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Fabrication and characterization of rigid magnetic monodisperse microspheres for protein adsorption

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Abstract

This article describes the fabrication of a rigid magnetic monodisperse bead (M-PGMA-TRI, 4.92 μm) with polyglycidyl methacrylate (PGMA) cross-linked by trimethylolpropane trimethacrylate (TRI). This was realized by adding a proper amount (2%, w/w) of TRI after 3 h of the dispersion-polymerization reaction with the monomer of GMA. The mono-sized microspheres were further processed to introduce magnetic granules by sulfonation and penetration-deposition approaches. The monodisperse bead (M-PGMA) without TRI addition was also fabricated for comparison. The morphology, size and magnetic characteristics of the microspheres were extensively characterized. The M-PGMA-TRI microspheres were nonporous, of smooth surface and superparamagnetic with a saturation magnetization of 13.0 emu/g. Recycled use of the material for protein adsorption exhibited stability of the magnetic properties of the M-PGMA-TRI, as compared to the significant loss of the saturation magnetization of the M-PGMA. The chemical stability of the M-PGMA-TRI was also confirmed by examining its protein adsorption and magnetic properties after incubation in various solutions such as acidic buffer (pH 2.2) for 24 h. The adsorption capacity of γ-globulin reached 287.2 mg/g and kept stable in the repeated adsorption/desorption/regeneration cycles. The results indicated that the introduction of 2% TRI was promising for producing rigid magnetic mono-sized microspheres for protein adsorption.

Keywords: Dispersion-polymerization; Penetration-deposition; Monodisperse; Magnetic polymer microsphere; Protein adsorption

1. Introduction

Polymeric magnetic microspheres (PMMs) refer to polymer beads containing a magnetic metal or its oxide core [1]. To date, PMMs have found wide applications in biotechnological and medical fields such as cell separation [2], enzyme immobilization [3] and protein purification [4–8] for their advantages in the ease of recovery, stable and robust physical and chemical properties, low cost, superior biological compatibility and variety of active functional groups [9–11]. Hence, fabrication of polymeric microspheres with high magnetic responsiveness, superparamagnetism and monodisperse or narrow size distribution has attracted much attention of researchers in recent years.

There are several ways of preparing the PMMs, usually including coating or encapsulation of magnetic particles with polymers [12] and monomer polymerization. The former is simple, but the resulted microspheres are usually polydisperse and of irregular shapes. The latter mainly includes suspension polymerization, emulsion polymerization and dispersion polymerizations. Generally, suspension polymerization [13] is suitable for preparation on a large scale, but the particle size distribution is wide in range from tens to hundreds of microns. Recently, Ma et al. [5] prepared micron-sized magnetic poly-(vinylacetate-divinyl benzene) microspheres, using a modified suspension polymerization approach. Their bead size was controlled within 10 μm, but the size distribution was still wide and a part of the microspheres lacked sphericity. In the case of products prepared by emulsion polymerization [14], however, there are still drawbacks such as weak magnetic responsiveness, low particle yield and severe flocculation of microspheres. So, dispersion polymerization is considered to be the most favorable approach to the preparation of monodisperse microspheres [15]. At present, however, the dispersion-polymerization method still needs to overcome some obstacles for its further development and wide application. For example, the manufacturing pro-

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cess is hard to control because of the inherent inhomogeneity of the reaction system, and the products often contain only a small loading of magnetic materials. Furthermore, conventional dispersion-polymerization has employed no or less amount of cross-linkers, usually not more than 0.2% (w/w) [16], to guarantee a monodisperse feature of the microspheres. For example, Ma et al. [17] prepared monodisperse superparamagnetic PGMA microspheres by dispersion-polymerization and penetration-deposition approach; no cross-linker was introduced in their procedure. Huang et al. [16] prepared poly(methyl methacrylate) microspheres with narrow size distribution with only 0.2% (w/w) cross-linker, while partial flocculation was found at 0.5% cross-linker addition. The lack of cross-linkers in the polymerization would weaken the physical and chemical stabilities of a magnetic microsphere and compromise its long-term and repetitive use. So, the main aim of the present work is the development of a dispersion-polymerization procedure at higher cross-linker concentration.

There have been efforts on introducing cross-linkers to the polymerization system to improve the properties of polymeric microspheres. For example, Horak [18] and Horak et al. [19] prepared magnetic polyglycidyl methacrylate (PGMA) and magnetic polyhydroxyethyl methacrylate (PHEMA) microspheres with cross-linker (ethylene dimethacrylate) in the presence of iron oxide by a modified dispersion-polymerization. It was found that the iron oxide existed both inside and outside the microspheres, with unfavorable particle morphology. Ugelstad et al. [20] developed a two-step swelling method for the preparation of magnetic microspheres, but the procedure was complicated and seemed difficult for application to large-scale preparations.

Hence, we have adopted the dispersion-polymerization [21] and penetration-deposition [17] approaches to synthesize monosized superparamagnetic polymer microspheres. The procedure was modified to be useful for the introduction of a cross-linker during the polymerization, thus leading to a rigid polymeric monodisperse superparamagnetic microsphere of high magnetic responsiveness. The microspheres were functionalized by coupling sulfonate groups and characterized for protein adsorption. The rigidity of the cross-linked product was compared with that prepared without using a cross-linker.

2. Experimental

2.1. Materials

Trimethylolpropane trimethacrylate (TRI) (analytical grade) was obtained from Aldrich (Milwaukee, USA). Pig γ-globulin, and sodium salt of ethylinediaminetetraacetic acid (EDTA) was purchased from Sigma (St. Louis, MO, USA). Glycidyl methacrylate (GMA) (99%) was a product of Shanghai Yuanji Company (Shanghai, China) and used without further purification. Polyvinylpyrrolidone (PVP K-30) (analytical grade) and 2,2′-azobis-(isobutyronitrile) (AIBN) (analytical grade) were received from Tianjin Damao Company (Tianjin, China). All other chemicals were of analytical grade from local sources.

2.2. Preparation of monodisperse polymeric microspheres

Poly(GMA-TRI) (PGMA-TRI) microspheres were prepared by a modified dispersion-polymerization method similar to that reported previously [21]. The polymerization was carried out in a 500-mL three-necked, round-bottom flask equipped with a condenser. At first, a mixture of 103 mL ethanol, 3 g PVP K-30 and 9 mL water was introduced into the flask and stirred for 10 min at room temperature. Next, nitrogen was purged for 10 min to exclude oxygen. Then, 9.3 mL GMA and 0.2 g AIBN were added into the flask while keeping stirring (200 rpm) at 70 °C for several hours (e.g., 3 h). Thereafter, a predetermined amount of TRI (e.g., 0.2 g) was introduced into the flask and the reaction continued to make the total reaction time reach 24 h. The resulting polymer beads were recovered by centrifugation and washed routinely with distilled water. Finally, the microspheres were lyophilized under vacuum. The product was denoted as PGMA-TRI.

The particle yield in the preparations was calculated by the weight ratio of the recovered microspheres to the theoretical output calculated based on the input of the monomers (including cross-linking agent if any).

2.3. Synthesis of cation exchanger

Sodium sulfite was used to react with PGMA-TRI microspheres to introduce sulfonate groups on the particles. The coupling of the negatively charged groups would also make it easy for the Fe^{2+} ions to impregnate into the microspheres in the preparation of magnetic beads in the following penetration-deposition procedure (see below in Section 2.4). In the reaction, 2 g of dried PGMA-TRI beads was added to 100 mL of 100 g/L Na_2SO_3 in an Erlenmeyer flask. Then, two droplets of isopropanol were added into the suspension. The suspension was shaken at 40 °C for 8 h in an incubator of 200 rpm. The microspheres were then recovered by centrifugation and washed with distilled water. The product, denoted as PGMA-TRI-SO_3^-, was lyophilized under vacuum.

2.4. Preparation of magnetic cation exchanger

Magnetic cation exchange resin was prepared by the penetration-deposition method [17] with some modifications. In brief, 2 g of PGMA-TRI-SO_3^- was added to 100 mL water in an Erlenmeyer flask. The suspension was stirred to make the microspheres disperse uniformly in the system. The solution was adjusted to pH 3 by adding 6 mol/L hydrochloric acid, and then 1.67 g $FeSO_4 \cdot 7H_2O$ was introduced. The system was purged with nitrogen for 30 min and shaken at 25 °C for 12 h. Then, 0.21 g of $NaNO_2$ and 25 mL of 25% (w/w) ammonia were added into the flask, and the system was kept shaking at 40 °C for another 2 h. The solid microspheres were then separated by magnetic sedimentation and washed by distilled water and 0.5 mol/L HCl for more than 20 times. The magnetic cation-exchange resin, named M-PGMA-TRI, was thus obtained.

Magnetic microspheres without cross-linking were also prepared without adding TRI in the dispersion-polymerization

described in Section 2.2 and treated by the same procedures described above. This microsphere was denoted as M-PGMA.

2.5. Flow hydrodynamic experiments

To test the mechanical strength of the microspheres, the magnetic resins (cross-linked or not cross-linked) was packed into a stainless steel column (50 mm × 4.6 mm) by a dry method. The flow hydrodynamic experiments were carried out on an Agilent 1100 HPLC system (Agilent Technologies, DE, USA). The backpressure of the column as a function of mobile-phase (distilled water) flow velocity was thus determined.

2.6. Protein adsorption experiments

Protein adsorption was performed in a batch adsorption system and the same batch of bead preparation was used in all the adsorption experiments. Unless stated otherwise, fresh beads were used for protein adsorption. γ-Globulin was used as a model protein to test the adsorption properties. Typically, 50 mg of lyophilized resin was mixed with 10 mL of adsorption buffer (0.1 mol/L acetate buffer, pH 5.0) solution with γ-globulin. Aqueous phase ionic strength was adjusted with NaCl. The experiment was conducted at 25 °C for 4 h in a shaking incubator at 200 rpm. The adsorbent was separated by magnetic sedimentation. The supernatant was assayed for residual protein concentration with an ultraviolet–visible spectrophotometer at 280 nm. The protein binding amount on the magnetic supports was calculated from mass balance [Eq. (1)].

$$q = \frac{(c_0 - c)v}{w} \quad (1)$$

where q is the protein binding amount (mg/g dry resin), c_0 the initial protein concentration (mg/mL), c the liquid phase concentration of protein in equilibrium with q (mg/mL), v the protein solution volume (mL) and w is the mass of the dry microspheres (g).

The effect of pH on γ-globulin adsorption to the M-PGMA-TRI was studied at the pH range of 2.6–8.0, with 0.1 mol/L acetate buffer (pH 2.6–5.0) and 0.1 mol/L phosphate buffer (pH 6.0–8.0). The experiments were carried out by the same procedures as described above. PGMA-TRI beads (without sulfonation) were used for protein adsorption at the same pH range to test the nonspecific adsorption (besides ionic exchange) of the resin material.

2.7. Stability of the magnetic monodisperse microspheres

To examine the chemical stability of the magnetic material, the M-PGMA-TRI beads were dipped in various solutions for different times. The solutions included 0.1 mol/L NaOH (pH 13), 0.1 mol/L glycine–HCl buffer (pH 2.2), 0.1 mol/L EDTA and 0.1 mol/L $MgSO_4$. The suspensions were incubated in a shaking incubator at 25 °C for different times. Then, the magnetic beads were recovered by magnetic separation and rinsed extensively. The regenerated beads were then used for γ-globulin adsorption. The adsorption experiments were carried out by the same procedures as described above.

To examine the stability of the magnetic adsorbents in long-term applications, their repetitive use was carried out by regenerating the microspheres after adsorption. In this experiment, 50 mg of lyophilized resin was mixed with 10 mL of 2.012 mg/mL γ-globulin solution in the adsorption buffer. The adsorbed protein was then dissociated from the separated solid phase by adding 10 mL of 1 mol/L NaCl solution in 0.1 mol/L acetate buffer (pH 5.0), and the solid phase was recovered by magnetic sedimentation and washed with the adsorption buffer for regeneration. The microspheres were magnetically separated again and used for the next adsorption/desorption/regeneration cycle.

In the above stability studies, the magnetic properties of the microspheres after treatment in different solutions or after repetitive use were measured as described in the following section.

2.8. Characterization

Particle size distribution was estimated by Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., Malvern, UK). In addition, the bead morphology was observed by environment scanning electron microscopy (ESEM, Model XL30ESEM, Philips, The Netherlands). The magnetic properties of the microspheres were measured by vibrating sample magnetometry (VSM) (Model 9600-1 VSM, LDJ Electronics, MI, USA).

3. Results and discussion

3.1. Determination of the time point and amount of cross-linker addition

We have discussed the necessities of introducing a cross-linker in the dispersion-polymerization to prepare monodisperse microspheres. However, it has been difficult to control the size distribution when a cross-linker was added in the polymerization system. For example, when over 0.2% cross-linker was added the resulting microspheres became obviously polydisperse with a wide size distribution [16]. Hence, first of all, we attempted to find a proper polymerization condition to produce cross-linked mono-sized microspheres. This was done by varying the addition amount as well as the addition time of TRI after the reaction had started with the monomer of GMA.

Table 1 lists the typical results we obtained when searching the conditions of cross-linker addition. It can be seen that

Table 1
Variations of particle size and dispersity with the addition amount as well as addition time of TRI after the reaction had started with the monomer of GMA

TRI content (%, w/w)	Addition time (h)	Particle size (μm)	Dispersity
0	–	5.13 ± 1.7	Monodisperse
2	0	0.601–6.691	Polydisperse
2	3	4.92 ± 1.5	Monodisperse
5	3	1.596–13.235	Polydisperse
20	3	1.102–12.345	Polydisperse

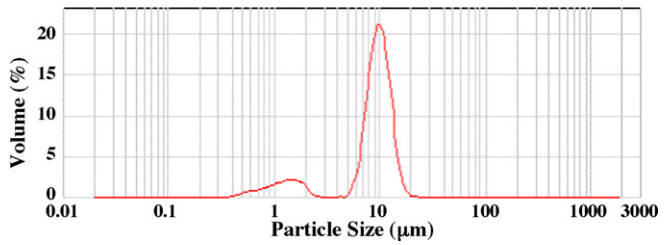

Fig. 1. Size distribution of the microspheres prepared by adding 20% TRI in 3 h after the reaction started.

the particle size distribution was significantly affected by the addition amount as well as the addition time of TRI after the reaction had started with GMA. The microspheres were polydisperse if 2% cross-linker was added at the beginning of the reaction, while they became uniform if the same amount of the cross-linker was introduced in 3 h after the reaction had started. The additions of 2% TRI to the reaction system at other time points were also attempted, but no another condition created mono-sized microspheres (data not shown). So, further experiments were carried out under the conditions of adding 5% or 20% of the cross-linker in 3 h after the reaction had started. As shown in Table 1, however, it was observed that the microspheres were all polydispersed at other conditions. Fig. 1 gives an example of the size distribution of the microspheres prepared by adding 20% TRI in 3 h after the reaction had started. Bimodal size distribution was observed when the cross-linker was over-introduced.

The differences in the dispersity of the microspheres obtained under different TRI adding conditions would be attributed to the effect of the cross-linker on the formations of the primary and secondary nuclei [17]. The cross-linker should be added when the nucleus formations have finished and the addition of the cross-linker must not initiate additional nucleus formations. So, the addition of TRI at improper time and/or amounts resulted in unexpected nucleus formation, leading to the polydispersity of the beads.

3.2. Mechanical strength and surface morphology of the monodisperse microspheres

In Table 1, two mono-sized microspheres produced with and without TRI addition are provided. It can be seen that the two media are similar in size. To compare their mechanical strength, the microspheres were separately packed into stainless steel columns (50 mm length, 4.6 mm i.d.), and the backpressure (Δp) as a function of flow velocity (u) was plotted (Fig. 2). It is obvious that the column packed with the cross-linked polymer beads (PGMA-TRI) had much lower pressure drop than that packed with the PGMA. Since the two resins had similar particle sizes, it is considered that the compressibility of the PGMA column was the main reason for its high backpressure. In other words, the cross-linked resin PGMA-TRI possessed much higher rigidity than PGMA. To confirm this speculation, the bed voidages were estimated from the Δp versus u plots. The backpressure of packed columns can be expressed by the Kozeny-Karman

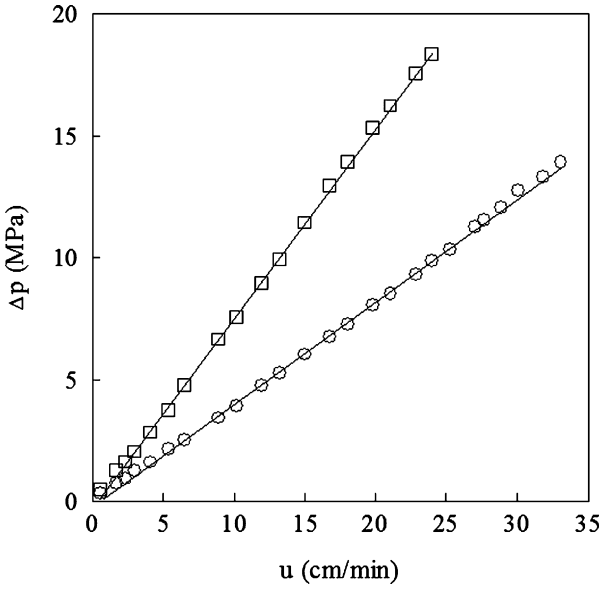

Fig. 2. Backpressure of the column (4.6 mm × 50 mm) as a function of superficial velocity of mobile-phase (deionized water). The columns were packed with (□) PGMA and (◯) PGMA-TRI. The solid lines are calculated from Eq. (2).

equation [22]:

$$\frac{\Delta p}{L} = 150 \times \frac{\mu u}{d_p^2} \frac{(1-\varepsilon)^2}{\varepsilon^3} \quad (2)$$

where L is the column length, μ the mobile-phase viscosity (Pa s), u the superficial velocity (m/s), d_p the mean particle diameter (m) and ε is the bed voidage. From Eq. (2) and the slopes of the plots, the bed voidages of the two columns were estimated at PGMA-TRI column: $\varepsilon = 0.344$; PGMA column: $\varepsilon = 0.296$. So, it becomes clear that the PGMA column had much lower bed voidage. It might be caused by the bead compressibility. The result has thus demonstrated the high mechanical strength of the PGMA-TRI beads and the necessity of cross-linking for preparing such a mono-sized microsphere. Preparations of the PGMA-TRI by addition 2% TRI to the dispersion-polymerization system have been repeated for more than 10 times and good reproducibility was achieved. The product yields were $82 \pm 4\%$ in the repetitive preparation experiments.

The two kinds of magnetic monodisperse microspheres were also characterized by ESEM observation (Fig. 3). Although the two media are similar in size (see Table 1), the cross-linked microspheres M-PGMA-TRI had smother surface morphology than the M-PGMA. This would be another reason for the lower backpressure of the PGMA-TRI column.

3.3. Magnetic properties of the monodisperse microspheres

The magnetic properties of the two magnetic monodisperse beads were measured by the VSM. As shown in Fig. 4, the magnetic beads showed typical sigmoid magnetization curves, which is a property of superparamagnetism. As listed in Table 2, the residual magnetizations of the M-PGMA-TRI and M-PGMA were as low as 0.346 and 0.598 emu/g, respectively, so the two magnetic materials can be regarded as superparamagnetic.

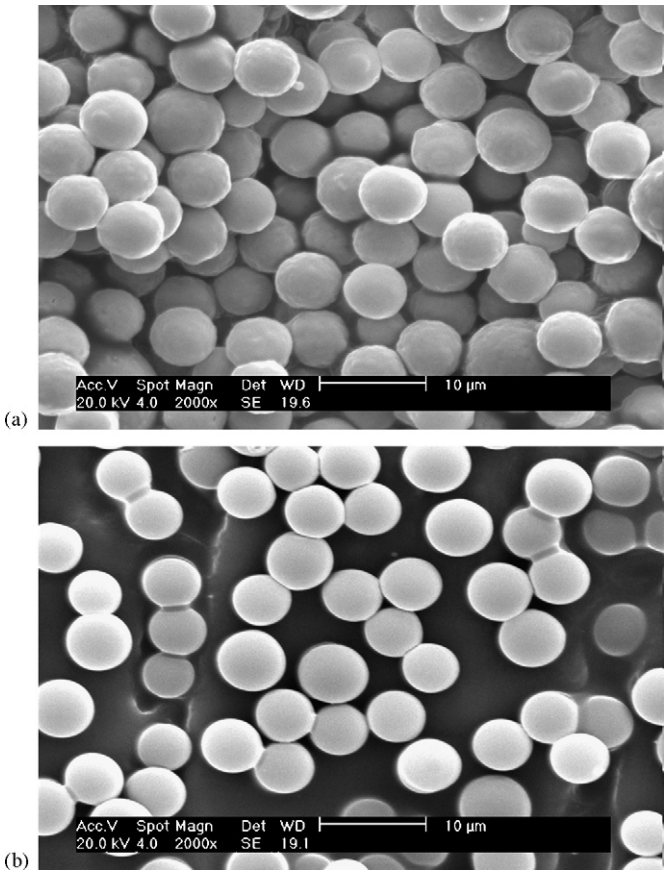

Fig. 3. ESEM photographs of (a) M-PGMA and (b) M-PGMA-TRI.

The saturation magnetization of the cross-linked bead reached 13.0 emu/g, higher or comparable to other resins produced by dispersion-polymerization or by suspension polymerization [17,23–25].

It is obvious from Fig. 4 and Table 2 that the M-PGMA had higher saturation magnetization than the M-PGMA-TRI. To examine the stability of the magnetic properties of the beads, we have used the microspheres for recycled protein adsorption (see Sections 2.7 and 3.4) in a shaking incubator. After five cycles of adsorption/desorption/regeneration, their magnetization curves were obtained once again, and the results are also illustrated in Fig. 4 and Table 2. As can be seen, the saturation magnetization of the M-PGMA-TRI changed little after the repetitive use, while that of the M-PGMA drastically decreased over 50%. The phenomenon implies that under the condition of shaking in acetate buffer, the magnetic granules inside the M-PGMA were prone to escape. It could be caused by the loose bead structure and instability of the skeleton of the uncross-linked polymer. In contrast, the M-PGMA-TRI was rigid and robust to keep the magnetic granules inside it in a long-term application.

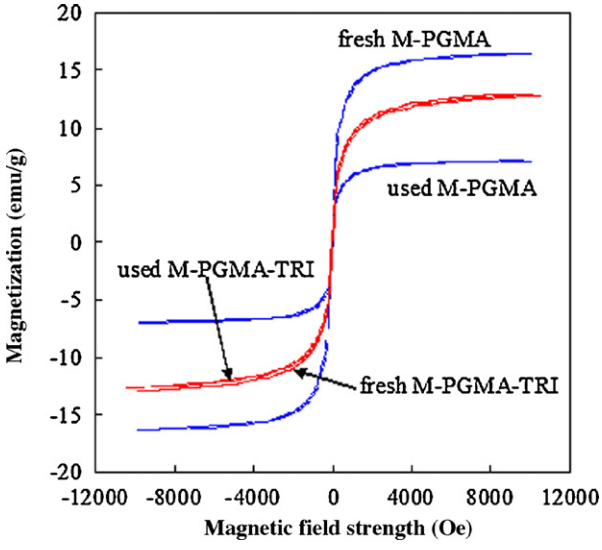

Fig. 4. Magnetization curves of the fresh and used (after five cycles of protein adsorption/desorption/regeneration) monodisperse magnetic microspheres (M-PGMA-TRI and M-PGMA) at room temperature.

Table 2
Properties of the M-PGMA-TRI microspheres

Magnetic bead	M-PGMA-TRI	M-PGMA
Fresh microspheres		
Saturation magnetization (emu/g)	13.0	16.4
Residual magnetization (emu/g)	0.346	0.598
Coercivity (Oe)	6.75	8.97
After five time repeated use		
Saturation magnetization (emu/g)	12.9	7.01
Residual magnetization (emu/g)	0.339	0.563
Coercivity (Oe)	6.71	10.97

After incubation of the M-PGMA-TRI in acidic and alkaline solutions, the magnetization curves were obtained once again. The result showed that the magnetic properties of beads did not change after 24-h incubation in 0.1 mol/L glycine–HCl buffer (pH 2.2), but the saturation magnetization decreased to 10.6 emu/g after 10-h incubation in 0.1 mol/L NaOH solution. This indicates that the magnetic beads were stable in the acidic solution. So, an acidic buffer would be favorable for the regeneration of the magnetic beads after protein adsorption.

3.4. Protein adsorption

M-PGMA-TRI has been used for protein adsorption studies. The ion exchange capacity was determined to be 4.2 mmol/g dry resin. At first, the time course of γ-globulin adsorption was examined by incubation of 0.05 g of lyophilized resin with 10 mL of 2.012 mg/mL γ-globulin solution. It was found that the binding amount increased rapidly in the initial adsorption period, reached 201.2 mg/g dry resin in 15 min and then increased slowly to about 279.0 mg/g dry resin after 1.5 h adsorption. This is similar to other reports related to protein adsorption to nonporous materials [26].

The adsorption isotherms of γ-globulin to the magnetic cation exchanger with sulfonate groups were then carried out at different salt concentrations. The results are indicated in Fig. 5. The solid lines were calculated by the Langmuir equation [Eq. (3)] by least-squares fitting.

$$q = \frac{q_m c}{K_d + c} \quad (3)$$

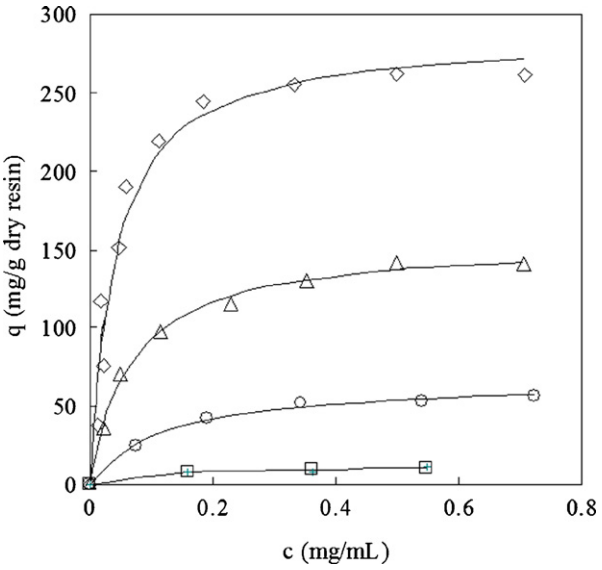

Fig. 5. Adsorption isotherms of γ-globulin onto the M-PGMA-TRI beads in 0.1 mol/L acetate buffer (pH 5.0) at 25 °C. (◊) no NaCl, (△) 0.3 mol/L NaCl; (◯) 0.5 mol/L NaCl; (□) 0.8 mol/L NaCl. Triplicate measurements were done and average values were indicated. The relative standard deviations were within 3.2%.

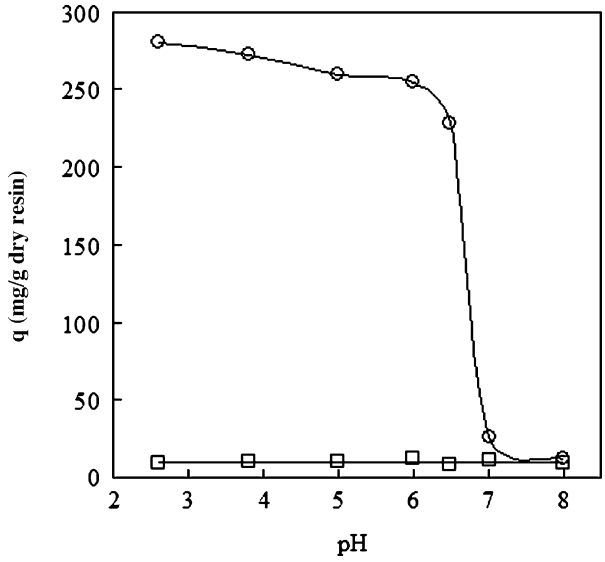

Fig. 6. Effect of pH on γ-globulin adsorption to (◯) M-PGMA-TRI and (□) PGMA-TRI. Triplicate experiments were done and average values were indicated. The relative standard deviations were within 4.1%.

where q_m is the adsorption capacity (mg/g dry resin) and K_d is the dissociation constant (mg/mL). It was determined that the adsorption capacity of the cation exchanger was as high as 287.2 mg/g dry resin when no sodium chloride was introduced to the buffer solution. This value was comparable to the highest value (282.7 mg γ-globulin/g dry resin) reported by Bayramoglu et al. for their GMA/MMA microspheres prepared by suspension polymerization [27].

Fig. 5 also shows that with the increase of NaCl concentration, the adsorption of γ-globulin decreased significantly. It is a typical feature of ion exchange adsorption. It also implies the lack of other protein adsorption mechanisms besides the electrostatic interaction, indicating that the bead surface was hydrophilic and suited as a protein adsorption medium. Because little protein was adsorbed at a salt concentration of 0.8 mol/L (Fig. 5), the adsorbed protein at low ionic strength would be dissociated in a high-ionic strength solution. So, in the recycling experiments of the magnetic beads, 1.0 mol/L NaCl in 0.1 mol/L acetate buffer (pH 5.0) was used as the eluent.

Fig. 6 shows the effect of pH on γ-globulin adsorption to the M-PGMA-TRI and PGMA-TRI. As can be seen, the amount of the protein adsorbed on the M-PGMA-TRI decreased slowly as the pH was increased from 2.6 to 6.0, and then drastically decreased when the pH was further increased above 7.0. This is because γ-globulin is negatively charged at pH higher than 7 for it has an isoelectric point of about 6.5.

Fig. 6 also shows that γ-globulin adsorption to the PGMA-TRI was lower than 10 mg/g in the pH range studied. Because PGMA-TRI was not sulfonated, this result has further indicated that the skeleton material of the M-PGMA-TRI was quite hydrophilic for low protein adsorption.

In order to test the stability of the M-PGMA-TRI in repetitive protein adsorptions, we have recycled the magnetic cation exchanger for γ-globulin adsorption. Fig. 7 summarizes the results of γ-globulin binding amount and elution recovery in the five recycled use. The recovery yields were kept at 92% to 93%. This is a high yield because batch elution with 1.0 mol/L NaCl solution was used for the desorption. Due to the incomplete desorption, the binding amount declined gradually, but it was still as high as 247 mg/g at the fifth adsorption, which was only 5.2% lower than the first use of the cation exchanger. The results indicated the stability of the M-PGMA-TRI for protein adsorption. Similar results were achieved with the M-PGMA: the binding amount to M-PGMA in the first use was 298.1 mg/g dry resin and decreased to 280.2 mg/g dry resin in the fifth test. Due to the magnetic instability of the uncross-linked material

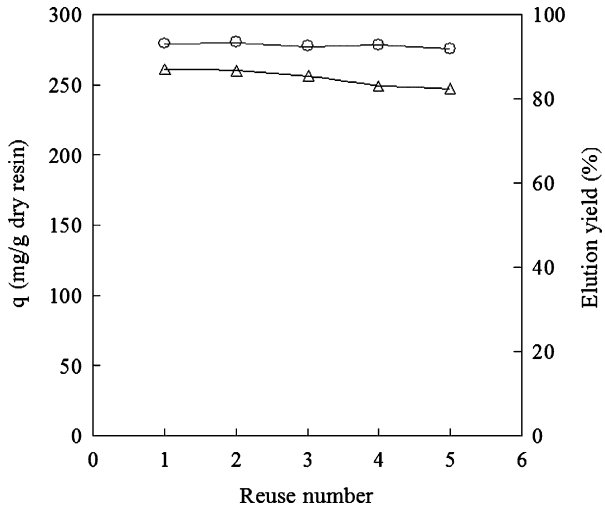

Fig. 7. Recycled use of the M-PGMA-TRI adsorbent for γ-globulin adsorption: (△) binding amount of γ-globulin; (◯) elution yield of γ-globulin by 1.0 mol/L NaCl in 0.1 mol/L acetate buffer (pH 5.0). Triplicate experiments were done and average values were indicated. The relative standard deviations were within 3.0%.

Table 3
The change of γ-globulin binding amount (mg/g dry resin) to the M-PGMA-TRI after the beads had been incubated in 0.1 mol/L glycinc–HCl buffer (pH 2.2) or in 0.1 mol/L NaOH for different times

Incubation time (h)	Glycine–HCl buffer (pH 2.2)	0.1 mol/L NaOH
0	259.5	243.7
1	254.7	221.0
5	261.3	193.2
10	260.1	145.9
24	259.1	150.2

Triplicate experiments were performed and average values were indicated. The relative standard deviations were within 4.9%.

(see Fig. 4 and Table 2), however, it would not be promising for magnetic separations.

In the studies of the chemical stability of the M-PGMA-TRI for protein adsorption, we found little change of γ-globulin binding amount after the beads were incubated in Mg^{2+} or EDTA for 24 h (data not shown). The results of γ-globulin adsorption after the beads had been incubated in acidic or alkali solutions for different times are listed in Table 3. It is obvious that the M-PGMA-TRI was stable in the acidic buffer, the same as observed for its magnetic stability reported above. However, by incubation in the alkaline solution, the protein adsorption amount of the cation exchanger decreased gradually with the incubation time, similar to the decrease of the saturation magnetization reported earlier. This indicates that the magnetic monodisperse microspheres cannot suffer from alkaline treatment in its regeneration as a protein adsorbent. In contrast, acidic buffer would be a promising medium for its regeneration.

4. Conclusions

This article has succeeded in the fabrication of rigid and robust magnetic monodisperse microsphere by using the dispersion-polymerization and penetration-deposition procedures. This was realized by adding the cross-linking agent of proper amount at a proper time point during the dispersion-polymerization. As a result, 2% (w/w) of the cross-linker TRI was introduced in the system that produced mono-sized microspheres. The microspheres were further processed to introduce magnetic granules by the penetration-deposition approach. The introduction of 2% of cross-linking agent was found enough to produce rigid and robust magnetic microspheres for protein adsorption because during the recycled use of the magnetic material its magnetic properties and protein adsorption ability have changed little. The magnetic mono-sized cation exchanger was also stable in acidic buffer of pH 2.2, so the magnetic adsorbent can be regenerated with an acidic solution after protein adsorption. The results indicate that the magnetic monodisperse microspheres are promising for protein separation and other applications involving a magnetic separation.

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