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Preparation, recognition characteristics and properties for quercetin molecularly imprinted polymers

Lanzhe Yu[a], Yanbin Yun[a,*], Weijun Zhang[a], Lihua Wang[b]

[a]*College of Environmental Science & Engineering, Beijing Forestry University, Beijing, China*
Tel. +86-132 4173 0890; email: y.yanbin@unsw.edu.au
[b]*Laboratory of New Materials, Institute of Chemistry, Chinese Academy of Sciences, Beijing, China*

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ABSTRACT

Molecular imprinting polymer (MIP) is a leading edge approach to extract quercetin (an important active ingredient of gingko). The preparation of MIP involved bulk polymerization using quercetin as the template molecule, acrylamide as the functional monomer, ethylene glycol dimethacrylate as the cross-linker in the porogens of chloroform and N,N-dimethylformamide. Afterwards, the synthesized MIP and non-imprinted polymer (NIP) were characterized by FTIR, SEM and BET. In addition, the obtained polymers were evaluated by adsorption isotherms and dynamic curves with respect to their selective recognition properties for quercetin. The experimental results showed that (1) there are quite differences between MIP and NIP in the aspects of surface morphologies, specific surface area and certain functional groups; (2) the quercetin-MIP exhibited a higher affinity for quercetin than the NIP; (3) a class of homogeneous recognition sites were formed in quercetin-MIP within the range of certain concentration by Scatchard analysis.

Keywords: Molecular imprinting; Flavonoids; Quercetin; Isothermal adsorption; Adsorption dynamics; Scatchard model

1. Introduction

Molecular imprinting technique (MIT) has emerged as an attractive synthetic approach to construct a polymer matrix with molecular recognition sites, which was introduced in 1972 by Wulff and Sarhan [1]. The molecular imprinting polymers (MIPs) involve co-polymerization of functional monomer molecules with cross-linking agents in the presence of template molecules to create three-dimensional network polymers. Removal of the template molecules yields vacant recognition sites complementing both chemically and sterically to the imprinting molecule structure. These recognition sites enable imprinted polymers to be the mimics of enzymes, receptors and antibodies for screening various kinds of compounds from a mixture with abundant interferences [2].

Quercetin (3,3,4,5,7-penta-hydroxy flavone), a typical active compound of the flavonoids family, is widely distributed in plants. Owning to quercetin's various bioactivities such as inoxidability, antiviral property, antitumor and adjusting immune function [3], considerable researches have been performed toward the sorption materials. MIP, however, could be potentially used as separation materials based on such features as the shape, size and functionality selectivity, strong affinity on rebinding target compounds, the relatively low cost for the preparation and the workability in organic solvents [4,5].

Up to now, many researches have been carried out to the MIT in order to achieve the selective interaction with flavonoids. Xie [6] developed non-covalent imprinting

*Corresponding author.

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and bulk polymerization based on quercetin to efficiently extract quercetin and kaempferol from the hydrolyzate of Gingko leaves, and demonstrated there was the feasibility of extraction of certain pharmacologically active constituents from plants by MIP. Employing the former MIP, the research group carried out the separation of two inhibitors of epidermal growth factor, namely butein and E-picetannol, from Caragana jubata [7]. They also utilized 2,2 - bis (hydroxymethyl) - butanol trimethacrylate (TRIM) as the cross-linker, polymerizing quercetin MIP to selectively extract template molecules from the plasma of rats fed the hydrolyzed extract of Gingko biloba L [2].

In this work, a selective and affinitive MIP was prepared for quercetin using a non-covalent imprinting approach. Characteristics of the polymer were evaluated by scanning electron microscope (SEM), Fourier transform infrared (FTIR) and BET. Scatchard method was used to assess the adsorption properties and recognition mechanism of quercetin-MIP.

2. Experimental

2.1. Materials

Quercetin was purchased from Beijing Chemical Works. Acrylamide (AM) and ethylene glycol dimethacrylate (EDMA) were supplied by Sigma-Aldrich (Milwaukee, WI, USA). 2.2-azobisisobutyronitrile (AIBN) was obtained from Shanghai Chemical Reagent Company. Methanol (MeOH), ethanol (EtOH), acetic acid and N,N-dimethylfomamide (DMF) were analytical reagents and purchased from Sinopharm Chemical Reagent Beijing Co., Ltd.

2.2. Polymer preparation

The template molecule quercetin (1 mmol) and the functional monomer AM (4 mmol) were dissolved in 5 ml of chloroform and 1.5 ml of DMF. After stirring for one hour, 20 mmol of cross-linker EDMA and 0.041 g initiator AIBN were added. The mixture was then polymerized by thermally initiation at 60°C oil bath under a nitrogen atmosphere for 24 h. The resulting polymer was grinded and sieved through 74 μm sieve. The particles were extracted with a mixture of MeOH / acetic acid (9:1, v/v) in Soxhlex extractor for 72 h to remove quercetin and rinsed with MeOH. Then, the MIPs were dried at 60°C under vacuum for 24 h. As a control experiment, non-imprinted polymer (NIP) in the absence of quercetin during the polymerization was also prepared and treated in the identical manner.

2.3. The morphologies of the quercetin-MIP and NIP

The surface morphologies of the polymers were observed using SEM (S-3400N, HITACHT, Japan). The quercetin-MIP and NIP were dried under vacuum prior to be coated with gold layer.

2.4. BET surface measurement

In order to remove adsorbed gases and moisture, the given amount of quercetin-MIP and NIP (0.14–0.15 g) were degassed at 200°C for a period of 3 h prior to analysis. A surface area and porosity analyzer (micromeritics ASAP 2020) was utilized for BET studies, determining polymer surface areas from multi-point N_2 adsorption isotherms.

2.5. FTIR measurements

FTIR spectroscopic measurements were performed on model Avatar 380 FTIR spectrometer (Thermo Nicolet Corporation, USA). The wave numbers of FITR measurement range were controlled from 500 to 4000 cm^{-1}, and collected at one data point per 2 cm^{-1}.

2.6. Kinetic experiments

To investigate the adsorption dynamics of the MIPs, the dry quercetin-MIP and NIP of 0.3 g were placed in 1 l conical flasks, respectively, and mixed with 1 l of 6.4 mg/l quercetin in ethanol solution. Then two conical flasks were oscillated at 40°C by SHZ-82 constant-temperature shaker (Guohua Instrument Factory, Jiangsu, China). The solutions of 2 ml were taken out from the conical flasks at different time intervals, such as 1 min, 3 min, 5 min, 15 min, 30 min, 1 h, 3 h, 5 h. After 15 min of high-speed centrifugation, the concentrations of quercetin in supernatants were determined by a spectrophotometer under wavelength of 370 nm.

According to the difference of quercetin concentration before and after specific adsorption, the binding amount of the polymers to quercetin was calculated as follows:

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

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

2.7. Isothermal adsorption experiments

In this process, 7 equivalent portions of the quercetin-MIP (50 mg) were placed in 100 ml conical flasks and filled by 50 ml quercetin solutions with different concentrations of 2–14 mg/l. Conical flasks were oscillated with constant temperature bath at 40°C for 5 h. The equilibrium binding capacity of quercetin was determined using spectrophotometer in accordance to the pre-step experimental method.

3. Results and discussion

3.1. Polymer preparations

In most of flavonoid compounds MIP synthesis, bulk polymerization is often considered as an effective approach. It involves thermal initiation, which AIBN is used as free radical initiator. Because the half-life of AIBN is around 10 h at 60°C, it is proper for the polymerization of MIP. EDMA, acting as cross-linker, was used to form a frozen spatial structure and enhanced the imprinted effect. Prior to polymerization, the mixture was thoroughly deoxidized in case of free-radical reaction quenching [8].

There are two vital influence factors of MIP recognizability: one is steric memory (size and shape of binding cavern), and the other is chemical memory (spatial arrangement of complementary functionality), both of which are correlative to the template-monomer interaction and porogens polarity. The porogens generally act as dispersion media and pore forming agents in the polymerization process [9]. It is generally believed that less polar solvent is desirable because it could not disturb the non-covalent cohesions, for instance, hydrogen-bonding interaction, between templates and functional monomers. However, another non-covalent interaction, such as hydrophobic interaction, electrostatic (ionic) interactions and π-π stacking effects [10], may be exploited in polar media and facilitated to binding sites cohesions. This experiment applied both lower polar solvent (5 ml of chloroform) and higher polar solvent (1.5 ml of DMF) as porogens to generate strong binding action and hole reaming effect.

The chemical compound AM is chosen as functional monomer because it is favorable for amino-group associating with templates in polar environment [11]. While, quercetin molecule contained a carbonyl group and five phenolic hydroxyl groups, forming strong hydrogen-bonding interaction with amino-group of AM. Thus, a stable receptor-donor combination between template and functional monomer was formed in the imprinted process as shown in Fig. 1.

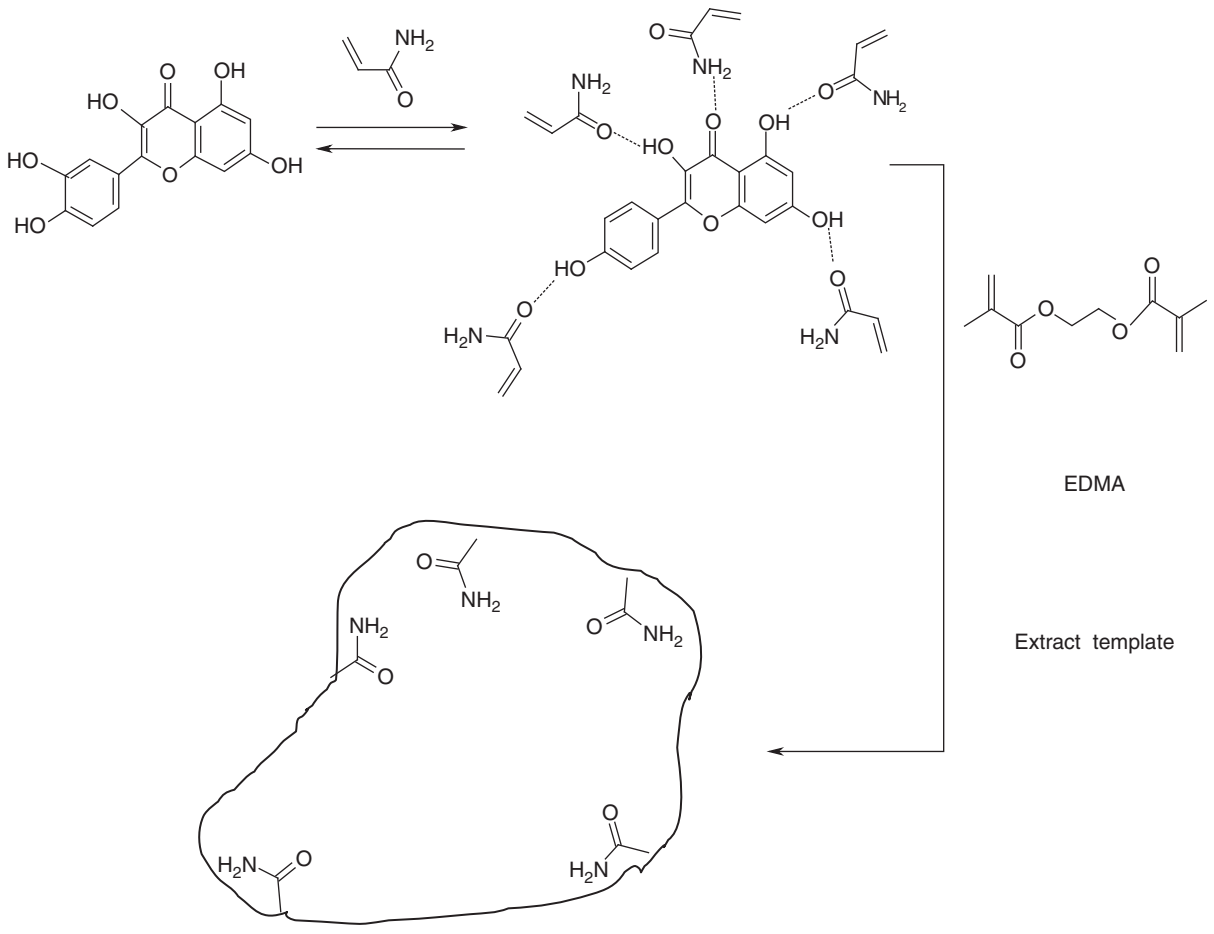


Fig. 1. Schematic representation of the quercetin-MIP.

3.2. FTIR analysis

To ensure the interaction among quercetin, AM, and EDMA, FTIR analysis was performed. As Fig. 2 (a) shows, the peak at 1729 cm^{-1} is attributed to C=O stretching vibration absorbance. Due to quercetin-MIP synthesized by the polymerized substance of AM and EDMA, the cross-linker may carry the repeated cross-linking units of EDMA. It exists a weak absorbance peak of C=C at 1637 cm^{-1}. This proves that a majority of AM takes part in the reaction, few ones remain stable. The broad peak from 3435 to 3449 cm^{-1} is assigned to the asymmetric stretching vibration of N–H in AM. Moreover, the stretching vibration peak of 1455 cm^{-1} is C–H_2 structure of AM, and C–O stretching vibration of asymmetric ester is found at 1155 cm^{-1}. For NIP shown in Fig. 2 (b), the characteristic signals are similar to quercetin-MIP. However, there are two different points: first, the intensity of the C=O (1729 cm^{-1}) stretching vibration peak is lower than that of quercetin-MIP; second, the strength of N–H (3435~ 3449cm^{-1}) stretching vibration peak is higher than that of quercetin-MIP. There are probably two reasons: the one is the polymerization of NIP being devoid of quercetin, leading AM remained and taking on strong peak of N–H; the other is that the quercetin, assembled with AM by hydrogen-bonded interaction, which is essential to the AM and EDMA co-polymerization. Consequently, stable imprinting cavities were formed by ordered distribution of functional groups containing C=O. This result confirmed that the quercetin-MIP possessed cavities creating abundance functional groups, behaving selective capabilities.

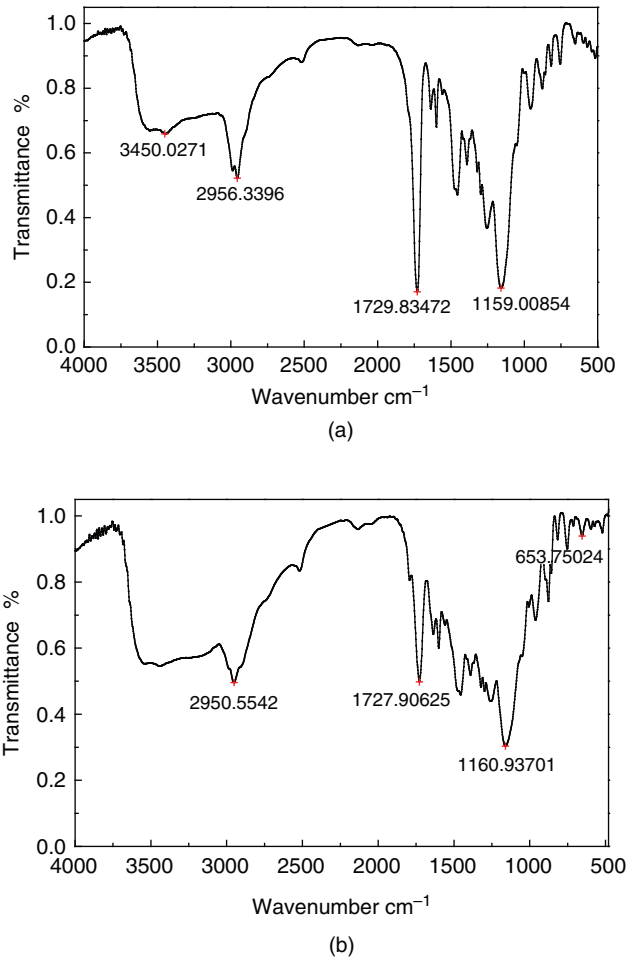


Fig. 2. FTIR spectra of quercetin-MIP removal of the template molecule (a) and NIP (b).

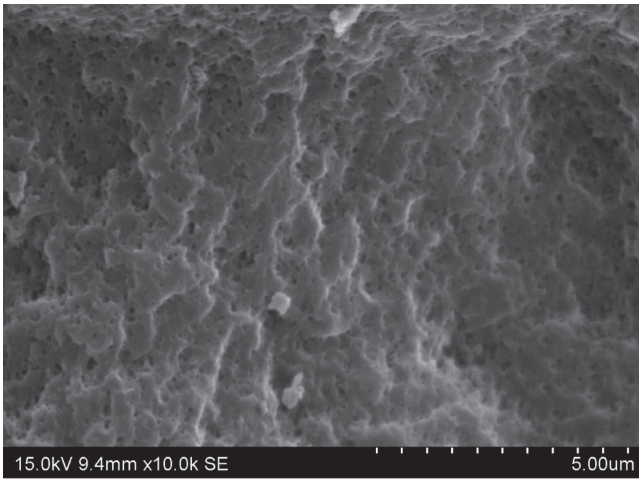
3.3. Surface morphologies

Comparing the images of quercetin-MIP and NIP in Fig. 3, the surface morphologies are quite different: though both have rough surfaces, the quercetin-MIP seems to be much denser with homogenous and well-distributed pores, as shown in Fig. 3(a). This difference may due to template-monomer complex packaged in the quercetin-MIP matrix. When removal of quercetin from the polymers, the structure of pore channels are formed in the quercetin-MIP network, which is obviously favorable for the template molecules transference and mass embedding, resulting in higher quercetin adsorption performance.

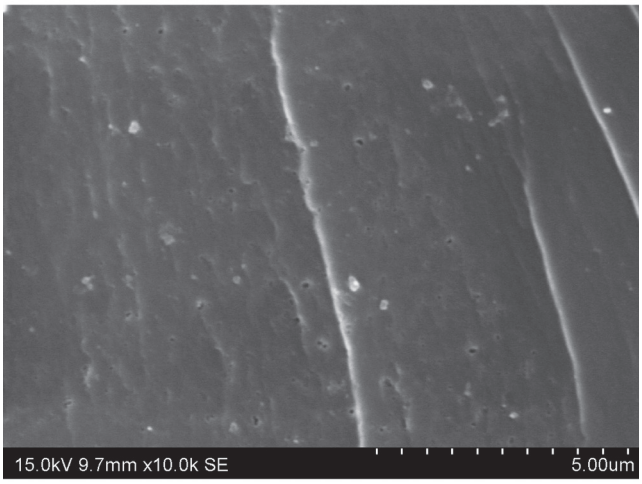
To further investigate the polymer's adsorption property, the surface areas of quercetin-MIP and NIP have been obtained via the evaluation of BET adsorption isotherms. The surface area of quercetin-MIP is 285.35 m^2/g compared to 213.01 m^2/g for the NIP. The result shows that the polymerization between imprinted and non-imprinted mixture exhibits significant differences in surface area. To some extent, the higher adsorptive capacity of quercetin-MIP is a consequence of increased overall surface area.

3.4. Dynamic adsorption on quercetin-MIP and NIP

Fig. 4 shows the kinetic curves for the quercetin adsorption onto quercetin-MIP and NIP. The kinetic curve in Fig. 4(b) indicates that the specific adsorption increased quickly and easily reached saturation in a short period of time (about 15 min), but the saturated adsorption amount is lower (stable around 236 μg/g). While the adsorption capability of quercetin-MIP, as shown in Fig. 4(a), increases from 55 to 700 μg/g with time extension and nearly reaches saturation state within 3 h. The massive recognition sites on the surface of MIPs lead to hyper-adsorption rate within 30 min. When the imprinted sites on the surface are occupied, it becomes difficult for quercetin to implant into quercetin-MIP. This may cause the adsorption to slow down. The various contributions of polymers on the template molecular recognition would be attributed



(a)



(b)

Fig. 3. Surface morphologies of quercetin-MIP (a) and NIP(b).

to the hydrogen bonds between template and monomer, and the comprehensive functions of resultant micro-environment and complementary cavity to the template molecules [12]. Although NIP presents similar trend to quercetin-MIP on the quercetin adsorption, the adsorptive capacity is much lower. This might be likely because of the non-specific adsorption of NIP caused by van der Waals force, due to the absence of imprinting procedure and thereafter lacking suitable recognition sites and imprinting cavities in the NIP [7]. However, Quercetin-MIP possesses numerous and precise binding sites, leading to specific adsorption. Evidently, the presence of complementary cavities to the template molecules in the polymers is vital for the specific adsorption.

3.5. Adsorption isotherm

The adsorption isotherm experiments were conducted in a series of quercetin ethanol solutions with different initial concentrations. It is shown in the Fig. 5 that the adsorption capacity increases from 267 to 1080 μg/g by the increasing initial concentration of quercetin from 2 to 14 mg/l. Nevertheless, NIP takes on the similar trend as quercetin-MIP, with adsorption amounts stable around 603 μg/g. Adsorption isotherms are important for describing how adsorbates will interact with adsorbents [13]. Thus, the correlation of equilibrium data analyzing by mathematical model is essential to practice adsorptive operation.

In studying the molecular imprinting technique, the Scatchard model is often employed to further analyze the binding isotherms, which reveals that the homogeneous binding sites form in the polymers. The data of quercetin-MIP and NIP obtained from adsorption isothermal experiments are plotted according to the Scatchard equation as follows [14]:

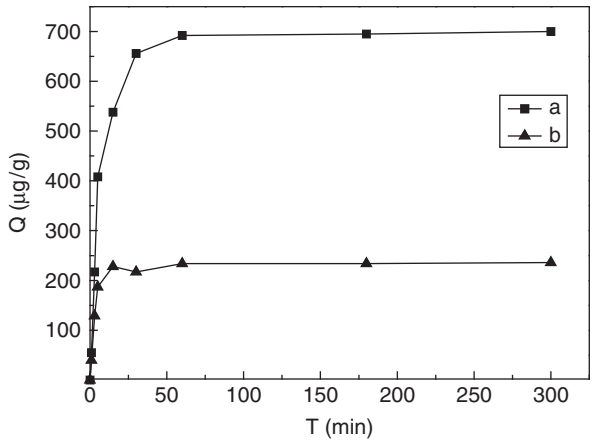


Fig. 4. Dynamic curves for the quercetin adsorption onto quercetin-MIP (a) and NIP (b).

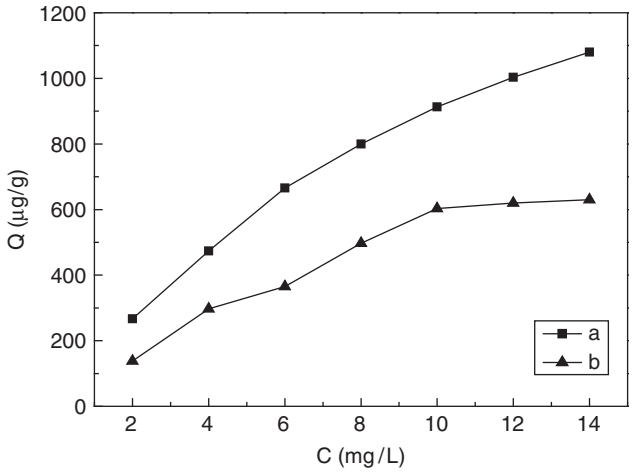


Fig. 5. Adsorption isotherm of quercetin-MIP (a) and NIP (b).

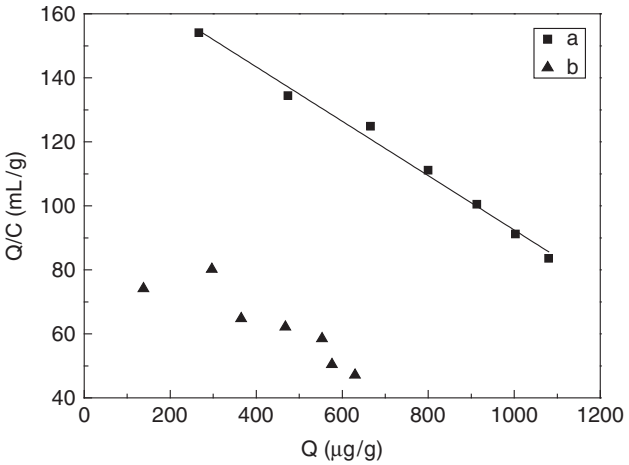


Fig. 6. Scatchard plots of quercetin-MIP (a) and NIP (b).

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

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).

Fig. 6 shows the Scatchard plots of the quercetin bond to the quercetin-MIP and NIP, respectively. It is clear that the relation curve between Q/C and Q becomes almost straight, the linear regression equation is, $Q/C = -0.085Q + 177.42$ ($R^2 = 0.9914$). According to the slope ($-0.085(1/K_d)$) and intercept ($177.42 (Q_{max}/K_d)$), Q_{max} and K_d are calculated to be 2.086 mg/g and 11.76 mg/l, respectively. This suggests that uniform binding sites for quercetin are formed in quercetin-MIP. In NIP, however, there are no equivalent selective sites for quercetin because the non-linear relationship. From the Scatchard model, it could be assumed that quercetin-MIP rebinding sites are mainly hydrogen bonding, while as for the NIP, van der Waals and electrostatic attraction are the major role for binding interaction.

4. Conclusions

Important aspects of this work included the utilization of acrylamide as functional monomer and combined polar solvents as porogen in bulk polymerization. Furthermore, the quercetin-imprinted polymers exhibit strong affinities to template molecules, such as fast adsorption dynamics and high adsorption capacity. The maximum adsorption capacity reached 2.086 mg/g. The fitted curve of Scatchard model revealed that quercetin-imprinted polymers possessed homogeneous binding sites for quercetin.

The molecular imprinting technique is still in a developing stage, and the performance of MIP may be further improved. Such as, employing novel cross linkers or functional monomers, inventing other synthetic methods are new channels of exploration to extract structurally similar component from plants.

Acknowledgements

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