

STUDY ON PREPARATION OF A NOVEL SILICA ADSORBENT AND ITS SELECTIVE SEPARATION APPLIED TO GENISTEIN

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Abstract - In order to selectively separate genistein from its crude solution, a novel silica adsorbent was prepared using genistein as the template molecule, γ -aminopropyltriethoxysilane as the functional monomer and tetraethyl orthosilicate as the cross-linker. It was analyzed by the BET, FTIR, TEM characteristics, the static adsorption experiment, and selective adsorption experiment. The results show that the silica adsorbent has high specific area, special selectivity and high adsorption capacity for genistein. It can recognize and bind genistein in aqueous solution, and lots of nanopores distribute on its surface uniformly. It is concluded that both the shape and size of the recognition sites matching the template molecule result in the performance of this adsorbent to bind genistein and that there are strong ionic and hydrogen bond attractions between the phenolic hydroxyl and the $-NH_2$.

Keywords: Silica adsorbent; Selective separation, Genistein.

INTRODUCTION

Genistein has a special anti-cancer and anti-oxidation properties among all kinds of soybean isoflavones, so it is broadly used in healthy food and its supplements. Common methods to extract it from crude solution include organic solvent extraction, macroporous resin adsorption, super critical fluid extraction, and microwave treatment. These processes have lots of drawbacks such as long extraction times, large quantities of solvents, high investment or complex operations. Especially, these methods can only separate isoflavones, but not the isolate component. Recently the molecular imprinting technique (MIT) has brought a great advantage to selective separation, while composite materials with nanometer and molecular level size show unique chemical and physical properties which

are different from macrosized level composites. For example, the theophyllin (THO) and caffeine (CAF) are extracted by the MIP film (molecular imprinted polymer film) and Xie Jianchun obtains the active compounds from the herbs using the same technology. If the adsorbent has a great deal of binding sites with molecular recognition function, it will achieve the objective to obtain the genistein among the isoflavones. If the diameter of the pores existing on the surface of the adsorbent is at the nanometer level, the adsorption performance will be improved. But the recognizing and binding processes are usually accomplished in non-polar solution and the preparation stuffs sometimes are organic materials. This means high cost and complex after-treatment. The purpose of this paper is to prepare a novel adsorbent which can recognize and bind genistein in the polar solution even in aqueous

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solution, so the cost will decrease and the efficiency will be enhanced, besides, our research team has provided a foundation for its accomplishment.

EXPERIMENTAL

Materials

The genistein was purchased from North China Pharmaceutical Group Corporation. The Rutin was gotten from Xi An HAUQI Co. Ltd. The tetraethyl ortho-silicate (TEOS) was gained from No.1 Reagent Chemical Factory, Tianjin, and was distilled and stored under nitrogen.

The adsorbents were characterized with the transmission electron microscope (TEM, Tecnai.G2.F20. FEI Corporation. Holland), fourier transform infrared spectrometer (FTIR, MAGNA. IR560. Nicolet Corporation. America), the specific area analyzer (BET, NOVA-2000. Quantachrome Corporation. American).

Adsorbent Preparation

0.1g genistein and 10ml γ -aminopropyltriethoxysilane were placed into a 150ml flask to prepare the precursor under slight agitation, then 20ml TEOS and 30ml H₂O were added to the mixture. After hydrolysis reaction and polymerization, a silica solid mixture appeared. Following filtration, the filter cake was dried at 105 °C. Finally the materials were extracted in acetone/water solution for 6h, and the novel silica adsorbent was obtained. For comparison, a blank adsorbent was prepared (without template molecule) under the same conditions. In this context, they were called silica adsorbent and blank adsorbent respectively.

The Adsorption Experiment in Static State

1g silica adsorbent was placed into 50ml genistein solution under different temperatures. Its concentration was 0.5mg/ml genistein in 30% ethanol aqueous solution. In this experiment, the concentration of genistein was analyzed by high performance liquid chromatography (HPLC) at the

fixed interval. The amount adsorbed can be calculated by formula (1). The same test was carried on blank adsorbent.

$$Q_e = \frac{(C_o - C_e) \times V}{m} \quad (1)$$

Q_e : the amount adsorbed, mg/g; C_o : the initial concentration, mg/ml;

C_e : the equilibrium concentration, mg/ml;

V : the solution volume, ml;

m : the quantity of adsorbent, g

Adsorption Dynamics of Genistein on the Silica Adsorbent

The adsorption experiment was repeated at different concentrations from 0.1~0.8mg/ml at 70 °C, and the same liquid volume of 50ml. The adsorption capacity was calculated by formula (1). The adsorption behaviors can be described with the Langmuir adsorption equation (Formula (2)).

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{\max}} + 1/bQ_{\max} \quad (2)$$

C_e : the equilibrium concentration, mg/ml;

Q_e : the equilibrium adsorption capacity, mg/g;

Q_{\max} : the max adsorption capacity, mg/g;

b : the Langmuir adsorption equilibrium constant, g/ml.

The Selectivity of the Adsorbent

Rutin is another natural material. Its structure is similar to genistein as can be seen from Fig.1. It was placed into the same solvent of the former experiment. The volume and concentration were 50ml and 0.5mg/ml respectively. The adsorption experiment was repeated and the temperature kept at 70 °C, and the capacity was calculated with formula (1).

The Characterization of the Silica Adsorbent

Advanced instruments were helpful for deep researches of the adsorbent. The BET, FTIR and TEM tests showed the adsorbent inner structure.

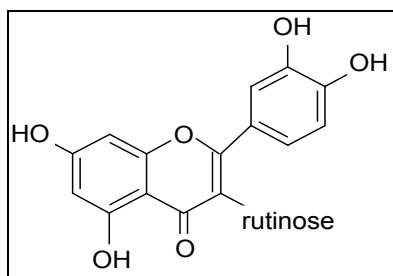


Figure 1: Molecular frame of rutin

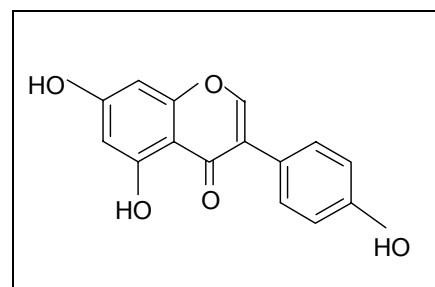


Figure 2: Molecular frame of genistein

RESULTS AND DISCUSSIONS

Amount of Genistein Adsorbed

Temperature is an important factor for genistein adsorption (Fig.3). 1g silica adsorbent is placed into 50ml of 0.5mg/ml. genistein solution. The experiment was fulfilled at different temperatures. When the temperature rises, the amount adsorbed increases. At 70 °C the amount adsorbed reached 10.8mg/g, which is higher than that at 50 °C and 90 °C, and the time to saturation was only 3h. This is due to the fact that the velocity of adsorption and desorption are very slow at low temperatures, so equilibrium is not reached and the adsorption is limited. When temperature rises, the adsorption is enhanced. But desorption process is also expanded, so very high temperatures will prejudice adsorption.

It can be concluded that this adsorbent can recognize and bind genistein in aqueous solution and 70 °C is the most favorable temperature.

The Adsorption Isotherms

In order to investigate the adsorption performance of the silica adsorbent, 1g silica adsorbent was placed into 50ml genistein solution and the experiment fulfilled at 70 °C, then the adsorption isotherm (Fig.4) was drawn with an initial concentration of genistein solution changing from 0.1 to 0.8mg/ml. Among this range, the data were regressed by Langmuir adsorption equation. Ultimately, the Langmuir equation is $\frac{C_e}{Q_e} = 0.00664 + 0.07251 C_e$, $R = 0.99961$. The new Q_e formed line is shown in Fig.5. The slope and intercept are equal to Q_{max} and $1/b Q_{max}$ respectively, and b and Q_{max} were calculated to be 10.92 and 13.79mg/ml. This means that the adsorption is consisted with the Langmuir adsorption theory. The reason is that the template molecules on the surface can be removed in the extraction process and one recognition site only binds one template molecule, so the adsorption must be a monolayer adsorption and must always happen on the adsorbent surface.

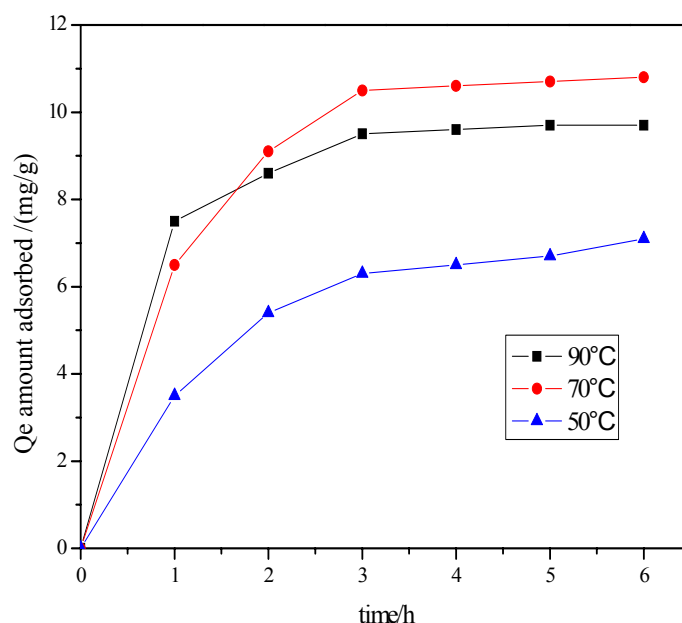


Figure 3: Amount adsorbed with time

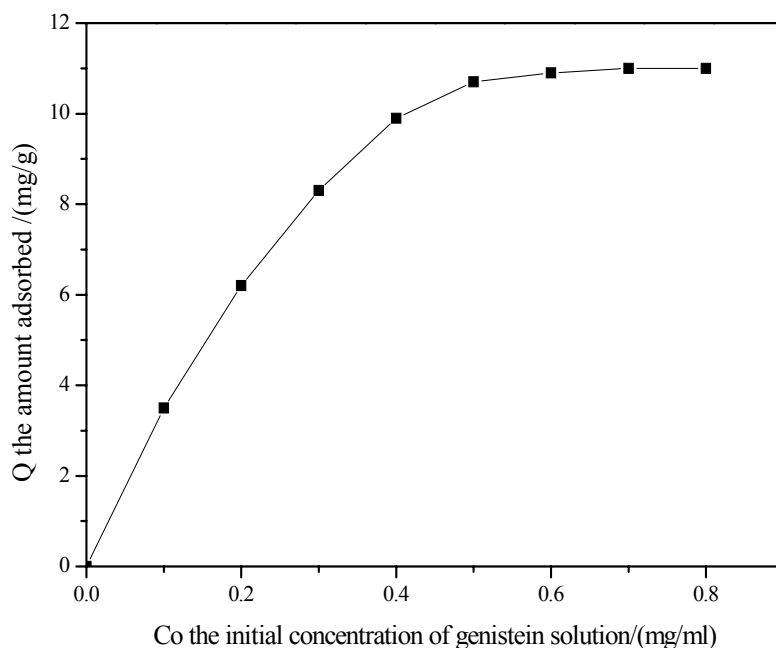


Figure 4: Curve of adsorption equilibrium under 70 °C

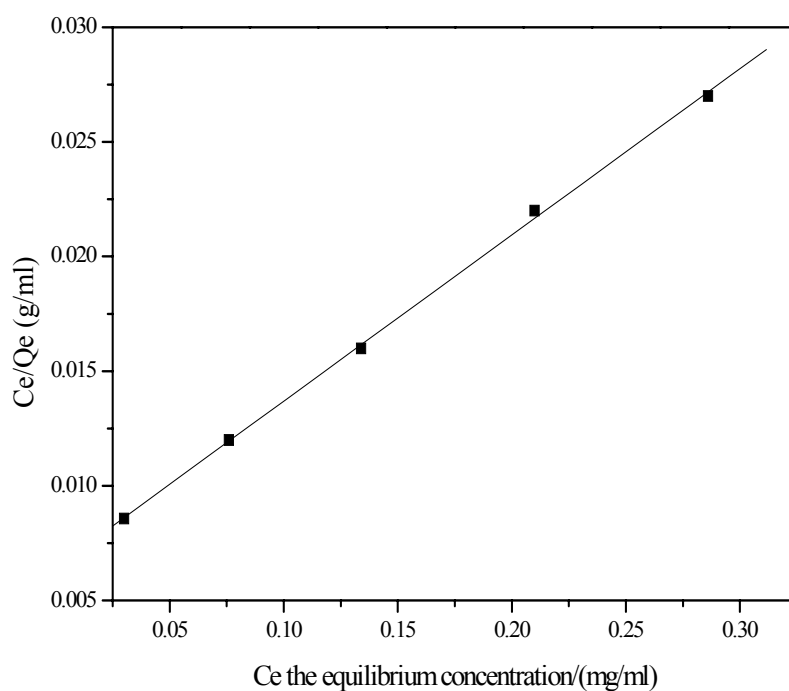


Figure 5: Adsorption isotherm according to the Langmuir equation

The Selectivity to Genistein

There is a big difference of adsorption capacity between the silica adsorbent and its blank counterpart when 1g silica adsorbent and 1g the blank adsorbent are put into 50ml genistein solution

respectively, whose concentration is 0.5mg/ml. The experiment was done at 70 °C. The figures in Table 1 show that the specific area of silica adsorbent is 201m²/g, which is much higher than that of the blank adsorbent. So it can bind more template molecule, as was verified in the adsorption experiment. The

adsorption capacity of silica adsorbent is more than four times greater than that of the blank adsorbent. So the silica adsorbent has a stronger affinity for the template.

The selective experiment was done at 70 °C. 1g silica adsorbent was placed into 50ml solution containing 0.5mg/ml genistein and 0.5mg/ml rutin. The experiment indicates that the silica adsorbent has a higher selective effect as can be seen in Table 2.

The equilibrium adsorption capacity for rutin was only 1.1mg/g, while the capacity for genistein reached to 9.3mg/g at the same conditions. As for the blank adsorbent, the final adsorption capacity for rutin and genistein were nearly equal, which were 1.03mg/g and 1.19mg/g respectively, so the blank adsorbent has no selectivity to genistein. The adsorption capacities of both adsorbents were a little inferior in the selective experiment when compared to the adsorption experiment, because there is a competition with rutin, which can take up some adsorption sites, so the capacity decreases.

The Characteristics of FTIR and TEM

Comparing the two lines in Fig.6, the peak of hydroxyl at 3400.3cm^{-1} obviously weakens. This proves that there is a strong ionic and hydrogen bond attractions between the phenolic hydroxyl and the $-\text{NH}_2$ of the functional groups. There is a new wide adsorption band at 1035.6cm^{-1} , which means that the Si-O-Si bond is formed.

The transmission electron microscope (TEM) is used to visualize the surface of the silica adsorbent, and the pores can be easily seen in Fig.7 as white dots in the picture.

Their diameter is about 30nm, and it remarkably expands the specific surface and the porosity of the adsorbent, contributing to the adsorption capacity of the adsorbent. The recognition sites exist in these pores, whose shape and size match the template molecule, so the molecular recognition occurs between the recognition sites and the template molecule by ionic and hydrogen bond attractions.

Table 1: Specific area and adsorption capacity of the silica adsorbent and the blank adsorbent

Material	Specific area (m^2/g)	Adsorption capacity(mg/g)
Silica adsorbent	201	10.8
Blank adsorbent	125	2.5

Table 2: Result of the selective experiment

Component	The initial concentration (mg/ml)	The adsorption capacity(mg/g)
Genistein	0.5	9.3
Rutin	0.5	1.1

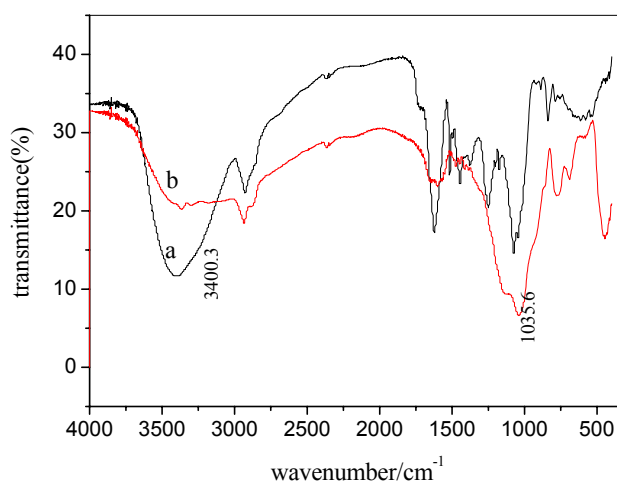


Figure 6: FTIR spectra of the silica adsorbent and the genistein (a: the genistein; b: the silicon adsorbent)

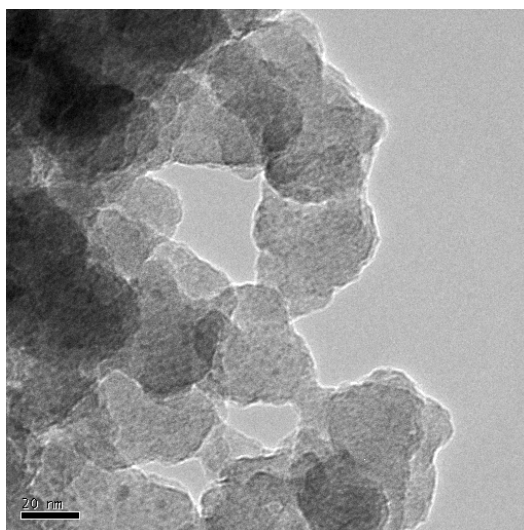


Figure 7: TEM image of the silica adsorbent

CONCLUSIONS

The silica adsorbent can be prepared in aqueous solution and the recognition and affinity processes can also be accomplished in water solution, so it is a great improvement to traditional molecular imprinting technique. This novel adsorbent has a special selectivity to genistein which can reach more than 4 times that of the blank counterpart, and lots of nanopores distribute on the surface of the silica adsorbent, which can help to enhance the adsorption capacity. The adsorption is consistent with Langmuir adsorption theory. So it is promising for selective separation of genistein.

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