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An Enhanced Adsorption in Puerarin Retention on Oligo-β-Cyclodextrin Grafted Poly(Glycidyl Methacrylate) Media

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Abstract The poly(glycidyl methacrylate) adsorbents P(GMA–EDMA) and P(GMA–DVB) were synthesized by the radical suspension–polymerization method and farther coupled by oligo-β-cyclodextrin (CDP) to obtain P(GMA–EDMA)–CDP and P(GMA–DVB)–CDP. The synthesized polymeric media were characterized by Fourier transform infrared (FTIR) spectrometer, scanning electron microscopy, and BET surface area. The adsorption of puerarin from aqueous solution onto the four media, i.e., P(GMA–EDMA), P (GMA–DVB), P(GMA–EDMA)–CDP, and P(GMA–DVB)–CDP, was studied. An enhanced adsorption of puerarin apparently presented on grafted media. The interaction between the polymeric media and the puerarin was researched by FTIR. The result shows that the adsorption efficiency on P(GMA–DVB)–CDP driven by multiple weak interactions is much higher than that on P(GMA–EDMA) driven by hydrogen bonding interaction only and on P(GMA–DVB) or P(GMA–EDMA)–CDP driven by two interactions.

Keywords Polymeric media · Weak interactions · Enhanced adsorption · Synthesis · Oligo-β-cyclodextrin

Introduction

In recent years, polymeric resins have been widely used in separation and purification of natural products and pharmaceuticals. The major causative factors are the favorable characteristics of polymeric adsorbents such as high chemical stability and mechanical intensity, recycled use for industrial operations, and low cost. There have been considerable studies of synthesis, properties, and applications about these functional polymers in

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literatures [1–6]. However, the adsorption selectivity of these media is generally low although they have a variety of functions, which has limited their application for preparation purposes. More precise separation with higher recovery of natural products or pharmaceuticals has become a requirement in the processing fields recently. To fulfill the appeal, much attention has been paid to the manufacture of fine-particle and uniform polymeric adsorbents [7] and controlling their pore structure [8]. On the other hand, modification of their surface chemistry is an important and useful method to improve the separation selectivity of polymeric matrices [9–11].

In our previous work, we have synthesized a novel medium D152–CDP through coupling functional pendant groups of oligo-β-cyclodextrin (CDP) on the commercial resin D152 [12]. Utilizing this promising matrix, we can efficiently obtain pure puerarin in a one-step chromatographic operation. The higher selectivity for puerarin on the coupled medium D152–CDP resulted from the introduction ligands of CDP compared with the initiative resin. It has been reported that the inclusion complex can form between β-cyclodextrin (CD) and phenolic compounds [13, 14]. We can deduce that inclusion complexation between CD and puerarin plays an important role despite other interactions existing in the process of puerarin separation from solution. In this paper, two poly(glycidyl methacrylate) adsorbents with different functional groups and their CDP-grafted media were synthesized. The static adsorption experiments were performed to test adsorption efficiency. Fourier transform infrared (FTIR) spectroscopy was used to investigate the interactions between the polymeric media and the puerarin, following recent trend that FTIR has been used as one of the major techniques to research the interactions between monomer and polymer or between polymers [15, 16]. The present work was aimed at achieving a better understanding on the interactions of puerarin and adsorbents. The result of this paper might be helpful to the design, synthesis, and choice matrix in the purification and separation of natural products or pharmaceuticals.

Experimental

Chemicals

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), divinylbenzene (DVB), and 2,2′-azobis-isobutyronitrile (AIBN), were purchased from Alfa Aesar company. These regents were used without further purification. Polyvinyl alcohol was purchased from Beijing Xudong Chemicals Factory, Beijing, China. β-CD was purchased from the Beijing Aoboxing Biotech Company Ltd., Beijing, China. It was recrystallized and vacuum dried at 110°C before use. Epichlorohydrin (EP) was obtained from Beijing Yili Fine Chemicals Factory, Beijing, China. Puerarin with purity higher than 98% and a crude extract powder of Radix puerariae with purity about 45% were bought from Luye Biology, Huainan, China. All other reagents were of analytical grade and obtained from Beijing Chemical Reagent Company, Beijing, China.

Synthesis of Polyacrylate Adsorbents and Coupled by CDP

The poly(GMA–EDMA) and poly(GMA–DVB) adsorbents (P(GMA–EDMA), P(GMA–DVB)) were synthesized by the radical suspension–polymerization method following the previously described procedure [17]. The polymerization monomers (GMA and EDMA or DVB, 60/40 *v*/*v*) and porogenic solvents (toluene and *n*-heptane, 60/40 *v*/*v*) were mixed in which the free radical initiator AIBN (1.0% *w*/*v* with respect to monomers) was dissolved. After degassing and thorough mixing in an ultrasonicator for 30 min, 20.0 ml of the mixture was suspended in 100.0 ml of 1.0% (*w*/*v*) poly(vinyl alcohol) aqueous solution. The suspension was transferred to a 250-ml three-necked round bottom flask submerged in a 45°C water bath. Nitrogen was introduced into the reaction vessel and the solution stirred under 500 rpm. Then the temperature was linearly raised from 45 to 65°C within 2 h and was kept at 65°C for 3 h, 75°C for 2 h, and finally 85°C for 2 h. After the resultant white resins were recovered by filtration and thoroughly washed with hot distilled water, they were placed in a Soxhlet extractor to remove the porogenic solvents with ethanol for at least 24 h. The resins were dried under vacuum and kept in a desiccator. The CDP and the media of P(GMA–EDMA)–CDP, P(GMA–DVB)–CDP were prepared according to the approaches reported in literature [18] and [12]. The major synthetic routes were displayed in Figs. 1 and 2.

Puerarin Adsorption Experiments

Static adsorption experiments were used to test the adsorption capacity of puerarin on the four polymeric media of P(GMA–EDMA), P(GMA–DVB), P(GMA–EDMA)–CDP, and P (GMA–DVB)–CDP. The amounts of 0.05 g dried media were put into flasks with a lid. After adding 10.0-ml puerarin sample solutions (purity 98%) with a concentration of 2.0 mg/ml, the flasks were shaken (150 rpm) on oscillator for 24 h at room temperature. The concentration of the puerarin remaining in the solution was measured by high-performance liquid chromatography (HPLC). The HPLC system was Shimadzu (Kyoto, Japan) HPLC apparatus equipped with a reversed-phase C18 column (250 mm×4.6 mm ID, 5 μm). The working calibration curve based on puerarin standard solutions was Y=4,852,307 X−143,956, R^2=0.9993, where Y and X are the peak area and the concentration of puerarin (mg/ml) respectively, which showed good linearity over the range of 0.01–1.5 mg/ml. The adsorption capacity of the puerarin on the four synthesized polymeric media was expressed using the adsorption efficiency in this paper. The adsorption efficiency was calculated according to the following equation:

$$\text{Adsorption efficiency }(\%) = \frac{C_0 - C_\mathrm{e}}{C_0} \times 100\%,$$

where C_0 and C_e (mg/ml) are the initial and equilibrium concentrations of puerarin in the solution, respectively.

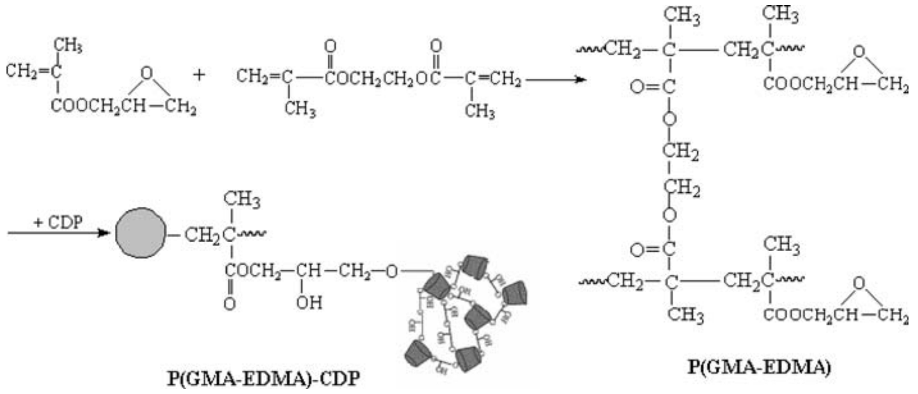


Fig. 1 Synthetic route of P(GMA–EDMA)–CDP

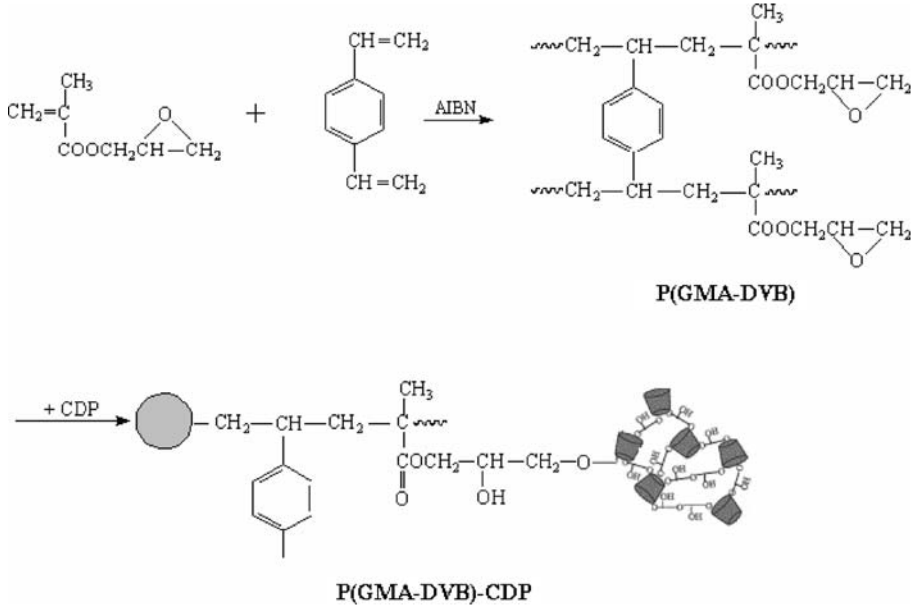


Fig. 2 Synthetic route of P(GMA–DVB)–CDP

Characterizing of the Media Structures and IR Study

The morphology of the synthesized polymeric adsorbents was obtained by scanning electron microscopy (Hitachi S4700, Japan). The BET surface area and pore structure parameters were determined with ST-2000 surface area and pore diameter measurement instrument (Beijing Beifen Instrument Company, China). Their FTIR spectra of the characteristic structures were recorded on Nicolet Nexus 670 (Thermo Nicolet, USA) with KBr disc. The IR spectra of the media after adsorbing puerarin were studied. The amount of grafting CDP on the synthesized media was determined in the light of reference [12] and [19]. The coupled resins were decomposed and the glucose concentration in hydrolysate solution was measured. The numerical value of the β-CD apparent grafting amount Q_s (μmol/g) was calculated from the following equation:

$$Q_s = \frac{C \times 50 \times 1,000}{180 \times 7 \times W}$$

where C is the glucose concentration in hydrolysate solution (μg/ml); W is the weight of media (mg). Then the amount of grafting CDP can be obtained.

Results and Discussion

Characterization of the Polymeric Media

The synthesized polymeric adsorbents were sieved, and the particle sizes of the four media used in the puerarin adsorption experiments ranged from 40 to70 μm. The other characteristics of the synthesized adsorbents are listed in Table 1. It can be found that the

Table 1 Characteristic properties of polymeric media P(GMA–EDMA), P(GMA–DVB), P(GMA–EDMA)–CDP, and P(GMA–DVB)–CDP.

Property	P(GMA–EDMA)	P(GMA–DVB)	P(GMA–EDMA)–CDP	P(GMA–DVB)–CDP
BET surface area (m^2/g)	126.3	145.2	98.5	102.7
Pore volume (ml/g)	0.1490	0.1991	0.1253	0.1789
Average pore diameter (nm)	55.2	79.7	49.6	67.5
Amount of β-CD (μmol/g)			54.3	48.7

values of surface area and pore diameter between P(GMA–EDMA) and P(GMA–DVB) have little differences; however, the differences are expanded between before and after coupled media P(GMA–EDMA) and P(GMA–EDMA)–CDP. Also, the same case happened on P(GMA–DVB) and P(GMA–DVB)–CDP. The phenomenon that the spatial structures of the coupled media become closer might result from the introduction of CDP. Figure 3 reflects the inner structures of the four synthesized media observed at magnifications of 10,000. The micrograph shows the highly reticular, three-dimensional porous structure within the media, which was formed by clusters of globules and numerous interconnected channels and pores. This feature of the synthetic media ensures the puerarin availably accessing pores and adsorbed on the media. From the SEM photographs (Fig. 3),

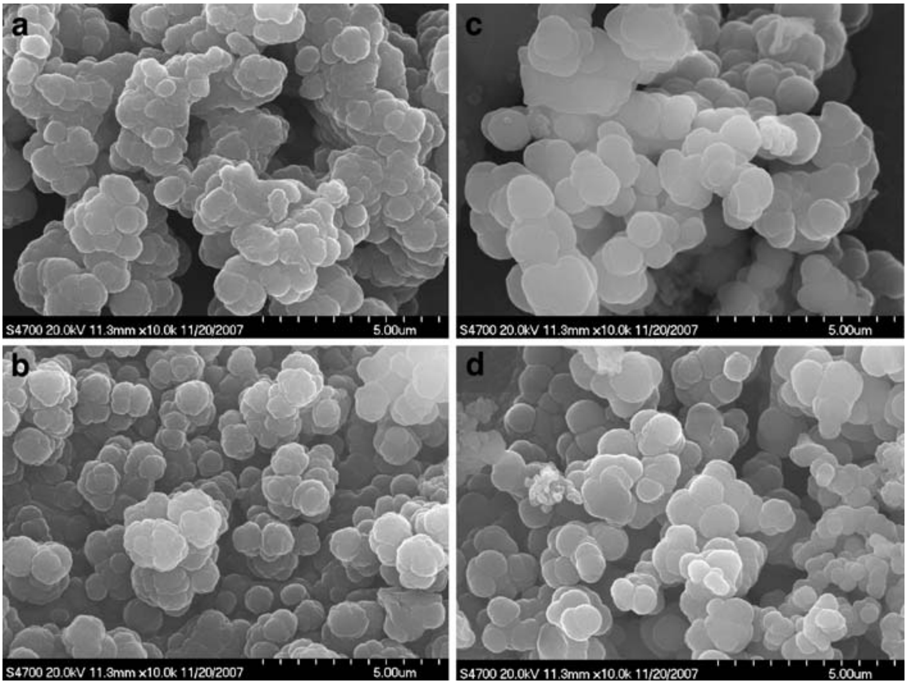


Fig. 3 Scanning electron micrographs of the four media. **a** P(GMA–EDMA), **b** P(GMA–EDMA)–CDP, **c** P (GMA–DVB), **d** P(GMA–DVB)–CDP

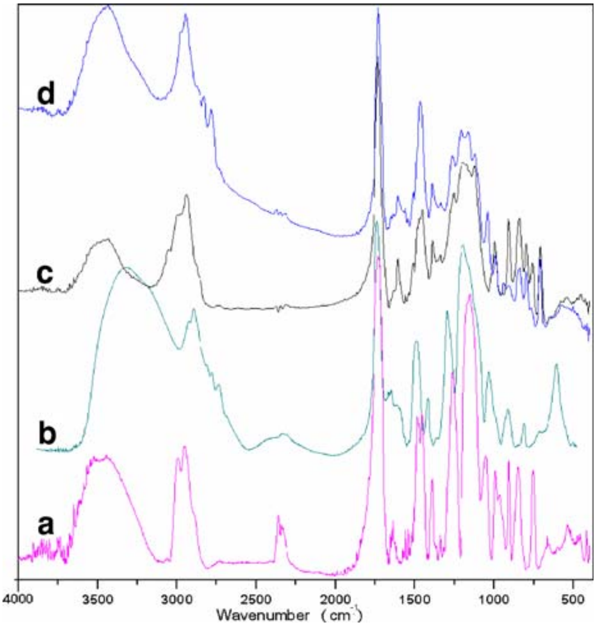


Fig. 4 IR spectra of the synthetic media. **a** P(GMA–EDMA), **b** P(GMA–EDMA)–CDP, **c** P(GMA–DVB), **d** P(GMA–DVB)–CDP

the slightly closer pore structures of the coupled media compared with their natives can be observed. The IR spectra of the four media were illustrated in Fig. 4. The adsorption peaks located around 1,738, 1,263, and 1,151 cm^{-1} in Fig. 4 are attributed to the carbonyl group band in the media. The adsorption peaks around 2,922 and 1,446 cm^{-1} in Fig. 4c and d are ascribed to the stretch vibration of $–CH_2–$ and C=C, which are the characteristic peaks of phenyl group. The strong absorption peaks at 3,448 and 1,680–1,610 cm^{-1} in the spectra of Fig. 4b and d were ascribed to the transform vibration peaks of the –OH, which indicated the introduction of CDP.

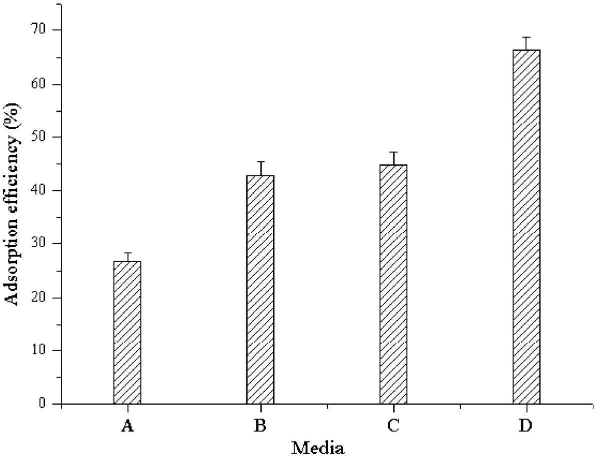


Fig. 5 Adsorption efficiency of puerarin on the polymeric media. **a** P(GMA–EDMA), **b** P(GMA–EDMA)–CDP, **c** P(GMA–DVB), **d** P(GMA–DVB)–CDP

Puerarin Adsorption Experiments

The result of the static adsorption experiments is shown in Fig. 5. Puerarin can be adsorbed and retained on all four media. The adsorption efficiencies of puerarin on P(GMA–EDMA) and P(GMA–DVB) were 27.1% and 44.8%, respectively. On the other hand, the adsorption efficiencies of the coupled media P(GMA–EDMA)–CDP and P(GMA–DVB)–CDP reached 42.8% and 67.1%, which were higher than their natives as expected. In addition, the P(GMA–DVB)–CDP matrix possesses the largest adsorption efficiency of puerarin. The above result was related with the interactions between the sorbate puerarin and the synthesized polymeric media. From the chemical structures of the polymeric media and puerarin, only hydrogen bonding between carbonyl group of P(GMA–EDMA) and a phenolic hydroxyl group of puerarin contributed to the adsorption process, whereas the driving forces of sorption of puerarin on P(GMA–DVB) included hydrophobic interaction between a phenyl ring of the adsorbent and a phenolic ring of puerarin and hydrogen bonding between carbonyl group of P(GMA–DVB) and a phenolic hydroxyl group of puerarin. If the two interactions occur synergistically, the total interaction should be much stronger than the same interaction occurring individually. This is the rational explanation that the adsorption efficiency of P(GMA–DVB) is larger than that of P(GMA–EDMA) in static adsorption experiments. Furthermore, with respect to their CDP-grafted media P (GMA–EDMA)–CDP and P(GMA–DVB)–CDP, in addition to the abovementioned interactions, the formation of inclusion complex between β-CD and puerarin is an important interaction involved in the adsorption process. The adsorption efficiency on these coupled media was significantly improved.

This result was also observed on column chromatography of the four synthesized polymeric media. The sieved media were respectively packed in columns (250×4.6 mm ID) by the slurry packing method. In a single run, the purity and recovery for puerarin separation from crude extracts (with purity about 45%) on the four media under the optimum mobile phase (acetic acid/water=7.0/93.0 (*v*/*v*)) are displayed in Table 2. It is discovered that the efficient purification puerarin can be obtained on P(GMA–DVB)–CDP medium with purity of 85.6% and the recovery of 95.1%.

The typical interaction between puerarin and the synthesized polymeric medium P (GMA–DVB)–CDP is demonstrated in Fig. 6, which would be helpful to elucidate the retention mechanism and the static adsorption experimental result. From Fig. 6, it can observed that, when the sorbate puerarin was absorbed on medium by one driving force such as hydrogen bonding or hydrophobic interaction, the molecules of puerarin and adsorbent were closer in space. This is beneficial to the occurrence and formation of other weak interactions between puerarin and the medium. Although the amount of grafting CDP on P(GMA–DVB)–CDP is lower than P(GMA–EDMA)–CDP (in Table 1), the retention capacity for puerarin on P(GMA–DVB)–CDP is the highest among the four

Table 2 Degree of purity and recovery of puerarin on the synthesized polymeric media.

Synthesized polymeric media	Purity (%)	Recovery (%)
P(GMA–EDMA)	61.5	93.3
P(GMA–DVB)	75.3	93.7
P(GMA–EDMA)–CDP	74.8	94.2
P(GMA–DVB)–CDP	85.6	95.1

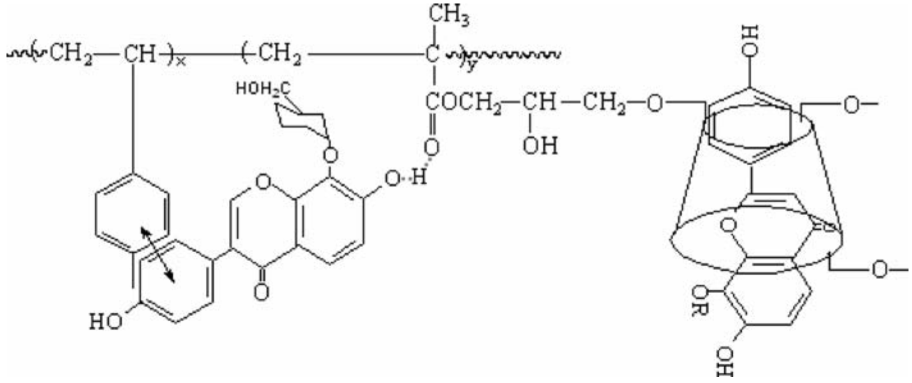


Fig. 6 Scheme for the interactions between P(GMA–DVB)–CDP and puerarin

synthesized polymeric adsorbents with the synergetic effect of multiple weak interactions (in Fig. 5).

Infrared Spectroscopy Study

FTIR is a particularly suitable method to determine the presence of specific interactions between various groups in polymer blends due to the force constants and is sensitive to both inter- and intra-molecular interactions [20]. Figure 7 show the infrared spectra of puerarin and the puerarin/P(GMA–EDMA), puerarin/P(GMA–EDMA)–CDP blends after static adsorption experiments. As mentioned above, in adsorption peaks around 1,100–1,700 cm^{-1} region of P(GMA–EDMA) spectrum, the peaks located at 1,738 and 1,151 cm^{-1} are assigned to the band of carbonyl stretching vibration and stretch vibration of epoxy bonds, respectively. In puerarin/P(GMA–EDMA) and puerarin/P(GMA–EDMA)–CDP spectra, it can be observed that the intensity of the free carbonyl band at 1,738 cm^{-1} decreases and shifts to lower frequency 1,719 cm^{-1}. It is expected that a large portion of

Fig. 7 IR spectra of the media after adsorption experiments. **a** Puerarin, **b** puerarin/P(GMA–EDMA), **c** puerarin/P(GMA–EDMA)–CDP

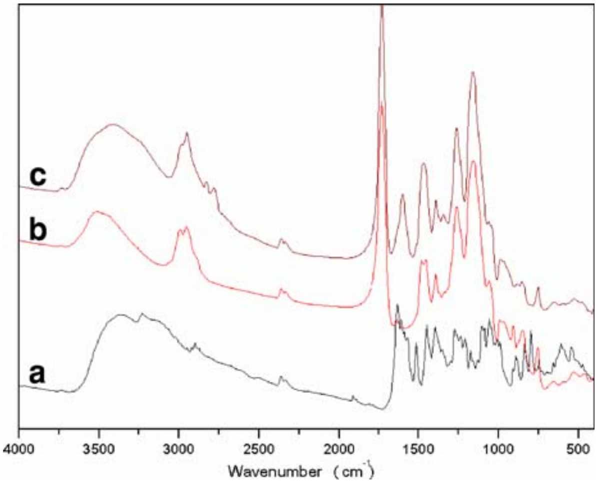
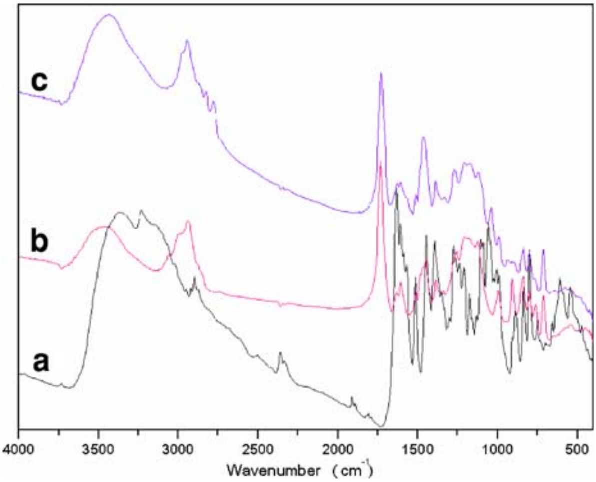


Fig. 8 IR spectra of the media after adsorption experiments. **a** Puerarin, **b** puerarin/P(GMA–DVB), **c** puerarin/P(GMA–DVB)–CDP



these free carbonyl groups is consumed by forming the hydrogen bond between the media and the puerarin [21]. Meanwhile, the band around 3,342 cm^{-1} appears in puerarin/P(GMA–EDMA)–CDP spectrum as the result of the decrease in the free hydroxyl band. It down shifts about 106 cm^{-1} and the intensity decreases a lot. These phenomena reveal that the interactions were formed between puerarin and P(GMA–EDMA) or P(GMA–EDMA)–CDP [21]. The IR spectra of the blends of puerarin/P(GMA–DVB) and puerarin/P(GMA–DVB)–CDP are displayed in Fig. 8. It can be also discovered that the intensity of narrow band at about 1,740 cm^{-1} corresponding to free carbonyl groups decreases. The broad band of P(GMA–DVB)–CDP hydroxyl groups around 3,528 cm^{-1} shifts to 3,340 cm^{-1}. These results resemble those for P(GMA–EDMA) and P(GMA–EDMA)–CDP. In addition, the infrared spectra of puerarin/P(GMA–DVB) and puerarin/P(GMA–DVB)–CDP in the phenyl-stretching region show a lower shift to 1,223 cm^{-1} and their intensities decrease. The differences of spectra of media before and after adsorption experiments indicate the formation of intermolecular interaction on the media surface [22]. The information of IR spectra in Figs. 7 and 8 are evidence to support the formation between the synthesized polymeric media and puerarin.

Conclusion

In this paper, several synthesized polymeric media were developed for adsorption and separation of puerarin from aqueous solution. The adsorption efficiency and affinity on P(GMA–DVB)–CDP towards puerarin were greater than on the other synthesized media P(GMA–EDMA), P(GMA–DVB), and P(GMA–EDMA)–CDP. The multiple weak interactions would contribute more to the adsorption process as acting simultaneously than that of acting individually. FTIR was a useful tool to investigate the weak interactions between puerarin and the synthesized polymeric media.

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