eluted solution was methanol-0.6 mol/L NaCl (80:20, v/v) and re-adsorpted paeoniflorin. Repeat the same operation 10 times.

RESULTS AND DISCUSSION

SEM of PF-MIPS and NIPs: After polymerization, the template was eluted after washing step, leaving the micro-environment as recognition sites in the MIPs. The SEM micrographs clearly showed that the pores were embedded in the network of MIPs as reported in Fig. 2, there were substantial differences in morphology between the PF-MIPs and NIPs. PF-MIPs have tougher structure with proper cavities and surface area than those of NIPs, mainly due to the existence of the binding sites of paeoniflorin[12].



(a) PF-MIPs



(b) NIPs

Fig. 2. Scaning electron micrographs of the polymers; (a) PF-MIPs (b) NIPs

Determination of adsorption equilibrium time of PF-MIPs: Bulk polymerization of MIPs resulted in the paeoniflorin recognition sites that are located inside the matrix structure of PF-MIPs. It took time for paeoniflorin to penetrate into the matrix. The adsorption equilibrium time was tested and the result shows that the unit adsorption of PF-MIPs tended to be stability about 0.5 h and the maximum binding capacity is 31.69 mg/g (Fig. 3).

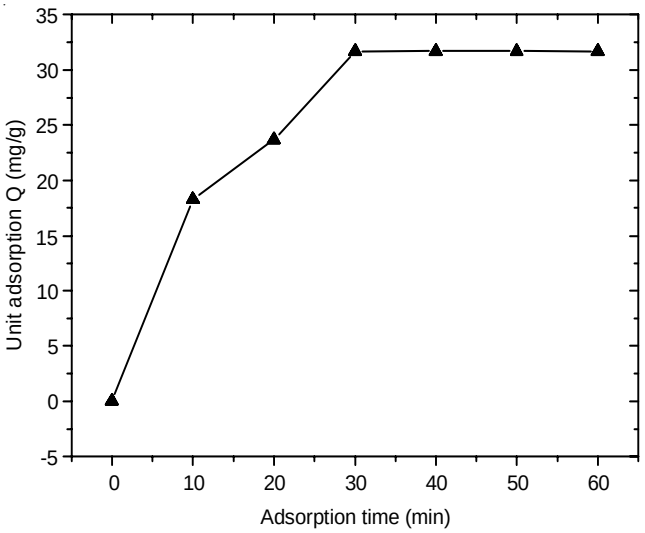


Fig. 3. Adsorption equilibrium time curve of PF-MIPs

Specific selectivity analysis of PF-MIPs: The qualified imprinted polymers not only have higher adsorption capability to the template, but also have specific recognition ability to paeoniflorin[13]. The results was presented in Fig. 4. Compared to albiflorin, the PF-MIPs have the significant higher adsorption quantity for paeoniflorin and albiflorin didn't disturbed the specific adsorption of PF-MIPs.

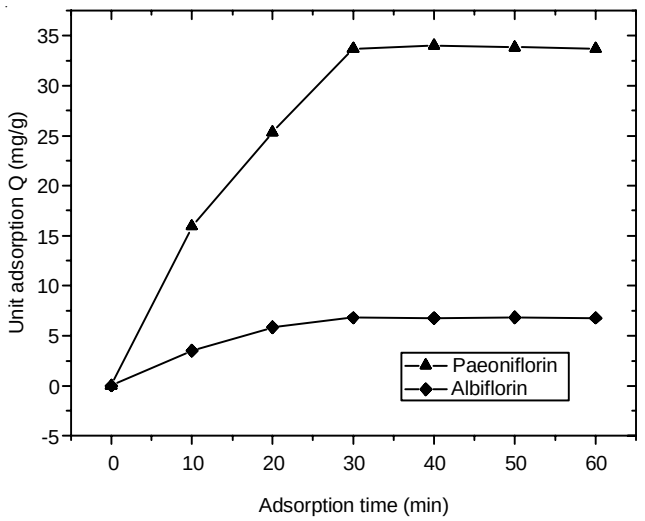


Fig. 4. Specific selectivity adsorption curve of PF-MIPs

Thus, the analysis of the specific selectivity further identified that PF-MIPs were well imprinted. The main mechanism for selectivity of MIPs could, probably, be attributed to the formation of the shape-selectivity cavity[14].

Scatchard analysis and adsorption isotherm of PF-MIPs

Scatchard analysis of PF-MIPs: The adsorption equilibrium studies were applied to depict the interaction of paeoniflorin and PF-MIPs. According to the scatchard adsorption experiment, the batch adsorption amount of paeoniflorin on PF-MIPs was shown in the Scatchard curve (Fig. 5), Q and Q/[C*] yield a straight line, the correlation coefficient R^2 was 0.9548 of the fitted curve, the binding site was equivalent in PF-MIPs. Analyzed the Scatchard curve, the theoretical maxium adsorption Q_{max} was 47.22 mg/g, the desorption constant K_d was 0.46 mg/mL, the specificity of PF-MIPs was well illustrated.

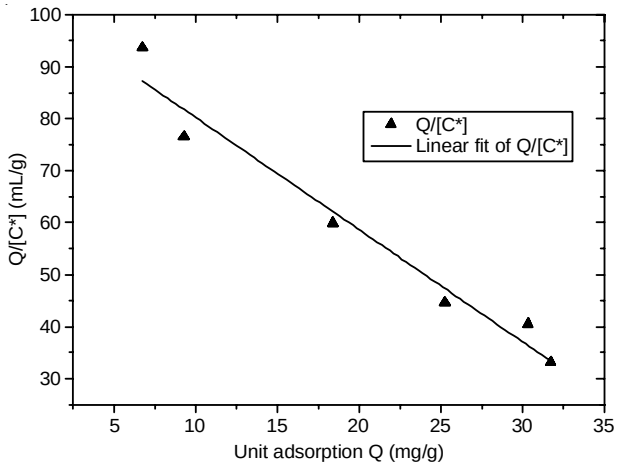


Fig. 5. Scatchard plot of PF-MIPs

Adsorption isotherm of PF-MIPs: The adsorption isotherm studies were applied to investigate the affection of temperature to adsorption capacity of PF-MIPs. Fig. 6 showed that the adsoption capactiy was enchanced differently by increasing the temperature, the adsorption process was endothermic process, it means that raising the adsorption temperature contributed to adsorption behavior of PF-MIPs.

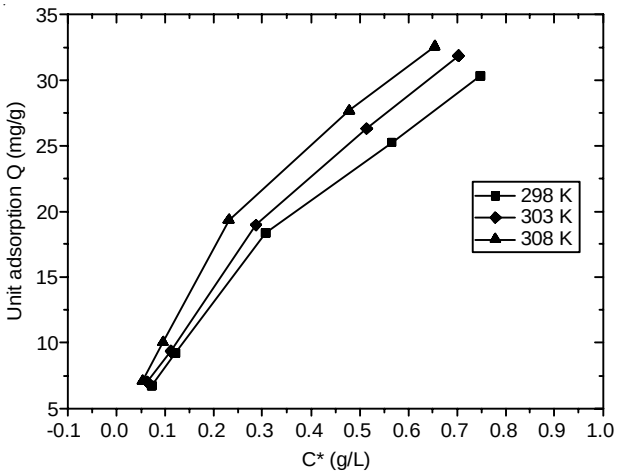


Fig. 6. Adsorption isotherm of paeoniflorin on PF-MIPs

Comparing the fitted curves of Langmuir and Freundlich models (Fig. 7 and Table-1), the correlation of regression equation for Langmuir model was smaller than Freundlich model. It indicated that the adsorption of paeoniflorin on MIPs followed Freundlich adsorption model.

TABLE-1
FITTED EQUATION OF LANGMUIR AND FREUNDLICH MODELS

Polymers	T(K)	Freundlich	R^2	n	K_L	Langmuir	R^2
PF-MIPs	298	log Q = 0.648 log (C*') + 0.275	0.997	1.543	1.883	C*/Q = 0.020C* + 0.010	0.984
	303	log Q = 0.655 log (C*') + 0.281	0.997	1.527	1.908	C*/Q = 0.019C* + 0.010	0.977
	308	log Q = 0.620 log (C*') + 0.404	0.994	1.613	2.533	C*/Q = 0.020C* + 0.007	0.992

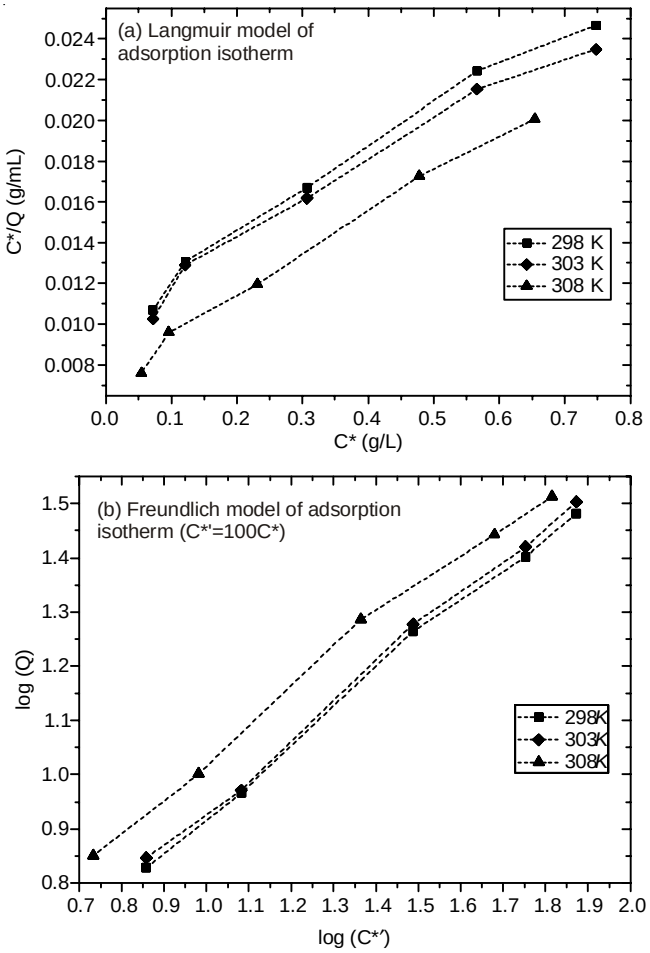


Fig. 7. Langmuir and Freundlich models of adsorption isotherm; (a) Langmuir (b) Freundlich

Reusability of PF-MIPs: In order to isolation paeoniflorin and reuse the PF-MIPs, the reusability of MIPs was studied. The result shows that the adsorped paeoniflorin being eluted out was 91 % and the adsorption capacity of PF-MIPs was able to reach 96 % in the next run. And after 10 times repeated experiments, the adsorption performance hadn't significantly decreased. We can use the PF-MIPs to isolate paeoniflorin from the mixture of paeoniflorin and plbiflorin or other herbal extracts with paeoniflorin.

Conclusion

Using β-cyclodextrin as functional monomer, epichlorohydrin as cross-linker, paeoniflorin as template molecule. The molecularly imprinted polymers for isolation of PF(PF-MIPs) microspheres were prepared by suspension polymerization. The specific matrix structure of MIPs was showed under the SEM. Results of imprinting experiment revealed that PF-MIPs have specific selectivity on paeoniflorin. The paeoniflorin binding site was found inside of PF-MIPs, with K_d was 0.46 mg/mL and Q_{max} was 47.22 mg/g, the adsorption process was endothermic process, the adsorption of paeoniflorin on MIPs followed Freundlich adsorption model. The PF-MIPs can be re-used to isolation paeoniflorin at least 10 times. A highly efficient method to harvest paeoniflorin from herbal extracts by molecular imprinting was provided, which can promote the process of modernization of Chinese medicine.

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