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Studies on preparing and adsorption property of grafting terpolymer microbeads of PEI-GMA/AM/MBA for bilirubin

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Abstract

Crosslinking copolymer microbeads with a diameter range of 100–150 μm were synthesized by suspension copolymerization of glycidyl methacrylate (GMA), acrylamide (AM) and *N*,*N*′-methylene bisacrylamide (MBA). Subsequently, polyethyleneimine (PEI) was grafted on the surfaces of the terpolymer microbeads GMA/AM/MBA via the ring-opening reaction of the epoxy groups, and the grafting microbeads PEI-GMA/AM/MBA were prepared. In this paper, the adsorption property of the grafting microbeads for bilirubin was mainly investigated, and the effects of various factors, such as pH value, ionic strength and grafting degree of PEI on the surface of grafting microbeads and the adsorption capacity of the grafting microbeads for bilirubin were examined. The batch adsorption experiment results show that by right of the action of grafted polyamine macromolecules PEI, the grafting microbeads PEI-GMA/AM/MBA have quite strong adsorption ability for bilirubin; the isotherm adsorption conforms to Freundlich equation. The pH value of the medium affects the adsorption capacity greatly, As in the nearly neutral solutions with pH 6, the grafting microbeads have the strongest adsorption ability for bilirubin, whereas in acidic and basic solutions their adsorption ability is weak. The ionic strength hardly affects the adsorption ability of the grafting microbeads. The grafting degree of PEI on the surfaces of the grafting microbeads also has a great effect on the adsorption capacity, and higher the grafting degree of PEI on the surface of the microbead PEI-GMA/AM/MBA, the stronger is the adsorption ability of the microbeads.

Keywords: Bilirubin; Polyethyleneimine; Grafting; Glycidyl methacrylate; Terpolymer microbead; Adsorption

1. Introduction

Bilirubin is a principal degradation product of heme catabolisma. The free bilirubin is a lipophilic endotoxin, and under normal physiological conditions, it is transported to liver by albumin for conjugation and subsequent excretion [1,2]. However, in certain pathological conditions such as jaundice, the amount of unconjugated bilirubin in blood increases and the elevated bilirubin level interferes with the normal functioning of the cellular machinery and eventually manifests systemic toxicity [1], and further may result in fatal kernicterus [3,4]. An effective treatment for hyperbilirubinemia is hemoperfusion treatment, and a successful hemoperfusion technique requires the absorbent to be specific, of high adsorption capacity and blood compatible and biocompatible [5,6]. The bilirubin molecules contain carboxyl and imine groups (as shown in Scheme 1) and the absorbent can adsorb bilirubin via electrostatic interaction or hydrogen bond interaction, so the reported resins, synthetic or natural, used for eliminating bilirubin are usually the polymer containing amine groups (they are usually protonized) and hydroxyl groups [5–8]. Moreover, for the synthetic resins, the amine groups on the macromolecular chains are generally obtained through chemically modifying matrix polymers [6]. Some researchers also utilized the lipophilic characteristic of bilirubin and adopted polymer immobilized β-cyclodextrin as adsorbents [5,9] because the central hydrophobic cavity of β-cyclodextrin have an inclusion ability for bilirubin compared to other lipophilic substances.

Polyethyleneimine (PEI) is a water-soluble polyamine. There are a mass of nitrogen atoms of amine groups on the macromolecular chains of PEI, and these nitrogen atoms of amine groups have a very strong protophile property; in aqueous solution, as pH < 10, these nitrogen atoms are mostly protonized, so PEI is a cationic polyelectrolyte [10,11]. As a

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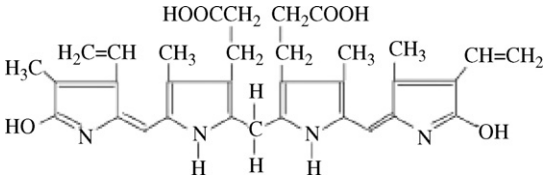


Scheme 1. Chemical structure of bilirubin.

functional polymer, the characteristics of PEI has attracted wide attention of scientists, and it has been used in many research fields, especially, in the biomedical field. PEI has many important applications, for example, it can be used in the separation and purification of biomacromolecules, in enzyme immobilizing, in biosensor constructions and in drug deliveries [12–15].

Methacrylates is a class of polymers with biocompatibility [16,17]. In this work, crosslinking terpolymer microbeads with glycidyl methacrylate (GMA) as main monomer and acrylamide as minor monomer were synthesized by suspension copolymerization, and subsequently PEI was grafted onto the surface of the crosslinking microbeads by utilizing the active epoxy groups of GMA. It is expected that the grafting microbeads should have better adsorption property for bilirubin because there is high density of amine groups on the grafted macromolecular chains of PEI, and the experiment facts confirmed this viewpoint. The designed and prepared biomedical adsorption microbeads in this research not only have excellent adsorption property for bilirubin, but also have biocompatibility, and the similar researches are reported scarcely although the PEI-grafting copolymer membranes containing glycidyl methacrylate used for bilirubin removal has been reported [7].

2. Experimental

2.1. Materials and instruments

Polyethyleneimine (PEI, Mr = $2 - 4 \times 10^4$, an aqueous solution with a content of 25 wt.%) was obtained from Qianglong Chemical Limited Company (Wuhan, China), and its concentration had been determined with UV method prior to use. Glycidyl methacrylate (GMA), acrylamide (AM) and α-α′-azoisobisbutyronitrile (AIBN) were obtained from Fluka, and the monomers was distilled under reduced pressure in the presence of hydroquinone and stored at 4 °C. *N*,*N*′-Methylene biscrylamide (MBA) was supplied from Xiangzhong fine chemical factory (Hunan Province, China) and used as received. Bilirubin (94% of content) was supplied from Dongzhu biological reagent Limited Company (Pingdingshan City, China). Bovine serum albumin (BSA) was obtained from the biochemical reagent company of Shanghai. Surfactant Span-80 (Sorbitan monooleate) and Petroleum ether were purchased from the chemical reagent factory of Tianjin (Tianjin City, China).

Used instruments were as follows: Shimadzu-8400S FTIR spectrometer (Japanese Shimadzu Company), Unic-2602 UV spectrometer (American Unic Company), DDS-11Ar digit conductivity meter (Leici Instrument Limited Company of Shanghai), PHS-2 acidimeter (The Second Analytical Instrument Factory of Shanghai), TG16-WS high speed centrifuge (Changsha Xiangyi Centrifuge Factory, China) and THZ-92 constant temperature shaker equipped with gas bath (Boxun Medical Treatment Equipment Factory of Shanghai), XSZ-4 optical microscope with micrometer (the Taiyuan Optics Instrument Factory, China).

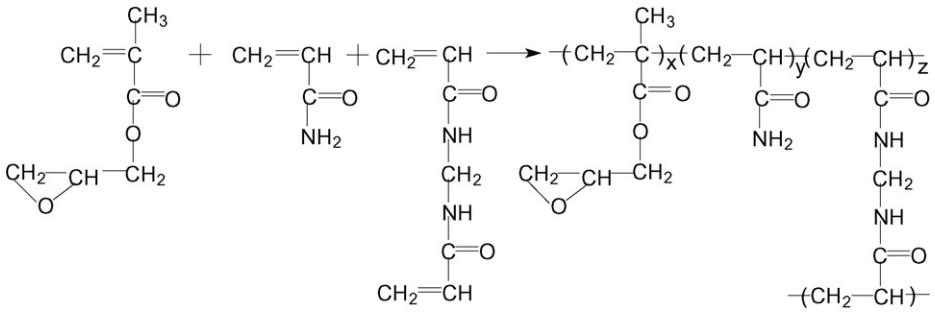
2.2. Synthesizing and characterizing of crosslinking and grafting microbeads

2.2.1. Synthesis and characteristic of microbeads GMA/AM/MBA

The crosslinking microbeads were prepared by suspension copolymerization of GMA, AM and MBA with AIBN as an initiator. The continuous phase consisted of 55 mL petroleum ether containing 0.456 g oil-soluble surfactant Span-80 which was used as suspension stabilizer, and the continuous phase was placed in a four-necked flask equipped with a mechanical stirrer, N_2 inlet and a condenser. 3.75 mL of GMA, 0.2 g of AM and 0.238 g of MBA were dissolved in 10 mL of mixed solvent of methanol and water (7:3, v/v), and the mixture was used as the dispersed phase in which the monomer feed ratio is equal to 10:1:0.5 (mole ratio). The dispersed phase was added into the continuous phase, the agitating speed was adjusted and the reaction mixture was stirred for 20 min to ensure good mixing of the two phases. The reactor was placed in a thermostat water bath, and when the mixture temperature was enhanced to 60 °C, a certain amount initiator (0.5 wt.%) was added, and under a nitrogen atmosphere, the copolymerization was carried out at the constant temperature of 60 °C for 6 h. After ending the reaction, the microbeads were filtered off, purified by extraction in a Soxhlet using acetone for 10 h. Finally, the product was dried under vacuum at 50 °C, and the white crosslinking microbeads GMA/AM/MBA were obtained. The chemical structure of the crosslinking microbeads was characterized, and their morphology and size (particle diameter) were confirmed by an optical microscope.

2.2.2. Preparation and characteristic of grafting microbeads PEI-GMA/AM/MBA

The crosslinking microbeads GMA/AM/MBA were placed in the mixture of dioxane and aqueous PEI solution (1:3, v/v), made to be sufficiently swelled, and then the grafting reaction was carried out at 85 °C for 36 h. After finishing the reaction, the microbeads were filtered out, washed repeatedly with distilled water and dried in a vacuum oven at 50 °C, and the grafting microbeads PEI-GMA/AM/MBA were gained. The FTIR spectrum was measured, the amount of amine groups on the surface of the grafting microbeads was determined by HCl-conductometric titration and the grafting degree (g/100 g) of PEI was further calculated. By varying the grafting reaction time, the grafting microbeads with different grafting degrees were obtained.



Scheme 2. Synthesis process of crosslinking microbead GMA/AM/MBA.

2.3. Determining adsorption property of grafting microbeads for bilirubin

2.3.1. Preparing bilirubin solution

Since bilirubin is decomposed by direct exposure to sunlight or any other light source, all preparation and adsorption experiments were performed under the condition of avoiding light. A certain amount of bilirubin was weighed, dissolved in a little of NaOH solution of 0.1 mol/L (3 mL), the solution was transferred into a volumetric flask of 100 mL, diluted to scale with phosphate buffer solution (pH = 7.4) with 0.1 mol/L (it was evaluated that the buffering capacity of the added phosphate buffer solution was over the amount of NaOH in bilirubin solution), and afterwards, the adsorption solutions of bilirubin with varying concentrations in series were prepared.

2.3.2. Measurement of adsorption dynamics

0.2 g of grafting microbeads PEI-GMA/AM/MBA was weighed, added into 10 mL of bilirubin solution, and the mixture was shaken on a shaker at a constant temperature of 30 °C. After a certain period, the mixture was centrifugally separated, the supernatant was sucked up and diluted, and the absorbance at 438 nm was determined. The repeated experiments were conducted but with different adsorption time. The absorption amount Q (mg/g) was calculated according to Eq. (1). The absorption amount Q as a function of time was plotted to determine the time needed for adsorption equilibrium (found to be about 150 min).

$$Q = \frac{(C_0 - C_1)V}{1000m} \quad (1)$$

where, C_0 and C_1 (mg/L) are the initial and final concentrations of bilirubin, respectively, V (mL) is the volume of the adsorption solution and m (g) is the mass of the grafting microbeads.

2.3.3. Determining adsorption isotherm

All the bilirubin adsorption experiments were conducted at 30 °C. The grafting microbeads with a fixed amount were added into the bilirubin solutions with different concentrations, respectively, the mixtures were shaken for 150 min, the concentrations of supernatants were determined, and the equilibrium adsorption amounts Q_e (mg/g) were calculated by Eq. (2).

$$Q_e = \frac{(C_0 - C_e)V}{1000m} \quad (2)$$

where, C_0 and C_e (mg/L) are the initial and final equilibrium concentrations of bilirubin, respectively, V (mL) is the volume of the adsorption solution and m (g) is the mass of the grafting microbeads.

2.4. Examining effects of various factors on adsorption capacity of grafting microbeads

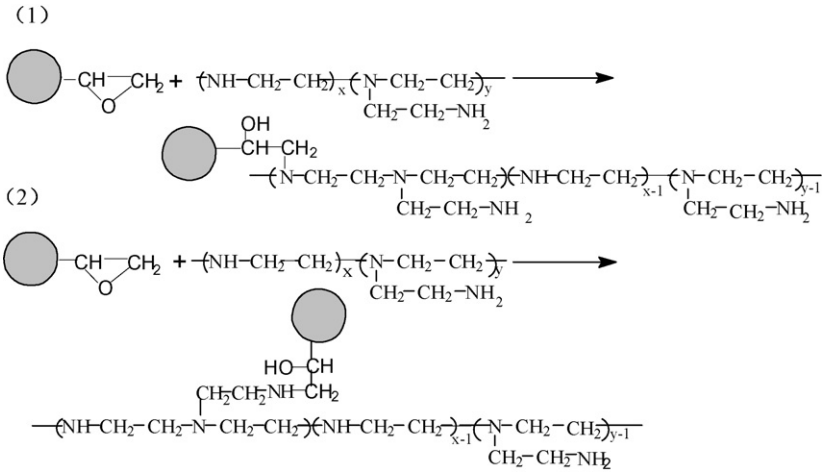
The isotherm adsorption experiments were performed by using the bilirubin solutions at various pH values, so that the effect of pH value on the adsorption capacity of the grafting microbeads for bilirubin was studied; the isotherm adsorption experiments were conducted by using the bilirubin solutions containing different NaCl concentrations, so that the effect of ionic strength on the adsorption capacity of the grafting microbeads for bilirubin was examined; the isotherm adsorption experiments were carried out in the presence of BSA whose concentration was half of bilirubin concentration, so that the effect of BSA on the adsorption capacity of the grafting microbeads for bilirubin was tested; the isotherm adsorption experiments were carried out by using the grafting microbeads with different grafting degree of PEI, so that the effect of the grafting degree of PEI on the adsorption capacity of the grafting microbeads for bilirubin was researched.

3. Results and discussion

3.1. Preparing process of crosslinking and grafting microbeads

In order to increase the biocompatibility of the product, a little of acrylamide was incorporated into the copolymer microbeads (10%, mole%) and methylene bisacrylamide was used as crosslinker. In the suspension copolymerization, methanol was used as the cosolvent of GMA, AM and MBA, but the solubility of MBA in methanol is limited. Based on this consideration, a little of water was added into methanol, and the optimal ratio of the mixed solvent is methanol: water = 7:3 (v/v). The process to synthesize the crosslinking microbeads GMA/AM/MBA is demonstrated in Scheme 2.

There are a lot of epoxy groups on the surface of the crosslinking microbeads GMA/AM/MBA. PEI macromolecules can be easily grafted onto the surface of the crosslinking microbeads in couple grafting manner via ring-opening reaction of epoxy



Scheme 3. Preparing process of grafting microbead PEI-GMA/AM/MBA.

groups, and the grafting microbeads PEI-GMA/AM/MBA are formed. The commercial PEI is usually branched, and there are primary, secondary and tertiary amine groups on its macromolecular chains and in the ratio of 1:2:1 [18]. According to the chemical structure of PEI, the couple grafting reaction can occur not only at the active sites of the primary amine groups but also at the active sites of the secondary amine groups, so the couple grafting reactions have two types, and they are given in Scheme 3.

3.2. Characterizing of chemical structures of polymer microbeads

Fig. 1 shows the infrared spectra of the crosslinking microbeads GMA/AM/MBA and grafting microbeads PEI-GMA/AM/MBA, respectively. In the spectrum of GMA/AM/MBA, the band at 1728 cm^{-1} is the characteristic absorption of ester carbonyl groups of GMA, and the bands at 906 cm^{-1} and 846 cm^{-1} represent the absorption of the epoxy groups of GMA. The band at 1648 cm^{-1} indicates the characteristic absorption of the primary acylamino of AM, and the band at 1147 cm^{-1} represents the characteristic absorption of the secondary acylamino of MBA. The appearances of these above bands reveal that the crosslinking copolymerization of GMA, AM and MBA has occurred and the crosslinking microbeads GMA/AM/MBA have been formed.

The FTIR spectrum of the grafting microbeads PEI-GMA/AM/MBA has some absorption bands different from those of the crosslinking microbeads GMA/AM/MBA. The most important absorption band at 1558 cm^{-1} representing N-H bending vibration absorption is due to polyethyleneimine grafted onto the crosslinking microbeads GMA/AM/MBA. Besides, the absorption of epoxy groups at 906 cm^{-1} and 846 cm^{-1} are greatly weakened, so the couple grafting of polyethyleneimine is further confirmed.

3.3. Adsorption behaviour of grafting microbeads PEI-GMA/AM/MBA for bilirubin

3.3.1. Dynamics curve of adsorption

In this research, first, the adsorption dynamics behaviour of grafting microbeads for bilirubin was studied, and Fig. 2 shows the adsorption rate curve. It is seen that there are relatively faster

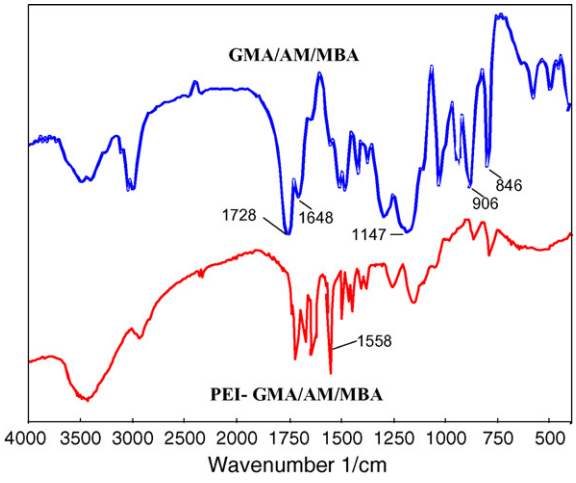


Fig. 1. FTIR spectra of two polymer microbeads.

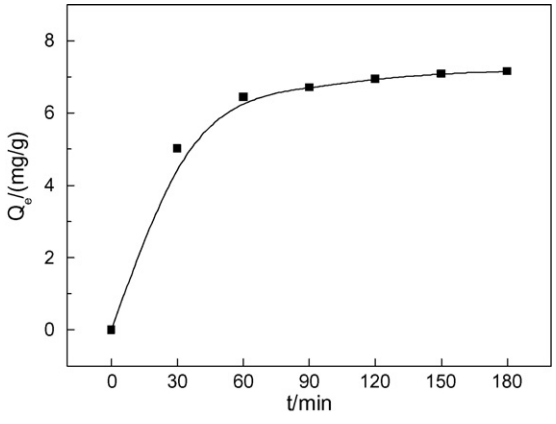


Fig. 2. Adsorption kinetic curve of grafting microbead for bilirubin. Preliminary concentration of bilirubin: 200 mg/L; pH = 7.4; temperature: 30 °C.

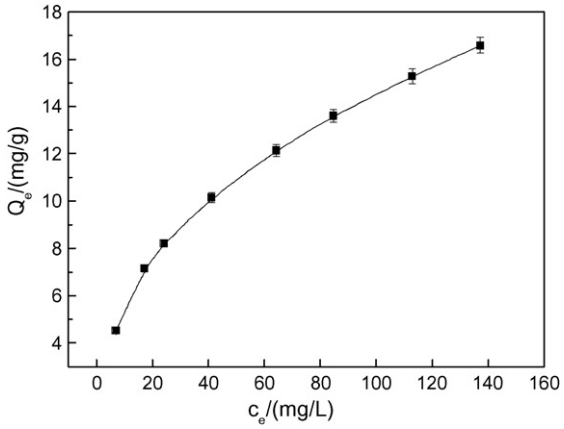


Fig. 3. Adsorption isotherm of PEI-GMA/AM/MBA for bilirubin. PEI grafting degree: 5.51 g/100 g; temperature: 30 °C; pH = 7.4; time: 150 min.

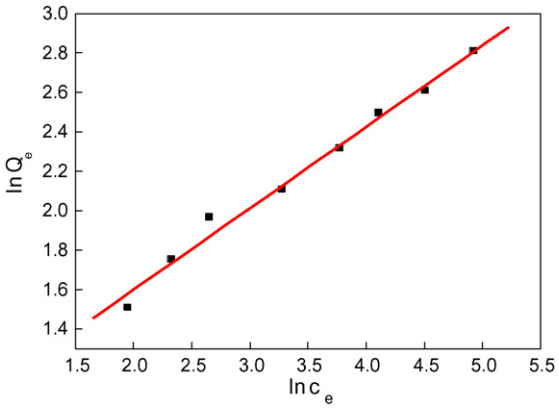


Fig. 4. Logarithm relationship between adsorption capacity and equilibrium concentration of bilirubin. Temperature: 30 °C; pH = 7.4; time: 150 min.

adsorption rates at the beginning, and then adsorption equilibrium is achieved gradually in about 150 min, so in the following adsorption experiments, all the adsorption periods are fixed as 150 min.

3.3.2. Adsorption isotherm

When using the grafting microbeads with a PEI grafting degree of 5.51 g/100 g, the relationship curve between the equilibrium adsorption amount and the equilibrium concentration of bilirubin at 30 °C is given in Fig. 3. With 500 mg/L of the initial concentration of bilirubin, finally the equilibrium concentration changes into 137 mg/L, and the equilibrium adsorption amount gets up to 16.59 mg/g, as shown in Fig. 3. The higher adsorption amount suggests that there is a strong affinity interaction between the adsorbent and adsorbate. Polyethyleneimine is a kind of cationic polyelectrolyte, whereas there are two carboxyls that can dissociate in bilirubin molecule, so by right of electrostatic interaction, the microbeads PEI-GMA/AM/MBA can produce a strong adsorption action against bilirubin. Freundlich isotherm and its logarithm form are Eqs. (3) and (4), respectively.

$$Q = kC^n \tag{3}$$

$$lg\, Q = n lg\, C + lg\, k \tag{4}$$

When the data in Fig. 3 are treated with linear regression according to the logarithm form of Freundlich equation, a straight line is obtained with a regression coefficient in 0.9937, as shown in Fig. 4. This fact indicates that the adsorption of bilirubin on the surface of the grafting microbeads fits tthe Freundlich isotherm, and implies a monolayer adsorption.

3.4. Effects of various factors on adsorption capacity

3.4.1. Effects of initial concentration of bilirubin

Fig. 5(a) shows the adsorption capacity increases rapidly with the increase of the initial concentration of bilirubin owing to the adsorption equilibrium moving. When the concentration of bilirubin changes from 100 mg/L to 500 mg/L, the adsorption amount enhances from 4.46 mg/L to 16.59 mg/g, and obviously, the initial concentration of bilirubin affects the adsorption capacity greatly.

3.4.2. Effects of pH value

Fig. 6 displays the relationship curve between the equilibrium adsorption amount and the initial concentration of bilirubin at different pH values. It can be clearly seen that the effect of pH values on the adsorption capacities of the grafting microbeads for bilirubin is very remarkable. By taking the data with an initial concentration of 200 mg/L in Fig. 6, the equilibrium adsorption amount as a function of pH value is plotted, as shown in Fig. 7.

From Fig. 7, the pH dependence of the adsorption capacity of the grafting microbeads for bilirubin is more clearly revealed. As the pH values of the medium are lower, the adsorption capacity is very small and increases rapidly with the increase of pH values; as pH reaches to 6, the adsorption capacity is the highest; afterwards, the adsorption capacity decreases with the increase of pH values. The changes of the adsorption capacities in different pH ranges reflect the variation of the interaction between the grafting microbeads and bilirubin.

Different from common cationic polyelectrolytes, the cationic degree of PEI is strongly affected by pH value. In acidic solution, 70% of N atoms in PEI molecules are protonized; in

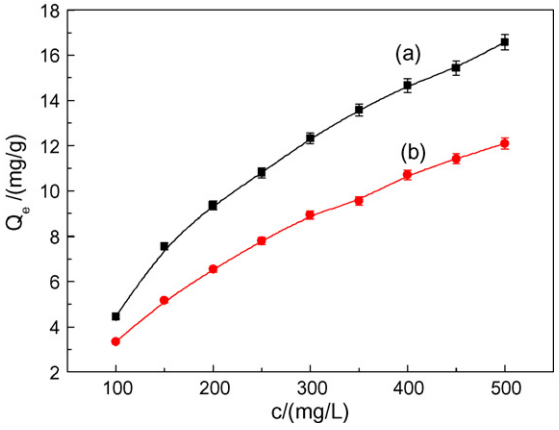


Fig. 5. Effect of initial concentrations of bilirubin on adsorption capacity. PEI grafting degree: 5.51 g/100 g; temperature: 30 °C; pH = 7.4; time: 150 min. (a) in absence of BAS; (b) in presence of BAS.

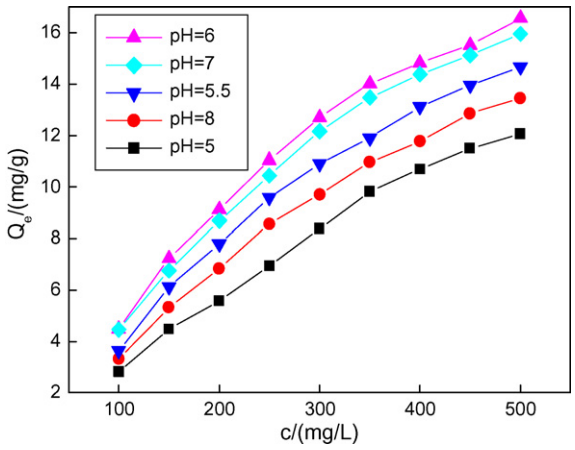


Fig. 6. Equilibrium adsorption amount as a function of initial concentration of bilirubin at different pH values. PEI grafting degree: 5.51 g/100 g; temperature: 30 °C; time: 150 min.

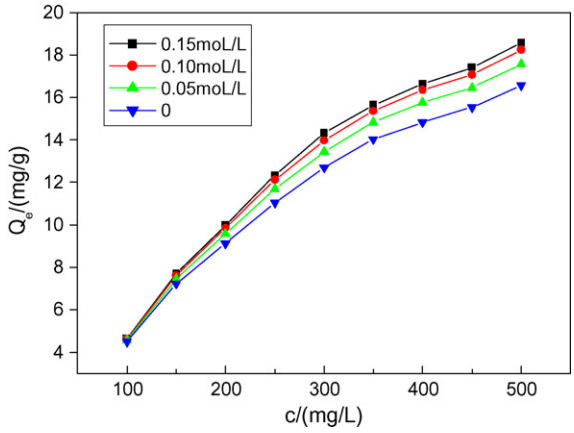


Fig. 8. Equilibrium adsorption amount as a function of initial concentration of bilirubin at different ionic strengths. PEI grafting degree: 5.51 g/100 g; temperature: 30 °C; time: 150 min.

neutral solution, protonized N atoms are about 60%; in basic solution of pH = 9, only 32% of N atoms are protonized; as pH = 10.5, the protonized degree is equal to zero [10]. On the other hand, in bilirubin molecule, the dissociation of two carboxyls (pK = 4.2–4.5) [7] strongly depends on the pH of the solution. So in different pH ranges, the existing forms of both the amine groups of PEI and carboxyls of bilirubin are different, resulting in the changes of the interaction between them.

In acidic solution, PEI has greater cationic degree, whereas the carboxyls of bilirubin exist as non-dissociating form. In addition, the imine groups of bilirubin are protonized, so the electrostatic repulsion between PEI and bilirubin makes the adsorption capacity of bilirubin on the grafting microbeads to be very low; along with the increase of pH, the dissociation degree of bilirubin carboxyls increases and the electronegativity of bilirubin molecules are strengthened, so the electrostatic attraction between PEI and bilirubin is gradually produced and reinforced, resulting in the increase of the adsorption capacity with pH rising. However, when pH > 6, the cationic degree of PEI decreases obviously and the electrostatic attraction between PEI and bilirubin reduces, resulting in the decrease of the adsorption capacity with pH rising. In basic solution, the cationic degree of PEI is very low, so the adsorption capacity of bilirubin on the grafting microbeads grows to be small.

3.4.3. *Effects of ionic strength*

Fig. 8 shows the relationship curve between the equilibrium adsorption amount and the initial concentration of bilirubin under the conditions of different ionic strengths. It can be observed that the adsorption capacities of bilirubin on the grafting microbeads increases slightly with ionic strength in a larger range of NaCl concentration, and this can be seen more vividly in Fig. 9, which comes from the data of 300 mg/L of bilirubin initial concentration in Fig. 7.

The result of the above experiment is different from that of other researches [6,7] in which the ionic strength always play a role to decrease the adsorption amount of bilirubin because of ionic atmosphere action. This research is of the opinion that the counteracting of the negativity influence of the ionic atmosphere and the positivity influence of the hydrophobic interaction leads to the above result. On one hand, at high ionic strength, the ionic atmospheres constituted by the counter ions produce

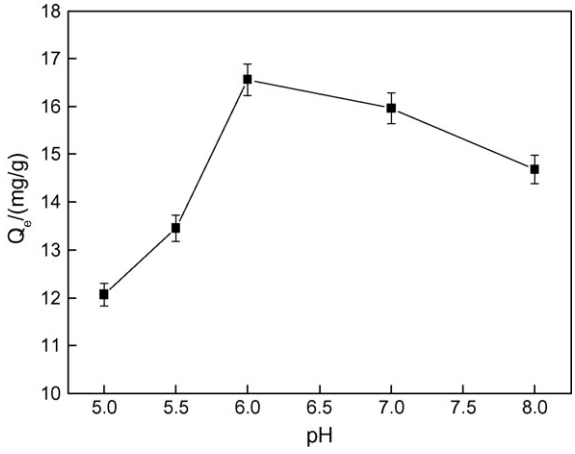


Fig. 7. Effect of pH on adsorption capacity. PEI grafting degree: 5.51 g/100 g; Initial concentration of bilirubin: 500 mg/L; temperature: 30 °C; time: 150 min.

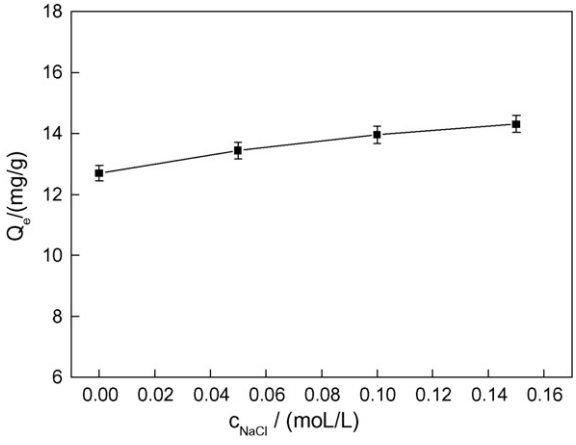


Fig. 9. Effect of ionic strengths on adsorption capacity. Initial concentration of bilirubin: 300 mg/L; temperature: 30 °C; time: 150 min.

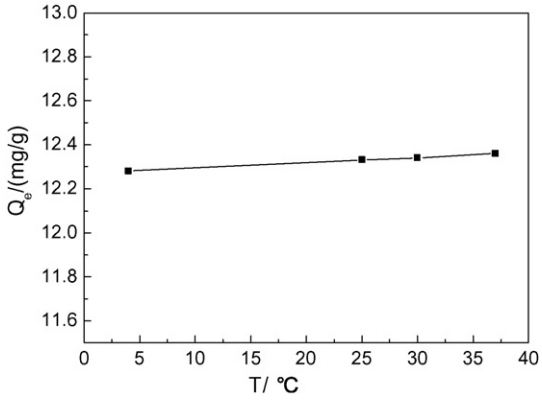


Fig. 10. Effect of temperature on adsorption capacity. Initial concentration of bilirubin: 300 mg/L; time: 150 min.

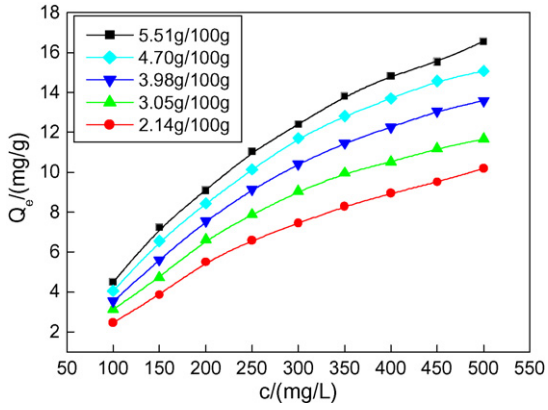


Fig. 11. Equilibrium adsorption amount as a function of initial concentration of bilirubin using grafting microbeads with different grafting degree of PEI. Temperature: 30 °C; time: 150 min.

shielding effects not only for the charges of protonized amine groups but also for the charges of dissociated carboxyls so that the electrostatic interaction between the grafting microbeads and bilirubin is weakened with the increase of the ionic strength. On the other hand, bilirubin molecule is lipophilic, and the matrix of the grafting microbeads is hydrophobic at a certain extent. High ionic strength will promote the hydrophobic interaction between them, so the positivity influence of the hydrophobic interaction makes the adsorption capacity of bilirubin on the grafting microbeads to be increased. As a consequence, the counteracting of the two opposite actions results in that the adsorption capacities of bilirubin change with ion strength mildly. Perhaps, the positivity influence of the hydrophobic interaction is slightly preponderant, so the adsorption capacities increase slightly with ionic strength.

3.4.4. *Effects of temperature*

The effect of temperature was studied under various temperatures, shown in Fig. 9.The adsorption capacity increases slightly with the increase of temperature. The result was similar to that of other related experiments [3,7]. The hypothesis for this phenomenon is that the hydrophobic interaction between the grafting microbeads and bilirubin was strengthened probably. The enhancement of temperature is in favor of hydrophobic interaction, and the contact surface area between the bilirubin molecules and grafting microbeads increase at higher temperatures, resulting in an increase in the affinity of the biomolecules for the adsorbent and in the increase of adsorption capacity (Fig. 10).

3.4.5. *Effects of BSA in the adsorption medium on the adsorption capacity*

Serum album is a natural carrier for bilirubin in the blood. Bilirubin in blood exists partially as complex with serum album. So the effect of BSA on the adsorption was studied. When the molar ratio of bilirubin to BSA in the adsorption medium was adopted as 2:1, the varying of the adsorption capacity with the initial concentration of bilirubin is shown in Fig. 5(b). It is obvious that the adsorption capacity of the grafting microbeads for bilirubin decreases to a certain extent because of the presence of BSA in the medium. This is the result of compete combination of BSA with the microbeads for bilirubin. However, the grafting microbeads PEI-GMA/AM/MBA still have a certain capability of complete adsorption for bilirubin in the presence of BSA. Thus, the grafting microbeads PEI-GMA/AM/MBA have higher affinity towards bilirubin, and are valuable for the removal of bilirubin.

3.4.6. *Effects of grafting degree of PEI*

Fig. 11 gives the relationship curve between the equilibrium adsorption amount and the initial concentration of bilirubin using the grafting microbeads with different grafting degrees of PEI. It is observed that the adsorption capacity differences for the grafting microbeads with different grafting degrees are great. By taking the data of 300 mg/L of bilirubin initial concentration in Fig. 11, the equilibrium adsorption amount as a function of the grafting degrees is plotted, as shown in Fig. 12, and the effect of grafting degree of PEI on the adsorption capacity of the grafting microbeads for bilirubin is more clearly displayed. It can be seen that the adsorption capacity increases with the enhancement of the grafting degrees owing to the increase of amino group amount on the surface of the grafting microbeads. Whereas as PEI grafting degrees are higher, the increase of the adsorption capacity becomes gentle, and the reason for this is

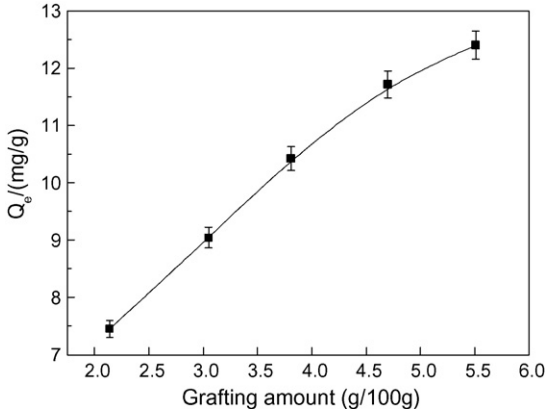


Fig. 12. Effect of PEI grafting degree on adsorption capacity. Initial concentration of bilirubin: 300 mg/L; temperature: 30 °C; time: 150 min.

that as the grafting degree of PEI is greater, the layer of PEI macromolecules on the microbeads becomes thick, and many amine groups cannot be in contact with bilirubin molecules due to wrapping and covering of PEI macromolecules with each other.

4. Conclusions

In this paper, crosslinking terpolymer microbeads of glycidyl methacrylate, acrylamide and *N*,*N*′-methylene bisacrylamide were synthesized by suspension copolymerization, then polyethyleneimine was grafted onto the surface of the crosslinking microbeads and the grafting microbeads PEI-GMA/AM/MBA with biocompatibility were successfully prepared. The grafting microbeads has excellent adsorption property for bilirubin by right of the electrostatic interaction between the protonized amine groups of polyethyleneimine and the dissociated carboxyls of bilirubin. The adsorption capacity is greatly affected by the pH of the medium, and in near neutral solution of pH = 6, the grafting microbeads have the highest adsorption capacity. Besides, the ionic strength nearly does not affect the adsorption property of the grafting microbeads prepared in this work, and this is obviously different from the reports of other literature. The grafting microbeads PEI-GMA/AM/MBA as biomedical adsorption microsphere are promising for adsorbing bilirubin through hemoperfusion technique. About the blood compatibility of the grafting microbeads, further study is needed to be carried out.

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