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Preparation and adsorption performance of molecularly imprinted polymers for Kaempferol

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ABSTRACT

Molecularly imprinting polymers (MIPs) are a class of highly cross-linked polymer with specific binding sites for a target molecule. In this study MIPs were prepared by precipitation polymerization, using kaempferol as the template molecule, methacrylic acid as the functional monomer, azodiisobutyronitrile as the initiator, and ethylene glycol dimethacrylate as the cross-linker in acetonitrile solvent. The synthesized kaempferol-MIP and nonimprinted polymer (NIP) were analyzed by infrared spectroscopy and scanning electron microscope. In addition, the obtained polymers were evaluated by adsorption isotherms and dynamic curves with their selective recognition properties for kaempferol. The experimental results show that (1) the imprinted polymers are spherical morphology; (2) the adsorption dynamic curves of kaempferol-MIP and NIP indicate that the specific adsorption increases with time extension and reaches saturation in a short period, and the kaempferol-MIP exhibits a higher affinity for kaempferol than the NIP; (3) the Scatchard analysis reveals that there exists a class of homogenous recognition sites in kaempferol-MIP; apparent maximal combination amount (Q_{max}) and equilibrium dissociation constant (K_d) were calculated to be 2140 μg/g and 5.77 mg/L, respectively.

Keywords: Molecularly-imprinted polymer; Kaempferol; Adsorption performance; Scatchard model

1. Introduction

Molecular imprinting technique (MIT) is based on the system used by enzymes for substrate recognition, which is called the "lock and key" model. The active binding site of an enzyme has a unique geometric structure that is particularly suitable for a substrate [1,2]. MIT was first proposed by Wulff and Sarhan (1972), and then, it was developed by the effort of Mosbach and coworkers in 1980s (1984). In a similar way, the MIP with specific binding sites has high adsorption properties for target molecules. MIPs have been widely applied in drug separation, food, and environmental testing, antibody or receptor analogs, sensors and many other fields in recent years [3–7].

Kaempferol (3,5,7-trihydroxy-4′-methoxyflavone), an important active ingredient of gingko, is widely

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found in leaves, fruits, vegetables, and grains. Owning to kaempferol's various bioactivities such as antiatherosclerosis, antioxidant ability, antitumor, and function of strengthening the capillary resistance, it has become a hot topic. Because of low concentration of kaempferol in nature, the complexity of samples, and the structural similarity to other flavonols, selective extract methods are necessary prior to analysis. So MIP could be potentially used as separation materials based on such features as the shape, size, and functionality selectivity, strong affinity on rebinding target compounds [8,9].

Up to now, many researches on synthesis and application of MIPs have been carried out. Xie [10] composed quercetin-MIP through bulk polymerization to extract quercetin from the hydrolyzate of Gingko leaves. Chen [11] synthesized MIP–puerarin to realize highly efficient separation of puerarin, and evaluated the MIPs' selectivity for puerarin by using solid-phase extraction. MIP-hydroquinone has also been used to analyze the environmental interest of hydroquinone. Since precipitation polymerization for the preparation of MIPs was first reported by Ye et al. [12] it has been widely used to recognize various compounds, such as amino acids, antibiotics and herbicides. Compared with bulk polymerization, MIPs prepared in the form of precipitation polymerization have regular size and shape.

In this study, the molecularly imprinted microspheres for kaempferol were prepared by precipitation polymerization. The characteristics of synthesized polymers were analyzed by infrared spectroscopy and scanning electron microscope (SEM). In addition, adsorption isotherms and dynamic curves were used to assess their selective recognition properties for kaempferol.

2. Materials and methods

2.1. Materials

Kaempferol was purchased from Beijing Chemical Works. Methacrylic acid (MAA) was provided by West Long Chemical Plant (China, AR). Ethylene glycol dimethacrylate (EDMA) was supplied by Sigma-Aldrich (USA, AR). 2.2-Azobisisobutyronitrile (AIBN) was obtained from Shanghai Chemical Reagent Company. Methanol (MeOH), ethanol (EtOH), acetic acid, and acetonitrile were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.

2.2. Polymer preparation

The template molecule kaempferol (0.5 mmol) and the functional monomer MAA (2.5 mmol) were dissolved in 40 ml of acetonitrile. After stirring for half an hour, 12.5 mmol of cross-linker EDMA and 0.1 g initiator AIBN were added. Then, the mixture was polymerized at 60℃ oil bath under a nitrogen atmosphere for 24 h. The imprinted polymers were extracted with a mixture of MeOH/acetic acid (9:1 v/v) in Soxhlex extractor for 48 h to remove kaempferol and rinsed with MeOH for 24 h. Then, the MIPs were dried at 60℃ under vacuum for 24 h. As a control experiment, NIP was also synthesized in the identical manner as the MIP but in the absence of kaempferol during the polymerization.

2.3. Morphologies of kaempferol-MIP and NIP

For morphology observation, the kaempferol-MIP and NIP were dried at 60℃ under vacuum and then attached to the silver paper and coated with a gold layer. The surface morphologies of the polymers were observed using SEM (S-3400N, HITACHT, Japan).

2.4. Infrared spectroscopic measurements

Infrared spectroscopic measurements were performed on model Avatar 380 FTIR spectrometer (Thermo Nicolet Corporation, USA). The wavelength of FITR measurement range was controlled from 400 to 4,000 cm^{-1}.

2.5. Kinetic experiments

To study the adsorption kinetics of the MIPs, the dry kaempferol-MIP and NIP of 0.03 g were added into 10 ml ethanol solution with kaempferol concentration of 10 mg/l, respectively. Then the mixtures were stirred at 150 rpm and reacted at room temperature. The solutions of 2 ml were taken out from the conical flasks at different time intervals, such as 0, 1, 5, 15, 30, 60, 180, and 300 min. After the solutions were centrifuged at high speed for 15 min, the concentrations of kaempferol in supernatants were measured by a spectrophotometer under wavelength of 370 nm.

The adsorption amount of kaempferol-MIP and NIP to kaempferol was calculated according to the difference of kaempferol concentration before and after specific adsorption. The calculation formula is as follows.

$$\text{Adsorption capacity } Q = \frac{(C_0 - C_e) \times V}{W}$$

where, C_0 is the initial kaempferol concentration (mg/L); C_e is the equilibrium kaempferol concentration at different time intervals (mg/L); V is the volume of kaempferol solution (L), and W is the weight of dry polymers (g).

2.6. Isothermal adsorption experiments

In this process, equivalent portions of the kaempferol-MIP and NIP (0.03 g) were placed in eight conical flasks and filled by 10 ml kaempferol solutions with different concentrations ranging from 0 to 14 mg/L. Then, the conical flasks were shaken at 30℃ for 24 h. The equilibrium binding amount of kaempferol was determined using spectrophotometer according to the prestep experimental method.

3. Results and discussion

3.1. Polymer preparations

Precipitation polymerization has been widely used to prepare microspherical MIPs because it is relatively simple experimentally and easy to get microsphere with regular size and shape. Firstly, the template molecular and functional monomer react through hydrogen bonding, electrostatic bonding and van der Waals' forces interactions in a suitable porogen. Cross-linker is added to form a frozen spatial structure and enhance imprinted effect, so that MIPs will remain functional for long periods of time. Then template is removed from the synthesized polymer to achieve specific binding sites. The imprinted process is shown in Fig. 1. The specific recognition characteristic of MIPs is attributed to the specific binding sites in the polymeric network. Generally speaking, MAA is most commonly used as a functional monomer, which can form hydrogen bond with kaempferol. So the interaction between the template and the functional monomer is of great importance to achieve highly efficient polymers.

3.2. Infrared spectroscopic measurements

As is shown in Fig. 2, MIP and NIP have similar absorption peak. It reveals that joining the template molecule does not change the type and location of groups. While, the adsorption peak of 3,445 cm^{-1} is N–H structure, and the peak at 1,729 cm^{-1} is attributed to C=O stretching vibration absorbance. Moreover, the stretching vibration peak of 1,455 cm^{-1} is C–H_2 structure of MAA. Compared with NIP, the adsorption peak of N–H at 3,445 cm^{-1} is strong. This may be because acetonitrile without reaction remains in polymers and takes on strong peak of N–H. The stretching vibration of C–O is found at 1,155 cm^{-1}. Template molecules have been completely removed from synthesized polymers, so MIP has similar absorption peak intensity at 1,155 and 1,729 cm^{-1} with NIP. Consequently, specific binding sites are formed by ordered distribution of functional groups containing C=O. This result confirms that the kaempferol-MIP possesses cavities creating abundance functional groups, behaving selective properties.

3.3. Surface morphologies

After MIPs and NIPs were sieved through 74 μm sieve, they were observed by SEM. The SEM images

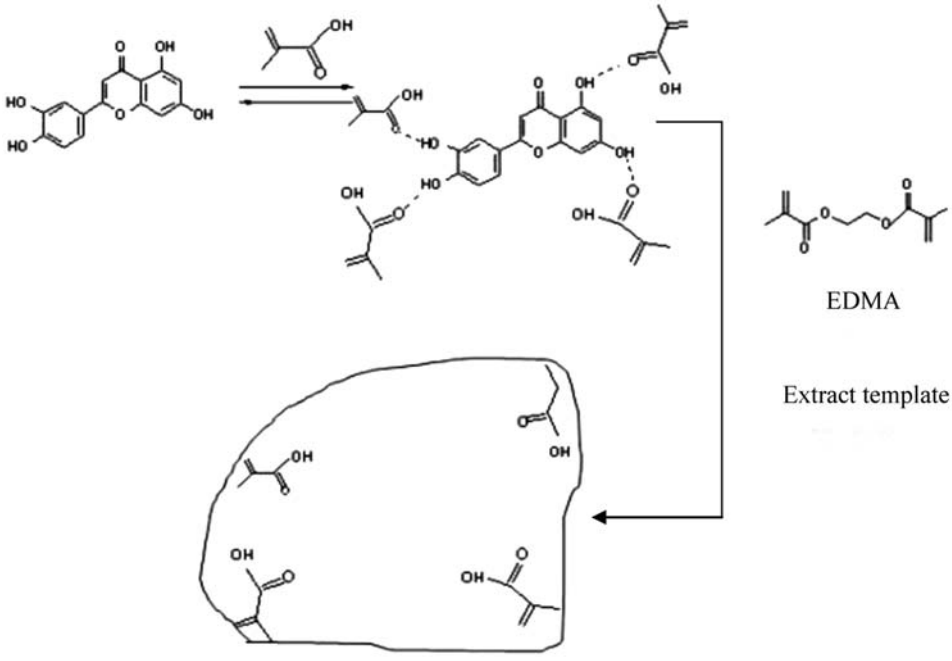

Fig. 1. Schematic representation of the kaempferol-MIP.

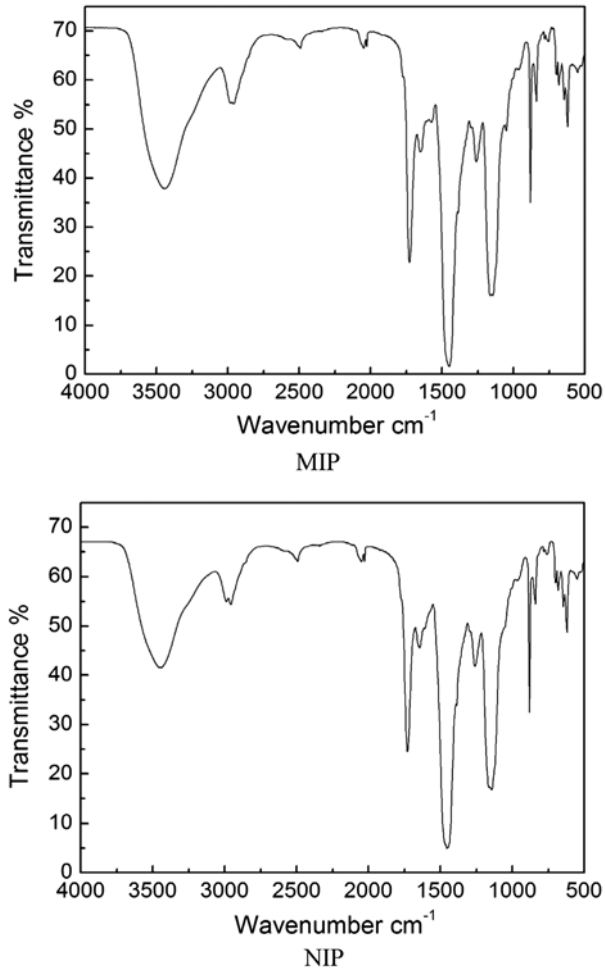

Fig. 2. FTIR spectra of kaempferol-MIP removal of the template molecule and NIP.

indicate the imprinted polymers are spherical morphology, and the diameter of MIP is smaller than NIP. This difference might be attributed to template–monomer complex packaged in the kaempferol-MIP matrix. Instability of the reaction conditions leads to the irregular size and shape of microsphere. As shown in Fig. 3, particles of MIP and NIP bond together, this may due to the hydrogen bonding and electrostatic interactions between particles. As removal of kaempferol from the polymers, the specific bonding sites are formed in the kaempferol-MIP, resulting in higher kaempferol adsorption performance.

3.4. Dynamic adsorption

The kinetic curves show that the specific adsorption capability of kaempferol-MIP increases from 517 to 1,361 μg/g with time extension. Also, adsorption easily reaches saturation in 60 min. In the early 30 min, the hyperadsorption rate is attributed to the massive specific binding sites on the surface of MIPs. While after 60 min, most of the recognition sites on the surface are occupied, so the adsorption almost reaches equilibrium. Although NIP shows similar trend to kaempferol-MIP on the kaempferol adsorption, the saturated adsorption amount of NIP, as shown in Fig. 4, is much lower (stable around 417 μg/g) than kaempferol-MIP. This might be attributed to the absence of template molecules in imprinting process and thereafter lacking suitable imprinting cavities in the NIP [13]. So NIP shows nonspecific adsorption for template molecules. Kaempferol-MIP shows a faster binding kinetics than polymers prepared by other traditional methods. This is attributed to the small and uniform size of MIPs microsphere, which leads the specific bonding sites more accessible for the template molecules. The presence of specific binding sites to the template molecules in the polymers is very important to the specific adsorption.

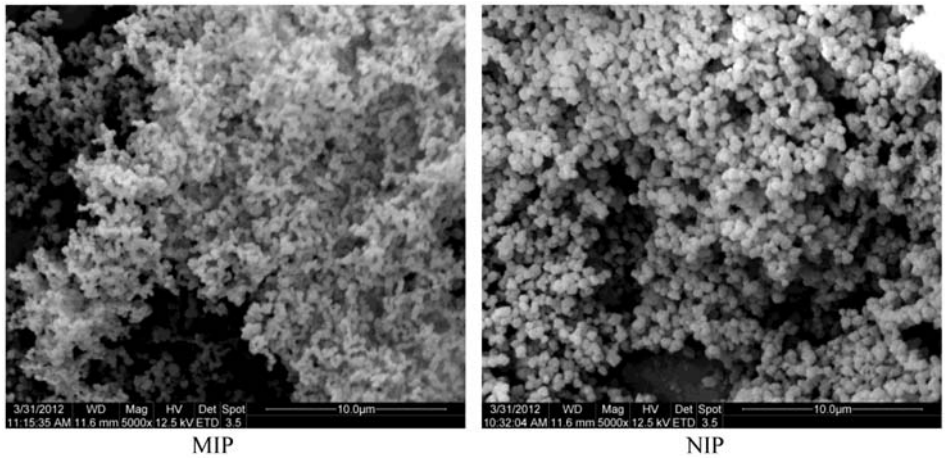

Fig. 3. Surface morphologies of kaempferol-MIP and NIP.

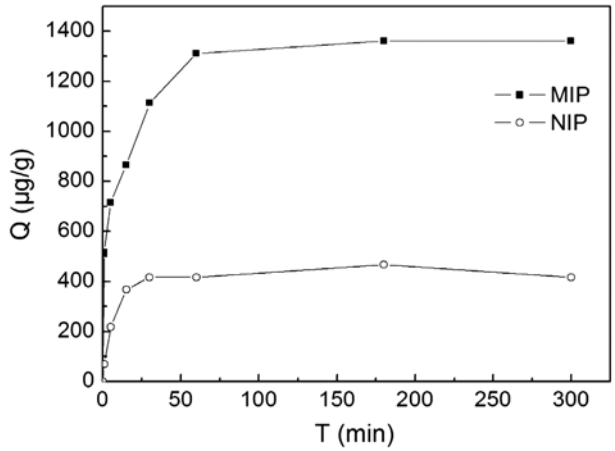

Fig. 4. Dynamic curves for the kaempferol adsorption onto kaempferol-MIP and NIP.

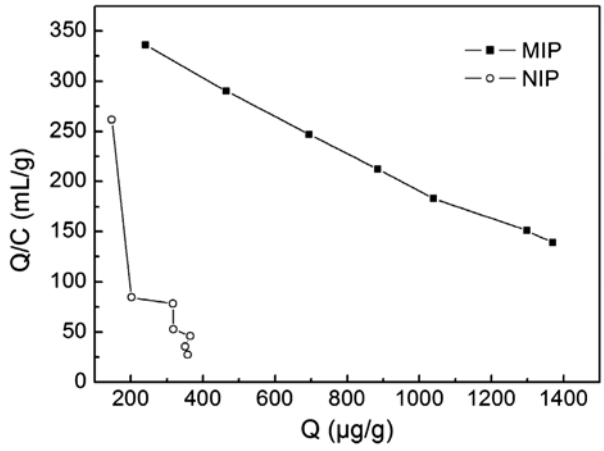

Fig. 6. Scatchard plots of kaempferol-MIP and NIP.

3.5. Adsorption isotherm

The adsorption amount of kaempferol-MIP, as shown in Fig. 5, increases from 241 to 1,370 μg/g with the increasing concentration of kaempferol. It is obvious that NIP shows similar trend to kaempferol-MIP. Comparing with kaempferol-MIP, the binding amount of kaempferol to NIP is much lower than that to MIPs.

Adsorption isotherms are usually used to describe how adsorbates interact with adsorbents [14]. So it is essential to analyze the correlation of equilibrium date by Scatchard model. The data of kaempferol-MIP and NIP obtained from adsorption isothermal experiments are plotted according to the Scatchard equation as follows [15]:

$$\frac{Q}{C_e} = \frac{Q_{max} - Q}{K_D}$$

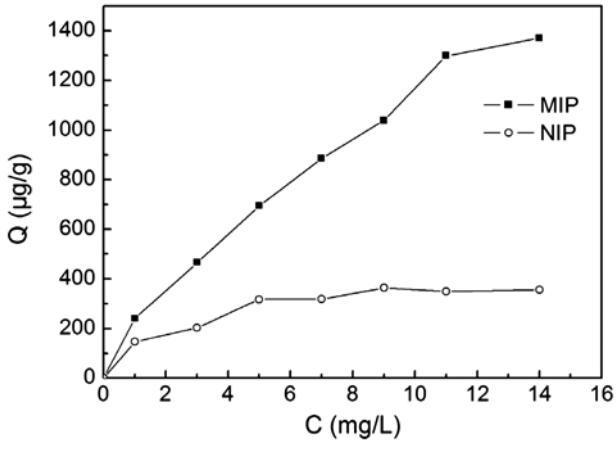

Fig. 5. Adsorption isotherm of kaempferol-MIP and NIP.

where, K_D is the equilibrium dissociation constant of the binding sites (mg/L); Q is the amount of template molecules bound to the polymer (μg/g); Q_{max} is the apparent maximal combination amount of binding sites (μg/g); and C is the equilibrium concentration of template molecules in solution (mg/L).

As shown in Fig. 6, the relation curve of kaempferol-MIP between Q/C and Q is almost straight, and the linear regression equation is as follows: $Q/C = -0.1733Q + 370.96$ ($R^2 = 0.9935$). According to the slope (–0.1733 ($1/K$)) and intercept (370.96 (Q_{max}/K_d)), Q_{max} and K_d are calculated to be 2.14 and 5.77 mg/L, respectively. This reveals that a series of specific binding sites for kaempferol are formed in kaempferol-MIP, and these sites mainly rely on hydrogen bonding. However, the Scatchard curve of NIP shows correlation of 0.7577, which reveals the adsorption of NIP to kaempferol belongs to nonselective adsorption. Also, van der Waals and electrostatic interaction play the major role in the adsorption of NIP.

4. Conclusions

This study mainly introduced the performance of kaempferol-MIP that was prepared by precipitation polymerization with MAA as functional monomer. The polymer particles show excellent uniform microsphere and the precipitation polymerization is proved to be a feasible method for preparation spherical MIPs. Moreover, the kaempferol-MIP exhibits high selectivity and recognition ability to template molecules, and the maximum adsorption amount reaches 2.14 mg/g. The Scatchard curves of kaempferol-MIP and NIP reveals that kaempferol-MIP possesses homogeneous binding sites for kaempferol, and that the NIP to kaempferol belongs to nonselective adsorption.

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