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Synthesis and characterization of superparamagnetic poly(urea-formaldehyde) adsorbents and their use for adsorption of flavonoids from *Glycyrrhiza uralensis* Fisch

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Abstract Superparamagnetic spherical poly(urea-formaldehyde) (PUF) adsorbents were synthesized and their selective adsorption for licorice flavonoids was investigated in this paper. The magnetite (Fe_3O_4) nanoparticles were prepared by co-precipitation of ferrous and ferric salts. Then the magnetic adsorbents with PUF shell were synthesized by reversed phase suspension polymerization. The spherical adsorbents have an average diameter of 50 μm and exhibit superparamagnetic characteristics. The saturation magnetization of the adsorbents was 15.1 emu/g. The sorption and desorption properties of licorice flavonoids on the adsorbents were studied. The result shows that the adsorbents have high adsorption capacity (about 16.7 mg/g (adsorbent)). The adsorption data of flavonoids generally obeys the Langmuir isotherm equation. The adsorption can reach equilibrium rapidly and depends strongly on the pH of the feed solution. The concentration of licorice flavonoids after desorption can reach 25.12% in the desorbed fraction with 75% ethanol solution, which is higher than the 21.9% of commercial macroporous resin XDA-1. HPLC showed that liquiritin, one of main flavonoids in the licorice, was retained in this fraction, while glycyrrhizic acid (GA) can be almost removed from this fraction.

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Abbreviations

a	adsorption equilibrium constant
A	adsorption ratio (%)
C_d	flavonoids concentration of desorption solution (mg/ml)
C_e	equilibrium concentration of flavonoids in the adsorption solution (mg/ml)
C_o	initial concentration of flavonoids in the adsorption solution (mg/ml)
D	desorption ration (%)
GA	glycyrrhizic acid
PUF	poly(urea-formaldehyde)
Q_e	adsorption capacity at adsorption equilibrium (mg/g adsorbents)
Q_m	saturation adsorption capacity (mg/g adsorbents)
V_d	volume of desorption solution (ml)
V_i	volume of initial solution (ml)
W	weight of the dry adsorbents (g)

1 Introduction

Flavonoids are polyphenolic compounds and the basic structures of this matter consist of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle. They are widely distributed in vegetables and plants such as licorice and ginkgo (Hatano et al. 2000; Fukai et al. 2002; Van Beek 2002). In recent years, these kinds of molecules have attracted the attention of many researchers because flavonoids display a remarkable array of

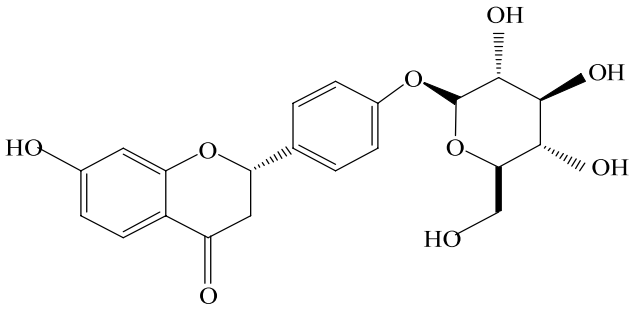

Fig. 1 Chemical structure of liquiritin

biological and pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, cardiovascular protection and so on (Tamir et al. 2001; Kimura et al. 2001; Yokozawa and Dong 1997). Licorice derives from *Glycyrrhiza uralensis* Fisch, which is one of Chinese traditional medical herbs. Compounds in licorice mainly include flavonoids and glycyrrhizic acid (GA) (Hu and Shen 1996; Niu et al. 2005). At present, hundreds of flavonoids have been reported. They are mainly included several categories: flavones, flavonols, flavnone, chalcone, isoflavone, isoflavan, isoflavene, isoflavanone and coumestan. Some measures should be taken to remove GA. Figure 1 is chemical structure of liquiritin, one of the main flavonoids in licorice. Rijke (Rijke et al. 2006) illustrated the analytical separation methods in detail. Macroporous resin adsorption is one of important and usual method (Fu et al. 2005).

Magnetic separation technology is another good selective to separate flavonoids from licorice. This method was introduced in the early seventies of 20th century and since then it has become one of the most attractive separation techniques. Recently, this technology was widely applied in the researches of bioseparation and bioengineering fields including cell isolation (Safarik and Safarikova 1999), enzyme immobilization (Dyal et al. 2003; Rittich et al. 2002), protein purification (Safirik and Safarikova 2004), DNA separation (Campo et al. 2005) and target drug (Saiyed et al. 2003) and also progressed rapidly in the other fields such as environmental inspection (Takafuji et al. 2004; Shan et al. 2005; Yang and Li 2001), catalysis (Zhang et al. 2003) and so on. The magnetic adsorbents are superparamagnetic, meaning that they respond strongly to magnetic fields, but retain no residual magnetism after the field is removed. Additionally, adsorbents with different active groups can be prepared by surface modification or macromolecule polymerization (Khng et al. 1998; Ding et al. 2001), which can greatly enhance the adsorptive selectivity to the interest material. The magnetic adsorbents not only use in a fixed bed mode but also use a batch system. The batch operation with magnetic adsorbents is better than fixed bed mode at small or analytical scale. The basic procedure in magnetic separation comprises three steps: (1) the selective binding of the interest material to the adsorbents, (2) the separation of the adsorbents bonded targets using a magnetic field and (3) the recovery of the bound molecular by elution.

Licorice extract is very complex systems which comprise large number of compounds. The key of the magnetic separation technology is to find appropriate ligands in order to improve the selectivity of flavonoids. Large number of amidogroups (-CONH-) exists on the surface of poly(urea-formaldehyde) (PUF) (Li et al. 2006), which can form strongly hydrogen bonds with flavonoids. Therefore, superparamagnetic PUF adsorbents were synthesized by reversed phase suspension polymerization in this paper. The properties of the adsorbents were characterized. The adsorption characteristics of the adsorbents for the separation of licorice flavonoids have been evaluated.

2 Experiments

2.1 Materials

Licorice root was obtained from Inner Mongolia Yili Science and Technology Industry Co. Ltd. (P.R. China). The calibration standard material rutin hydrate ($C_{27}H_{30}O_{16}\cdot xH_2O$, MW 610.52, 95%) was purchased from Sigma-Aldrich (USA). Liquiritin ($C_{21}H_{22}O_9$, MW 418.39, 98%) was purchased from National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine (P.R. China). Glycyrrhizic acid monoammonium salt trihydrate ($C_{42}H_{65}O_{16}\cdot 3H_2O$, MW 894.03, 98%) was purchased from the Acros Organics (USA). Trifluoroacetic acid was obtained from Beijing Jinglong Chemical Reagents Co. Ltd. (P.R. China). Acetonitrile was purchased from Tianjin Fucheng Chemical Reagent Plant (P.R. China). All solutions were filtered through 0.45 μm membranes (Jinteng, P.R. China) before HPLC. All other reagents used are available commercially and were analytical grade such as urea, formaldehyde, glycerol, ferrous chloride tetrahydrate ($FeCl_2\cdot 4H_2O$), ferric chloride hexahydrate ($FeCl_3\cdot 6H_2O$), liquid paraffin, benzene dichloride, span80, ammonia, hydrochloric acid, sodium hydroxide and ethanol etc.

2.2 Synthesis and characterization of superparamagnetic PUF adsorbents

2.2.1 Synthesis of superparamagnetic Fe_3O_4 nanoparticles

Superparamagnetic Fe_3O_4 nanoparticles were prepared by the chemical co-precipitation method according to the document (Ma et al. 2006). The brief procedure is as follows: 0.2 mol of $FeCl_3\cdot 6H_2O$ and 0.1 mol of $FeCl_2\cdot 4H_2O$ were dissolved in 300 ml deionized water under the protection of nitrogen gas with vigorous stirring. Then 60 ml of 25%

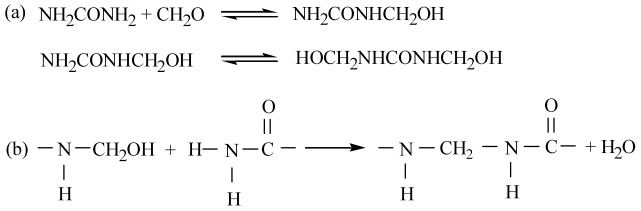

Fig. 2 The reaction scheme of the formation of PUF polymer. (**a**) The formation of PUF pre-polymer. (**b**) The formation of PUF polymer

ammonium hydroxide was added quickly into the solution when the temperature reached at 80 °C. After about one hour reaction, the paramagnetic magnetite precipitates were obtained and the supernatant can be removed by magnetic decantation. The precipitates were washed several times with deionized water to remove the unreacted chemicals.

2.2.2 Synthesis of superparamagnetic PUF adsorbents

Preparation of pre-polymer solution: 52.5 g of urea and 166.5 g of formaldehyde (37 wt%) were mixed in a 500 ml three-neck round-bottomed flask connected to a reflux condenser and equipped with a mechanical stirrer. The pH of solution was adjusted to 8–9 with sodium hydroxide after the urea dissolved. The temperature was kept at 90 °C for 2 hours and then the PUF pre-polymer solution was obtained. Figure 2a shows the reaction scheme of the formation of PUF pre-polymer.

Fe_3O_4 nanoparticles prepared above were dispersed into 100 g of pre-polymer solution by sonication for 20 min. The pH of the mixture was then adjusted to 3–4 with hydrochloric acid. Then the mixture was poured into the dispersion medium, which was composed of 180 ml of paraffin, 60 ml of dichlorobenzene and 4.8 ml of emulsifier (span 80). The reaction lasted for 2 hours at 40 °C temperature under continuous stirring. Then the magnetic PUF particles were obtained and washed with alcohol for several times by magnetic decantation. Figure 2b shows the condensation reaction scheme of PUF.

2.3 Adsorption of flavonoids from *Glycyrrhiza uralensis* Fisch

2.3.1 HPLC analysis

HPLC analysis of sample was carried out on an Agilent 1100 series HPLC system. The chromatographic column was Zorbax ODS (150 mm × 4.6 mm, 5 μm). The UV detector was set at the wavelength of 254 nm considering the maximum absorbed wavelength of flavonoids and glycyrrhizic acid (Zhou et al. 2004). The mobile phase was different proportion of water (A), containing 0.1% trifluoroacetic acid solution and acetonitrile (B). Gradient elution was used in this experiment. The gradient system was: 0–10 min, 20–40% of B; 10–15 min, 40% of B; 15–16 min, 40–50% of B; 16–17 min, 50–20% of B; 17–25 min, 20% of B. The HPLC analysis was processed at 0.8 ml/min of flow rate and at 25 °C temperature. 20 μl of sample was injected into the chromatographic column.

The calibration curve of liquiritin showed good linear relationship over the range of 0.01–0.32 mg/ml, and the regression equation was $y = 9475.6x + 18.738$ ($R^2 = 0.9981$), where y is the peak area of liquiritin and x (mg/ml) is the concentration of liquiritin.

The regression equation of GA was $y = 9030x + 196.96$ ($R^2 = 0.9728$), where y is the peak area of GA and x (mg/ml) is the concentration of GA. A good linear relationship was obtained over the range of 0.02–0.32 mg/ml.

2.3.2 Determination of concentration of total flavonoids

The concentration of total flavonoids was measured using a UV-visible spectrophotometer (Lambda Bio 40, Perkin Elmer, USA) (Lu et al. 2003). The procedure is as follows: the sample solution was added to a 10 ml flask and 50% ethanol solution was added to these flasks to become 5.0 ml. Then 0.3 ml of 5% $NaNO_2$ solution was added. After 6 minutes, 0.3 ml of 10% Al $(NO_3)_3$ solution was added. And then after 6 minutes, 4 ml of 20% sodium hydroxide was added. At last, 50% ethanol was added to these flasks and made to the volume. The solution was allowed to stand for 15 min at room temperature, and the absorbance at 510 nm was determined. The concentration of total flavonoids was calculated using rutin as the calibration standard. A good linear relationship was obtained over the range of 0.0020–0.1000 mg/ml. The regression equation was: $y = 11.4x + 0.0229$ ($R^2 = 0.999$), where y is the absorbance at 510 nm, x is the concentration of total flavonoids (mg/ml).

2.3.3 Preparation of crude licorice extracts

100 g of licorice sample was minced and extracted with 1000 ml of a solution of ethanol/water (70:30, v/v) for one day by dipping method. Then the extraction solution was filtered. After filtration, the clear extract was concentrated to one tenth of the original volume by removing the ethanol solution in a rotary evaporator at 50 °C. At last, the deionized water was added to the extract until the volume reached to 500 ml.

2.3.4 Adsorption and desorption studies

The procedure of adsorption experiments were performed as follows: 4 g of magnetic PUF adsorbents and 100 ml of sample solution were taken into a flask. The adsorption was

then carried out for some time in a water-bath shaker at the speed of 135 rpm and at 25 °C. The supernatant solution was decanted by magnet during the adsorption process. The adsorption capacity can be calculated by measuring the concentration of supernatant solution and initial solution.

The adsorption properties were evaluated under different conditions including the pH value (pH values are 4, 6, 8 and 10), initial solution concentrations and the adsorption time. After adsorption equilibrium was reached, the adsorbates were desorbed for 2 hours using 5%, 25%, 50%, 75% and anhydrous ethanol solutions, respectively. Then the desorbed solutions were analyzed by HPLC or UV-visible spectrophotometer.

2.3.5 Calculation of adsorption and desorption capacity

The following equations are used to quantify the adsorption and desorption capacities:

Adsorption capacity equation:

$$Q_e = \frac{(C_o - C_e) \times V_i}{W} \times 100\% \quad (1)$$

Desorption ratio equation:

$$D = \frac{C_d \times V_d}{(C_o - C_e) \times V_i} \times 100\% \quad (2)$$

Equation (1) means the weight of flavonoids adsorbed with 1 g adsorbents; (2) refers to the percentage of flavonoids which is desorbed from adsorbents by ethanol eluent

Where Q_e is the adsorption capacity at adsorption equilibrium (mg/g adsorbents); D is the desorption ratio (%); C_o and C_e are the initial and equilibrium concentration of flavonoids in the adsorption solution, respectively (mg/ml); C_d is the flavonoids concentration of desorption solution (mg/ml); V_i is the volume of initial solution (ml); V_d is the volume of desorption solution (ml); W is the weight of the dry adsorbents (g).

3 Results and discussion

3.1 Properties of superparamagnetic PUF adsorbents

Superparamagnetic Fe_3O_4 nanoparticles were prepared by co-precipitation method. Figure 3 shows the SEM (JSM-6700F, JEOL, Japan) image of the magnetic Fe_3O_4 nanoparticles. It can be seen that the nanoparticles are spherical in shape with an average size of about 15 nm. It is known that magnetic particles less than about 25 nm will exhibit superparamagnetism (Lee et al. 1996). Therefore, the prepared magnetic Fe_3O_4 nanoparticles have superparamagnetic properties. These particles seriously aggregate together mainly due to the magnetic dipole attraction.

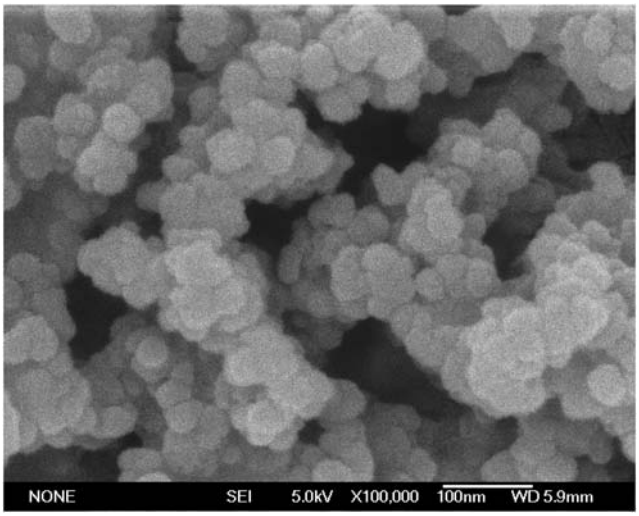

Fig. 3 SEM image of magnetic Fe_3O_4 nanoparticles

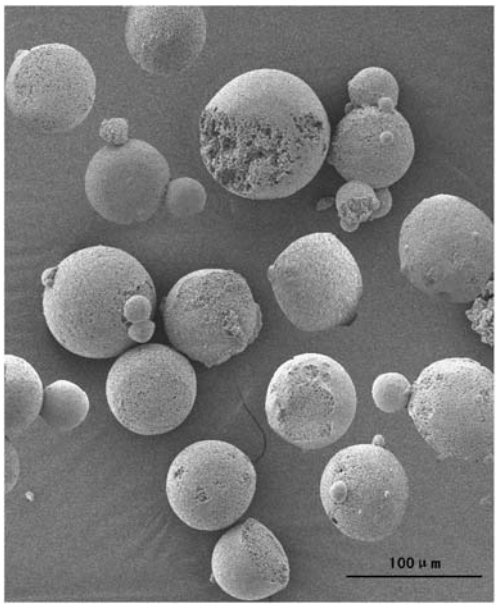

Fig. 4 SEM photograph of Fe_3O_4/PUF particles

Then magnetic Fe_3O_4 nanoparticles were coated with UF polymer. Figure 4 shows a typical SEM photograph of the magnetic PUF particles. It can be seen that these particles are well dispersed without aggregation. They are spherical in shape and the average size was about 50 µm in diameter. Many researchers have tried to investigate the interaction between the coating polymer and the Fe_3O_4 nanoparticles. Deng et al. (Deng et al. 2002) believed that the interactive mechanism might be the interaction between the lonepair electrons of N atom with the 3d orbital of Fe atom, to form a coordinate bond. Li et al. (2004) believed that the hydrogen bond play an important role. This paper thinks that these two interactions both exist based on the structures of Fe_3O_4 and PUF polymer. Magnetite nanoparticles prepared by the co-

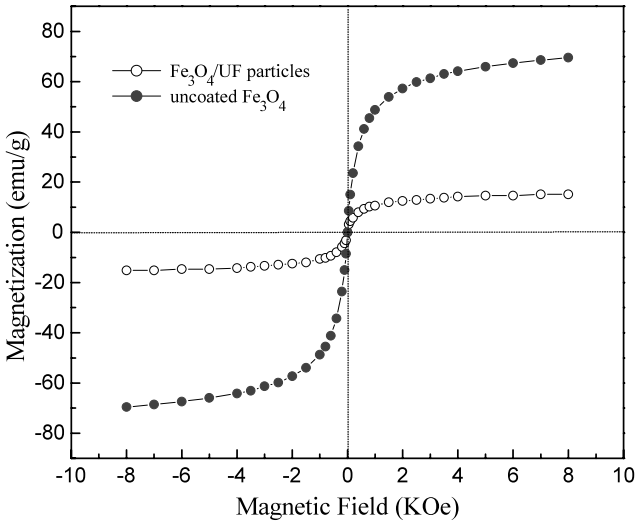

Fig. 5 VSM of uncoated Fe_3O_4 nanoparticles and Fe_3O_4/UF particles

precipitation method have extensive hydroxyl groups on the surface. So it is easy to form the hydrogen bonds between the PUF polymer and Fe_3O_4 nanoparticles. In addition, the lone-pair electrons of the N atom in PUF polymer are able to interact with 3d orbit of Fe atom to form coordinate bond. Technically, it is quite difficult at present to detect directly the interactions mentioned above, and the more accurate interactive mechanism of these interactions still needs further investigation.

The magnetic properties of these adsorbents were analyzed by vibrating sample magnetometer (VSM, Model-155, Digital Measurement System, Inc). Figure 5 shows the magnetization curve of uncoated Fe_3O_4 nanoparticles and PUF adsorbents at room temperature. The saturation magnetizations of uncoated Fe_3O_4 nanoparticles and magnetic PUF adsorbents are 69.53 emu/g and 15.1 emu/g, respectively. The PUF layers decrease the magnetization of uncoated Fe_3O_4 nanoparticles. However, the saturation magnetization of the adsorbents is enough to make the adsorbents easily separated from solution. In addition, the magnetization curve exhibits zero remanence and coercivity, which proves that uncoated Fe_3O_4 nanoparticles and PUF adsorbents both have superparamagnetic properties. So the adsorbents are able to respond to an external magnetic field without any permanent magnetization. When external magnetic field was removed, the adsorbents can be redispersed rapidly.

3.2 Adsorption of flavonoids from *Glycyrrhiza uralensis* Fisch

3.2.1 Effect of pH value

As shown in Table 1, with the increase of pH value, the adsorption capacity and adsorption ratio both decrease greatly.

Table 1 Effect of the pH value on adsorption of flavonoids (initial concentration, 0.413 mg/ml)

pH value	Adsorption ratio (%)	Adsorption capacity (mg/g adsorbents)
4	43.30	14.29
6	43.34	14.32
8	17.51	5.77
10	4.92	1.62

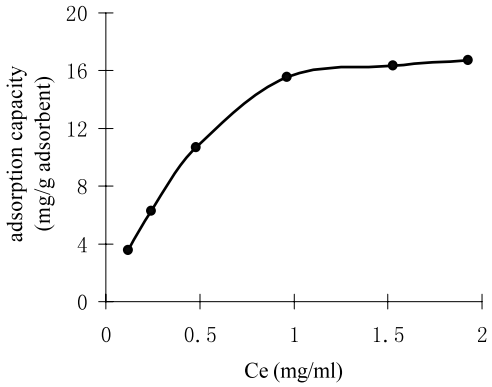

Fig. 6 Adsorption isotherm at room temperature for flavonoids on magnetic PUF adsorbent

Therefore, the adsorption strongly depends on pH value of the feed solution. Additionally, large number of amidogroups (-CONH-) exists on the surface of the adsorbents. H-bonding interactions easily occur not only between oxygen (-CO-) of adsorbent and the hydroxyl groups of flavonoids but also between imine groups (-NH-) of adsorbents and the oxygen of flavonoids. All these show that hydrogen bonds play an important role in the adsorption process. The phenolic hydroxyl groups on the flavonoids dissociate to form the anions $R\text{-}O^-$ with addition of OH^- ions (from alkali). Consequently, the H bonding interactions are reduced because of decreases of the proton-donating capacity of hydroxyl groups on the flavonoids (Saravanan and Subramanian 2005).

From Table 1, it is concluded that the optimal pH value is about 6. The pH value of initial extract is about 6.5. Thus, for all later experiments the pH value wasn't adjusted.

3.2.2 Adsorption isotherms

As shown in Fig. 6, the adsorption reached the saturation plateau when the initial concentration of flavonoids was 0.9632 mg/ml. so the concentration of flavonoids in the feed solution was selected at about 1.0 mg/ml.

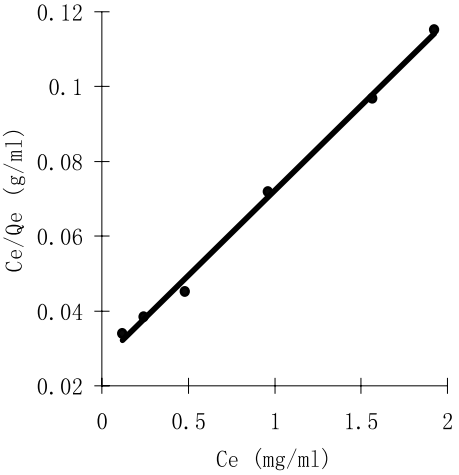

Fig. 7 Langmuir plot for the adsorption of flavonoids

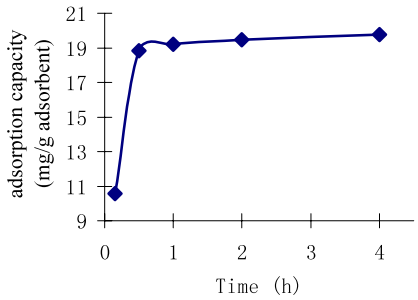

Fig. 8 Adsorption kinetics curve for flavonoids onto magnetic UF adsorbent

The adsorption isotherm of flavonoids is consistent with the Langmuir behavior. Langmuir isotherm can be plotted using the following equation:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{a \times Q_m} \tag{3}$$

where Q_e is the adsorption capacity at adsorption equilibrium (mg/g adsorbents); C_e is the flavonoids concentration at adsorption equilibrium (mg/ml); Q_m is the saturation adsorption capacity (mg/g adsorbents); a is the adsorption equilibrium constant.

The values of $1/Q_m$ and $1/(a \times Q_m)$ can be obtained from the intercept and slope of Fig. 7. The Q_m value is 22.27 mg/g adsorbents and the value of a is 1.7677. So the adsorption isotherm of flavonoids is: $Q_e = 22.27C_e/(C_e + 0.5657)$.

3.2.3 Adsorption kinetics

The adsorption equilibrium of flavonoids onto adsorbent is attained in about 30 min from Fig. 8. The possible reason of rapid equilibrium is that the adsorbent is non-porous type.

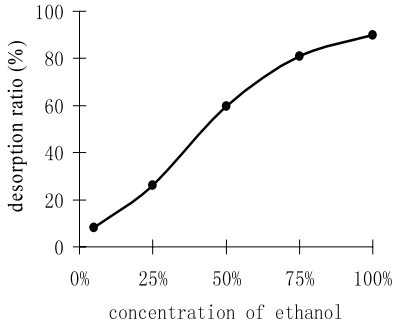

Fig. 9 Desorption ratio of flavonoids onto PUF adsorbent

Table 2 2 Purity of flavonoids and GA in the crude licorice extract and 75% desorbed fraction

	Crude extract	75% desorbed fraction	XDA-1
Total flavonoids	3.69%	25.12%	21.9% (Fu et al. 2005)
Liquiritin	2.1%	9.4%	–
Glycyrrhizic acid	4.24%	0.44%	–

The non-porous structure allows adsorption only on the surface, and there is negligible intraparticle diffusion within the adsorbent. While it takes 4 hours to equilibrate for licorice flavonoids onto macroporous resin XDA-1 (Fu et al. 2005). So the adsorbents prepared in this study can largely save the adsorption time comparing with the macroporous adsorbents.

3.2.4 Desorption

As shown in Fig. 9, the desorption ratio of flavonoids was 89.9% when the eluent was anhydrous ethanol, which indicates that ethanol solution was a better choice as eluent. This also indicates that hydrogen bonds play an important role in the adsorption process because ethanol can compete in the formation of hydrogen bonds with flavonoids. Ethanol has many advantages as eluent solution such as low toxicity, easy to recycle because of low boiling point and so on.

The desorbed solution was dried by vacuum drying method. Table 2 shows the purities of flavonoids and GA in the licorice extract and 75% desorbed faction. Although 100% fraction gives better recovery, the purity of flavonoids will decrease greatly. The 75% fraction was selected just considering the recovery and purity. The flavonoids purities of 50% and 75% desorbed fraction were 15.95% and 25.12%, respectively, while the crude licorice extract was 3.69%. The purity of flavonoids absorbed by macroporous resin XDA-1 was 21.9% (Fu et al. 2005). This demonstrates that the adsorbent has good selectivity to flavonoids in the licorice extract.

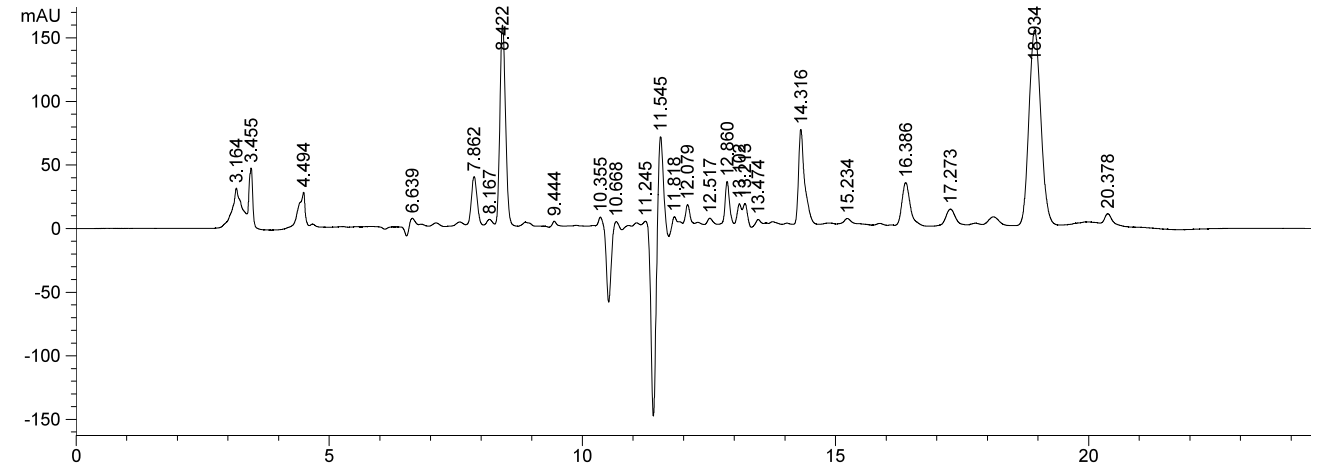

Fig. 10 HPLC analysis of crude licorice extract monitored at 254 nm

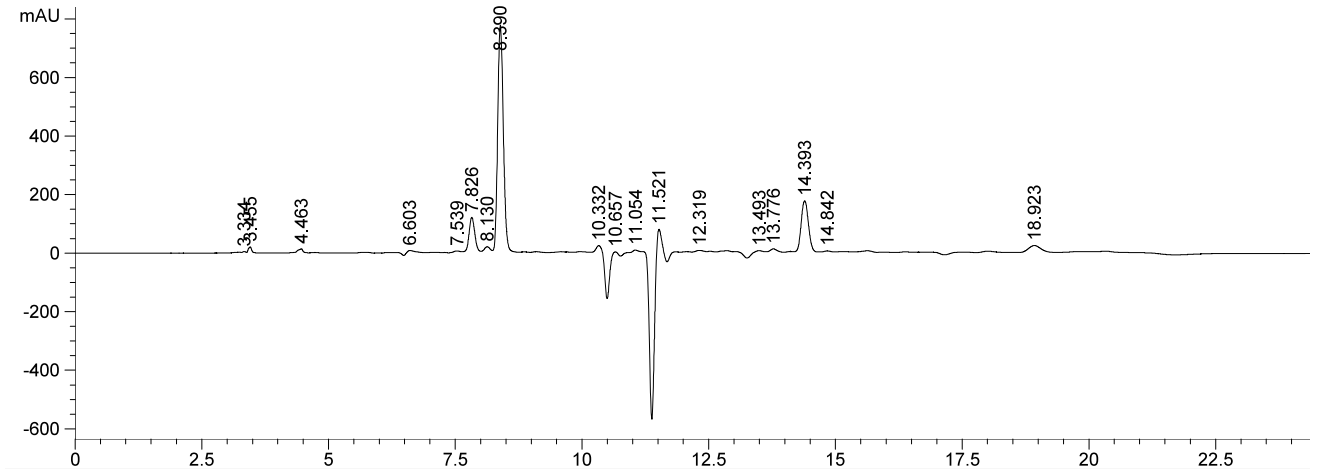

Fig. 11 HPLC analysis of 75% fraction of desorbed solution monitored at 254 nm

Figure 10 is HPLC analysis of crude licorice extract. The retention time of 8.4 min is the peak of liquiritin, one of main flavonoids in the licorice. The retention time of 18.9 min corresponds to glycyrrhizic acid. As shown in Fig. 11, liquiritin (about 9.4% purity) remained in the 75% desorbed fraction, while the GA is almost totally removed from crude licorice extract. The possible reason is that GA is easier to form the hydrogen bonds with the adsorbent than flavonoids. So it is more difficult to desorb GA than flavonoids from the adsorbent.

4 Conclusions

In this study, novel superparamagnetic PUF adsorbents were synthesized by reversed phase suspension polymerization. The adsorbents have superparamagnetic properties and high-magnetic saturation (15 emu/g). The adsorbent exhibited high adsorption selectivity to flavonoids in licorice extract. The desorbed fraction with 75% ethanol solution has 25.12% purity, which is higher than the 21.9% of commercial macroporous resin XDA-1. The GA can be effectively removed from the licorice extract. Magnetic separation method has the advantage of easy operation, rapid separation, easy handling and low nonspecific adsorption. Therefore, the magnetic separation method has great potential in the study of natural products.

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