Journal of Molecular Catalysis B: Enzymatic 63 (2010) 50–56

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Superparamagnetic aminopropyl-functionalized silica core-shell microspheres as magnetically separable carriers for immobilization of penicillin G acylase

Bianfang Shi, Yanqin Wang*, Jiawen Ren, Xiaohui Liu, Ye Zhang, Yanglong Guo, Yun Guo, Guanzhong Lu*

Lab for Advanced Materials, Research Institute of Industrial Catalysis, East China University of Science and Technology, Meilong Load 130, Shanghai 200237, PR China

ARTICLE INFO

Article history:
Received 7 July 2009
Received in revised form 4 December 2009
Accepted 4 December 2009
Available online 4 January 2010

Keywords:
Superparamagnetic
Core-shell
Immobilization
Penicillin G acylase

ABSTRACT

The superparamagnetic Fe_3O_4 microspheres about 300 nm diameter were prepared by the solvothermal method. After treated with chlorohydric acid, it was coated with aminopropyl-functionalized amorphous silica by the condensation of tetraethylorthosilicate (TEOS) and γ-aminopropyltriethoxysilane (APTES) through Stöber modified method. FT-IR, elemental analysis and TEM were used to characterize the aminopropyl-functionalized silica-coated magnetic microspheres, and then they were first used as magnetic separation carriers for immobilization of penicillin G acylase (PGA). The results showed that the amino content of the carriers has a little influence on the apparent initial activity, while the immobilization method and the shell thickness have more obvious influence on the apparent initial activity. The immobilized PGA (IMPGA) obtained through covalent attachment almost has no leaching and can retain above 78% of activity after 10 consecutive operations and exhibits higher resistance to thermal stability. More interesting, the silica-coated magnetic microspheres show high saturation magnetization and the obtained IMPGA can be separated quickly using an external magnetic field.

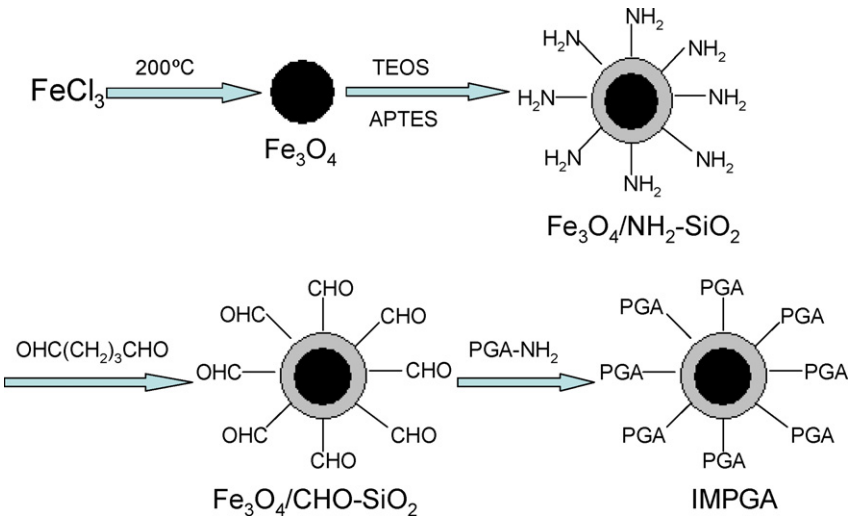
1. Introduction

Penicillin G acylase (PGA), which has been one of the most important industrial biocatalysts since its discovery in 1960, is widely used in the production of 6-aminopenicillanic acid (6-APA), 7-aminodeacetoxycephalosporanic acid (7-ADCA), and the semi-synthetic β-lactam antibiotics [1]. Therefore, as a solution enzyme, an efficient recovery and reuse for PGA is a prerequisite for its economic industrial applications. To immobilize PGA on a solid carrier is a good solution and it has attracted much attention. Many carriers, both organic and inorganic have been used for the immobilization of enzyme [2–9]. Thus, reported works using organic microbeads offers good enzyme accessibility and separability, but these composite particles greater than 1 μm may experience significant attrition [10]. Exploitation of enzyme carriers of even smaller size is worthwhile since a support reaching nanometric regime will theoretically give no attrition problem and will favor the binding capacity [11]. It is noted that such small composites, if used, are almost impossible to separate in a bioreactor by conventional means. In recent years, magnetic carriers attracted people's immense interest because of its easy separability from products using an external magnetic field.

Reported magnetic carriers are mainly magnetic polymeric microspheres in past decade and magnetic silica composites [12–16]. Generally, magnetic carriers are prepared by encapsulating inorganic magnetic particles (usually magnetite or maghemite) in organic polymers, such as polystyrene and poly(alkyl acrylate), or with inorganic silica [17]. Consequently, magnetic polymer microspheres combine the excellent properties of polymer microspheres (i.e., ease of surface modification and high dispersibility) with the unique magnetic responsibility of magnetic particles. The magnetic polymer microspheres reported in most previous work showed poor responsibility to an applied magnetic field, which hampered the application of magnetic polymer microspheres as a fast and efficient separation tool. And also the polymeric materials have a low reusability and create the problems in disposal. Herein, we present a simple way to prepare superparamagnetic aminopropyl-functionalized silica core-shell microspheres with high-magnetization and used these magnetic microspheres as carriers for immobilization PGA. The procedure for preparation of superparamagnetic aminopropyl-functionalized silica core-shell microspheres and immobilization of PGA is shown in Scheme 1. First, the Fe_3O_4 microspheres were prepared with a solvothermal reaction [18]. Secondly, the Fe_3O_4 microspheres were coating with aminopropyl-functionalized silica by the co-condensation of tetraethylorthosilicate (TEOS)

* Corresponding authors. Tel.: +86 21 64253824; fax: +86 21 64253824.
E-mail addresses: wangyanqin@ecust.edu.cn (Y. Wang), gzhlu@ecust.edu.cn (G. Lu).

1381-1177/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2009.12.003



Scheme 1. The procedures for preparation of $Fe_3O_4/NH_2–SiO_2$ and immobilization of PGA.

and γ-aminopropyltriethoxysilane (APTES) in base solution using modified Stöber method [18–21] and the superparamagnetic aminopropyl-functionalized silica core-shell microspheres ($Fe_3O_4/NH_2–SiO_2$) were obtained. Thirdly, the amino groups of the $Fe_3O_4/NH_2–SiO_2$ reacted with one aldehyde group of glutaraldehyde and obtained magnetic aldehyde-functionalized silica core-shell microspheres ($Fe_3O_4/CHO–SiO_2$). And last, the aldehyde groups of $Fe_3O_4/CHO–SiO_2$ reacted with amino groups of PGA and obtained immobilized penicilline G acylase (IMPGA). Using this $Fe_3O_4/NH_2–SiO_2$ microspheres as the carriers of immobilized PGA is endowed with the following advantages: (1) high-magnetization makes the carrier and obtained IMPGA be easily separated from the reaction mixture and lower operational cost, (2) higher and more uniform surface coverage of amino groups favor covalent binding PGA on the carriers, and (3) superparamagnetic property and the screening effect of the silica layer make the carrier and the obtained IMPGA can be readily and stably dispersed in water.

2. Experimental procedures

2.1. Chemicals

γ-Aminopropyltriethoxysilane (APTES) was purchased from Aldrich, Shanghai, China. Penicillin G acylase (PGA, 740 IU/ml) was purchased from Zhejiang Hiader Co. Ltd., Hangzhou, China. Penicillin G potassium salt was bought from Shandong Lukang Pharmaceutical Co. Ltd., Jining, China. Tetraethylorthosilicate (TEOS) and glutardialdehyde were bought from Shanghai Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. $FeCl_3·6H_2O$, ammonium hydroxide and ethanol were analytical grade and used without further purification.

2.2. Synthesis of Fe_3O_4 microspheres and synthesis of $Fe_3O_4/NH_2–SiO_2$ microspheres

The magnetic microspheres were prepared through a solvothermal reaction [18]. The core-shell $Fe_3O_4/NH_2–SiO_2$ microspheres were prepared according to the previously reported method [18–21], but using TEOS and APTES as the silica precursors. Briefly, 0.20 g of Fe_3O_4 particles (~300 nm in diameter) were treated with 0.1 M HCl aqueous solution (100 ml) by ultrasonication, and then homogeneously dispersed in the mixture of ethanol (160 ml), deioned water (37 ml) and concentrated ammonia aqueous solution (5.0 ml, 25 wt.%), followed by the addition of tetraethylorthosilicate (TEOS) and γ-aminopropyltriethoxysilane (APTES), the total amount of the TEOS and APTES kept at 0.37 g, the amino content was adjusted by changing the amount of TEOS and APTES. After stirring at room temperature for 6 h, the $Fe_3O_4/NH_2–SiO_2$ microspheres were separated and washed with ethanol and water several times until the pH value of the supernatant was 6–7, and then dried at 60 °C for 24 h. The obtained samples are denoted as $Fe_3O_4/NH_2–SiO_2$-*x*, *x* represents the weight percentage of APTES to total silica source. When the total amount of TEOS and APTES is increased to 0.94 g, the obtained samples are denoted as $Fe_3O_4/NH_2–SiO_2$-*x*-a.

2.3. Activation of $Fe_3O_4/NH_2–SiO_2$ with glutaraldehyde and immobilization of PGA

Two methods were used to immobilize PGA on the carriers, one was the covalent attachment and another was physical adsorption. The difference between these two methods was the activation of carriers with or without glutaraldehyde. The detailed process of the immobilization of PGA molecules on the carriers was the same as our previous works [22]. Particularly, all IMPGA were separated from reaction mixture by an external magnet and all the supernatant were collected and diluted with phosphate buffer for assay of the protein. The resultant IMPGA were placed in the refrigerator at 4 °C for the subsequent activity assays.

2.4. Activity assay of IMPGA

The apparent initial activity of IMPGA (A(IU/g carrier)) and free PGA (A(IU/ml)) were determined by titrating phenylacetic acid (PAA) with NaOH aqueous solution, which is a by-product in the hydrolysis reaction of penicillin G potassium salt [3]. The detailed process was described in reference [22]. Different from our previous work, per gram dry carrier was used to calculate the apparent initial activity in this work.

Other parameters used to characterize the performance of the carrier are the loading of protein (*Q* (mg protein/g carrier)) and immobilization yield (IMY (%)), which are described as follows, respectively:

$$Q\,(\text{mg protein/g carry}) = (Q_1 - Q_2)/m$$
$$\text{IMY}\,(\%) = (Q_1 - Q_2)/Q_1 \times 100\%$$
$$\text{Specific activity}\,(\text{IU/mg}) = A/Q$$

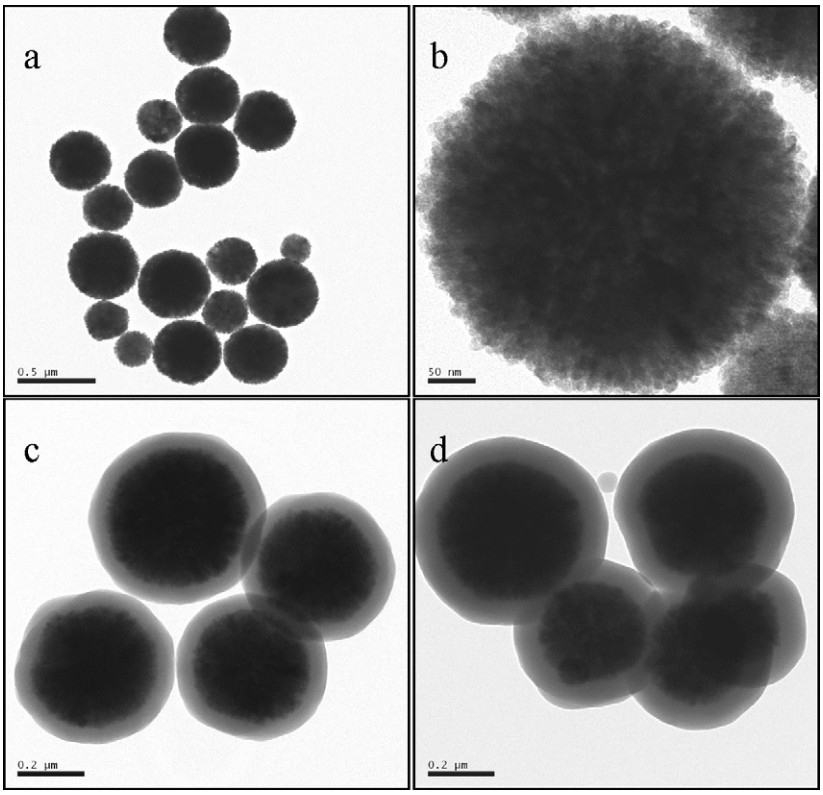


Fig. 1. TEM images of (a) and (b) magnetic Fe_3O_4; (c) $Fe_3O_4/NH_2–SiO_2$-10% and (d) $Fe_3O_4/NH_2–SiO_2$-10%-a.

where m [g] is the weight of carrier used for immobilization; Q_1 [mg] is the total amount of protein before immobilization; Q_2 [mg] is the total amount of protein after immobilization.

2.5. Measurement of enzyme loading kinetics of PGA on the carriers

The effect of PGA concentration on the PGA loading on carriers was carried out as follows: a series of flasks contained 0.1 g of carriers and 1.2 ml of PGA diluted solution with different PGA concentration were placed in a water bath shake and stirred at 30 °C for 24 h, then the supernatant was recovered and the protein concentration was detected with the well-known Bradford assay [23].

2.6. Reusability assay

The reusability of immobilized PGA was examined by conducting the activity measurement of immobilized PGA at 37 °C at time intervals of 30 min. After each activity measurement, the immobilized PGA was separated magnetically. Then, 15 ml of buffer (pH 8.0) and 15 ml of potassium salt (4 wt.%) were added to the immobilized PGA in sequence and the next activity measurement was carried out. Here the activity is expressed in relative units [%] where the apparent initial activity is set at 100%.

2.7. Characterization

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku D/max-2550/PC X-ray diffractometer (Japan). The TEM images were obtained on a TECNAI 20S-TWIN microscope. FT-IR spectra were recorded on a Nicolet Nexus 670 FT-IR spectrometer. The analysis of C, H and N elements was determined by Elementar Vario EL III element analyzer and the amino content of $Fe_3O_4/NH_2–SiO_2$ microspheres was quantitatively calculated from the N element content. Nitrogen adsorption–desorption isotherms were measured at 77 K with a Micromeritics ASAP 2020 M surface area and pore size analyzer. Magnetic measurement was carried out on SQUID magnetometer at room temperature.

3. Results and discussions

3.1. Preparation and Characterization of Fe_3O_4 and $Fe_3O_4/NH_2–SiO_2$ microspheres

The magnetic microspheres and the magnetic silica composites were characterized by XRD patterns and the results are similar with previous report [18]. All detected diffraction peaks can be indexed as face centered cubic (fcc) Fe_3O_4 (JCPDS card No. 19-629). From the XRD pattern of the magnetic silica microspheres, the broad diffraction peak centered at about 23° is due to the amorphous silica.

Fig. 1a and b shows the representative TEM images of the synthesized magnetic microspheres. We can calculate that the average diameter of Fe_3O_4 microspheres is about 300 nm, which is well in accordance with the previous reports [18,19]. The TEM images shown in Fig. 1c and d reveal that the core-shell structured $Fe_3O_4/NH_2–SiO_2$-10% and $Fe_3O_4/NH_2–SiO_2$-10%-a magnetic microspheres with the shell thicknesses of about 48 and 80 nm are successfully prepared. They also indicate that the shell thickness increase with the increase of the total amount of TEOS and APTES. According to FT-IR spectra and the solid state ^{29}Si MAS NMR and ^{13}C CP/MAS NMR spectra (not shown here), we conclude that aminopropyl groups connected with the silicon by one-pot condensation of TEOS and APTES, this was also confirmed by our previous works [22].

Fig. 2 shows the magnetic hysteresis loops of magnetite microspheres and aminopropyl-functionalized magnetic silica micro-

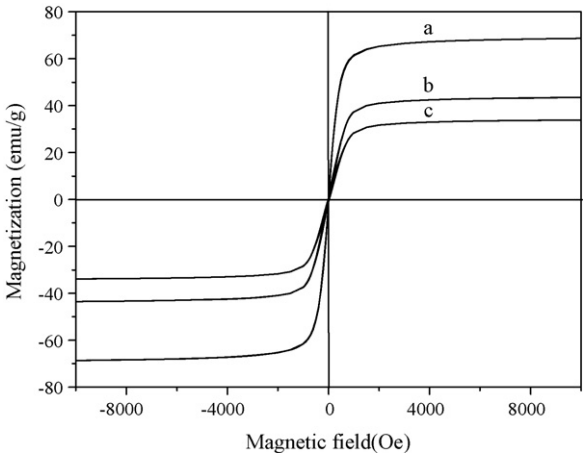


Fig. 2. The hysteresis loops of (a) Fe_3O_4; (b) Fe_3O_4/NH_2–SiO_2-10% and (c) Fe_3O_4/NH_2–SiO_2-10%-a at 300 K.

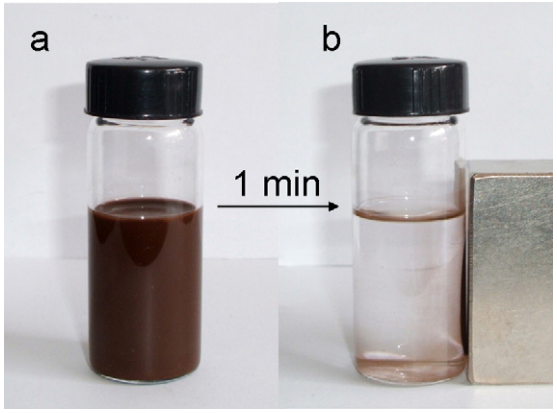


Fig. 3. Separability of Fe_3O_4/NH_2–SiO_2-10% by placing an external magnetic field. The time from state (a) to state (b) is within 1 min.

spheres. From the magnetization curve of magnetite microspheres, we can see that the saturation magnetization (Ms) of the magnetic microspheres is 68.7 emu/g. Similar to magnetite microspheres, the samples Fe_3O_4/NH_2–SiO_2-10% and Fe_3O_4/NH_2–SiO_2-10%-a showed higher saturation magnetization (Ms) of 43.5 and 33.9 emu/g, respectively. The difference among the Ms value can be due to the different magnetite content in the magnetic silica microspheres. Additionally, from the hysteresis loops of the magnetite microspheres and the magnetic silica microspheres, we find that all samples exhibit superparamagnetic properties [16]. Fig. 3 shows that the magnetic silica microspheres can be quickly separated to the wall of the container using a magnet within 1 min, and the IMPGA are also can be quickly separated and collected (the figure is similar with Fig. 3 and is not shown here).

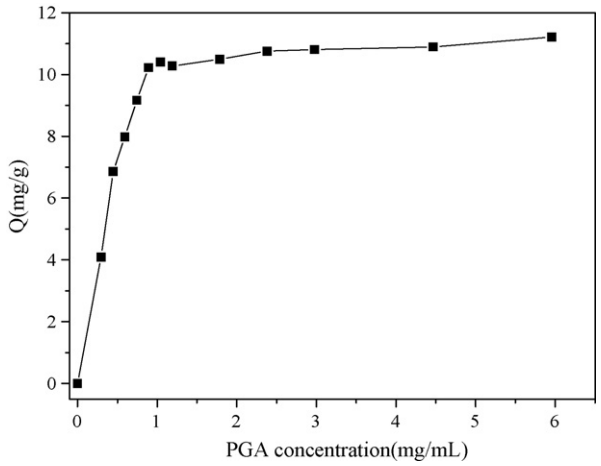


Fig. 4. The influence of PGA concentration on the loading of PGA on magnetic Fe_3O_4/SiO_2–NH_2-10%.

Other properties of the Fe_3O_4/NH_2–SiO_2 are shown in Table 1. From Table 1, we can find that all these samples show lower specific surface area because of larger size of the microspheres. The amino content of carriers is increase with the increase of the weight percentage of APTES, while the increase rate slower. This can be explained by the large steric hindrance of aminopropyl groups. In other words, if aminopropyl groups were incorporated into the silica shell, further condensation on the surface can be hindered by the aminopropyl groups exposed on the surface of the nanoparticles [16].

3.2. Immobilization of PGA on Fe_3O_4/NH_2–SiO_2 with different amino content and shell thickness

Table 1 shows the influence of immobilization method, amino content and shell thickness on the performance of the immobilized PGA, all these data were obtained by averaging three times' determined results. For comparison of the covalent attachment of PGA and adsorption immobilization of PGA, sample Fe_3O_4/NH_2–SiO_2-10% is used as an example. From Table 1, we can find that when PGA molecules are immobilized on sample Fe_3O_4/NH_2–SiO_2-10% with adsorption immobilization (the sample is denoted as Fe_3O_4/NH_2–SiO_2-10%-ads), the IMPGA shows very lower apparent initial activity and immobilization yield (IMY%), it is due to the low surface area of sample Fe_3O_4/NH_2–SiO_2-10% and the weak van der Walls' force or hydrogen bond interaction between PGA molecules and carriers, so PGA molecules can be easily washed out from the carrier. However, when PGA molecules are attached on the carrier by strong covalent bond, the IMPGA shows higher activity and IMY%. Therefore, the covalent attachment is more effective than adsorption immobilization in our study and glutaraldehyde plays an important role in the attachment of PGA molecules. The influence of weight percentage of APTES on the apparent initial activity

Table 1
The properties of Fe_3O_4/NH_2–SiO_2 microspheres and performance of immobilized PGA.

Samples	IMY (%)	Specific activity (IU/mg protein)	Initial apparent activity (IU/g carrier)	Amino content (mmol/g)	S_{BET} (m^2/g)
Fe_3O_4/NH_2–SiO_2-10%-ads	2.04	32.9	48	–	–
Fe_3O_4/NH_2–SiO_2-10%	16.2	29.3	339	0.51	7.6
Fe_3O_4/NH_2–SiO_2-20%	18.3	27.7	362	0.83	11.2
Fe_3O_4/NH_2–SiO_2-30%	19.5	26.4	369	1.01	9.0
Fe_3O_4/NH_2–SiO_2-40%	21.2	25.4	384	1.10	10.7
Fe_3O_4/NH_2–SiO_2-10%-a	14.1	28.2	285	0.57	19.5

Notes: These data were obtained by averaging three times' determined results.

of IMPGA obtained by covalent attachment with the help of glutaraldehyde is displayed in Table 1. We can find that the amino content has a little influence on the IMY% and apparent initial activity of IMPGA. When the weight percentage of APTES in the reaction mixture is increased from 10% to 40%, the apparent initial activity only increased about 13%. In our previous work [22], a series of samples with different amino content were prepared by condensation of TEOS and APTES in W/O microemulsion and we concluded that the apparent initial activity decreased with the increase of amino content. That was because the surface area decreased remarkably with the increase of the volume percentage of APTES, which played a key role in influencing the surface available amino content, the IMY% of carrier and the apparent initial activity of IMPGA. However, in this work, all samples have larger particle size and lower surface area, and the influence of surface area on the IMY% and the influence of pore size on diffusion can be ignored. As we discussed above, glutaraldehyde played an important role in the attachment of PGA molecules, therefore, the surface amino content is the key factor on the IMY% and the apparent initial activity. The specific activity of free PGA is 41.4 IU/mg protein, while much lower specific activity is obtained for the $Fe_3O_4/NH_2–SiO_2$ than free PGA, which can be explained that the more active sites of PGA are not available for taking part in the reactions when PGA was immobilized on supports by covalent attachment [24]. On the other hand, nearly all of the PGA molecules by the covalent attachment method are directly exposed to the solution and can therefore be easily damaged. When the total weight of TEOS and APTES is increased to 0.94 g and the percentage of APTES is kept 10%, the shell thickness of sample $Fe_3O_4/NH_2–SiO_2$-10%-a is about 80 nm, which showed lower apparent initial activity than that of $Fe_3O_4/NH_2–SiO_2$-10% whose shell thickness is about 48 nm. The bigger microspheres lead to lower surface amino content and lower IMY%.

3.3. The influence of PGA concentration

To determine the immobilized loading of PGA protein on sample $Fe_3O_4/SiO_2–NH_2$-10% (Q (mg/g)), the equilibrium adsorption experiments were used. The free PGA concentration in the supernatant can be measured by means of UV–vis spectrophotoscopic measurements, and then the immobilized loading on the carriers can be calculated. Fig. 4 shows the immobilized loading of PGA on the carriers as a function of PGA concentration. It can be observed that the immobilized loading of PGA protein increase with the increase of the PGA concentration initially, and then reached saturation at the PGA concentration of 0.89 mg/ml. At this concentration, nearly all PGA molecules are attached on the carriers. Therefore, it can be estimated that the immobilized loading of PGA protein on $Fe_3O_4/SiO_2–NH_2$-10% is about 11.21 mg/g when the PGA concentration is 5.96 mg/ml.

3.4. Effect of temperature on the activity of immobilized PGA

To determine the effect of temperature on the apparent initial activity, the activity of the free and immobilized PGA was determined by measuring the apparent initial activity in buffer solutions (pH 8.00) at different temperature. Here the activity is expressed in relative units [%], where the maximal activity value at a certain temperature is set at 100%. The results are shown in Fig. 5. At low temperature level, the enzyme activity of both the free PGA and the immobilized PGA increases with the increase of temperature. At high temperature level, the enzyme activity of both the free PGA and the immobilized PGA decreases sharply with the increase of temperature. Possible explanation is that with the increase of temperature the enzyme molecules outspreaded acutely and ultimately lost their biocatalytic activity. When the reaction temperature is at 45 °C, the free PGA and the immobilized

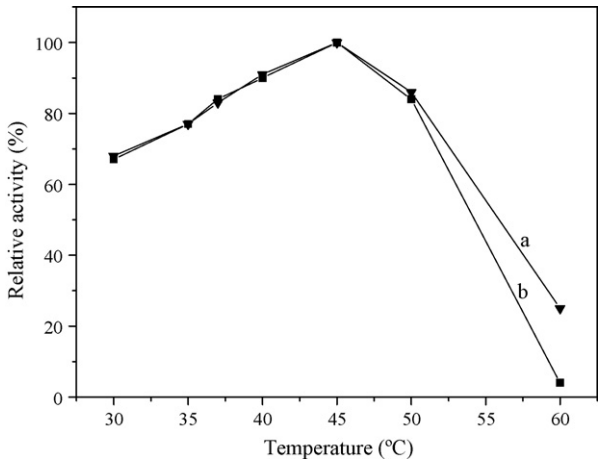


Fig. 5. The effect of reaction temperature on the activity of (a) immobilized PGA and (b) free PGA The highest activity of free PGA and IMPGA are 894 IU/ml and 421 IU/g carrier, respectively.

PGA shows highest activity which are 894 IU/ml and 421 IU/g carrier, respectively. Also, it is observed that the residual activity of the immobilized PGA was about 20% at 60 °C, however, the free activity lose its activity completely at 60 °C. This means that the immobilized PGA has higher resistance to the change of temperature than the free PGA.

3.5. Effect of pH on the activity of immobilized PGA

The effect of pH on the activity of the free PGA and the immobilized PGA was assayed in the pH range of 5.0–10 at 37 °C. In general, immobilization would result in the shift of optimal pH of the enzyme [25,26]. Typical results are illustrated in Fig. 6. When the optimum pH values is 7.0 for free PGA and 7.5 for IMPGA, the highest activity for free PGA and IMPGA are 784 IU/ml and 368 IU/g carrier, respectively. Both the free PGA and the immobilized PGA keep higher activity in the alkaline condition. The optimum pH value of immobilized PGA shifts with 0.5 units to the alkaline region after covalent immobilization. This result may probably be attributed to the stabilization of PGA molecules resulting from multipoint immobilization on the magnetic microspheres. It is well known that the immobilization of enzyme on a carrier can cause significant changes in the catalytic behavior of the enzyme. The pH optimum value of an immobilized enzyme shifts to a higher or

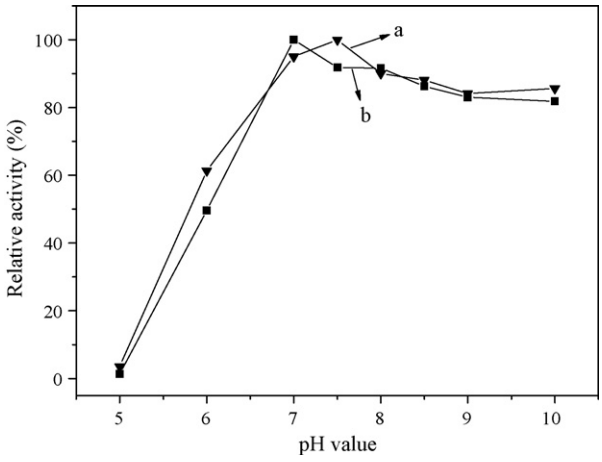


Fig. 6. The effect of pH value on the activity of (a) immobilized PGA and (b) free PGA. The highest activity of free PGA and IMPGA are 784 IU/ml and 368 IU/g carrier, respectively.

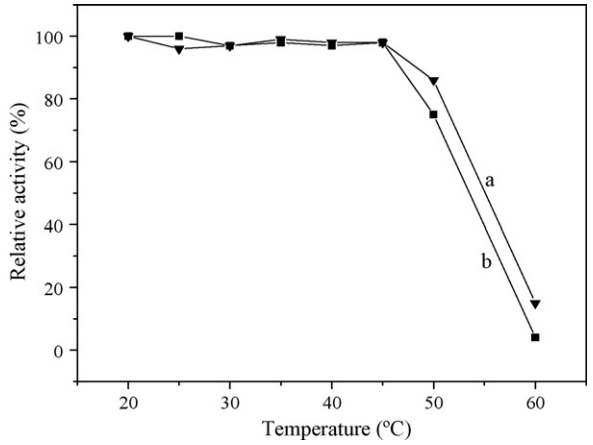


Fig. 7. Activity of (a) the immobilization PGA and (b) free PGA as a function of temperature, incubated in 0.1 M phosphate buffer (pH 8.0) at different temperature for 1 h. The highest activity of free PGA and IMPGA are 728 IU/ml and 332 IU/g carrier, respectively.

lower pH, which depends on surface charges and structure of the carrier [27,28].

3.6. Thermal stability of immobilized PGA

The thermal stability of the immobilized PGA is one of the most important criteria with respect to applications. In order to test the thermal stability of immobilized PGA, the relative activity was tested with a comparison of thermal stabilities of free and immobilized PGA at different temperature (20–60 °C) in phosphate buffer (pH 8.0) for 1 h. The results are shown in Fig. 7, and in this figure, the highest activity for the free PGA and the immobilized PGA are 728 IU/ml and 332 IU/g carrier, respectively. Both curves exhibit a similar trend; when the incubated temperature is below 45 °C, both the free PGA and the immobilized PGA show higher thermal stability. However, the immobilized PGA is more stable than the free one at higher incubated temperature. These results indicate that PGA is more stable it is immobilized on the carrier. The increase in the thermal stability may be due to the stabilization of the weak intramolecular forces and the prevention of the autolysis of the PGA. In this work, the activity assay were performed at 37 °C, therefore, another thermal stability test was performed by incubating the free PGA and immobilized PGA at 37 °C for different time (0.5–7 h), and we have concluded that both the free PGA and the immobilized PGA have no obvious activity loss with the increase of incubated time.

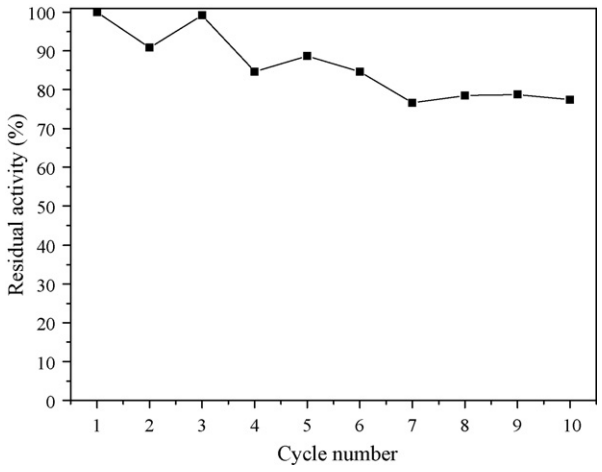


Fig. 8. Reusability of PGA immobilized on $Fe_3O_4/NH_2–SiO_2$-10%. The apparent initial activity for immobilized PGA is 352 IU/g carrier.

3.7. Reusability

The reusability of PGA immobilized on $Fe_3O_4/NH_2–SiO_2$-10% is shown in Fig. 8. The apparent initial activity of IMPGA is 352 IU/g carrier and the activity retained above 78% residual activity after 10 consecutive operations, which indicates that PGA immobilized on Fe_3O_4/NH2–SiO_2-10% performed a good reusability.

4. Conclusions

The superparamagnetic high-magnetization aminopropyl-functionalized silica core-shell microspheres with different amino content and different shell thickness were successfully synthesized and first used as carriers for immobilization PGA. Through systemically studying the influence of various factors (such as amino content, shell thickness and immobilization method) on the activity of IMPGA, we found that the amino content of the carrier had a little influence on the apparent initial activity, while the shell thickness had more obvious influence on the apparent initial activity. The covalent attachment was more effective than adsorption method in our study, and glutaraldehyde played an important role in the attachment of PGA molecules. More importantly, the silica-coated magnetic core microspheres showed high saturation magnetization, which make the obtained IMPGA can be quickly separated by a magnet. Furthermore, the immobilized PGA retained the activity and exhibited higher resistance to thermal stability. The immobilized PGA obtained through covalent attachment almost has no leaching and can retain above 78% of activity after 10 consecutive operations.

Acknowledgements

This work was supported financially by the 973 Program of China (No. 2004CB719500), National Natural Science Foundation of China (No. 20673037), New Century Excellent Talents in University, China (NCET-05-415), Commission of Science and Technology of Shanghai Municipality (08JC1407900) and Shuguang Program of the Commission of education, Shanghai (05SG33), China.

References

[1] A.I. Kallenberg, F. Van Rantwijk, R.A. Sheldon, Adv. Synth. Catal. 347 (2005) 905.
[2] G.W. Wuyun, G.Z. Lu, D.Z. Wei, Y.L. Guo, Y.S. Wang, Chinese J. Catal. 24 (2003) 219.
[3] P. Xue, G.Z. Lu, Y.L. Guo, Y.S. Wang, Chem. J. Chinese Univ. 25 (2004) 361.
[4] L.M. Van Langen, M.H.A. Janssen, N.H.P. Oosthoek, S.R.M. Pereira, V.K. Švedas, F. Van Rantwijk, R.A. Sheldon, Biotechnol. Bioeng. 79 (2002) 224.
[5] M.H.A. Janssen, L.M. Van Langen, S.R.M. Pereira, F. Van Rantwijk, R.A. Sheldon, Biotechnol. Bioeng. 78 (2002) 425.
[6] J. He, X.F. Li, D.G. Evans, X. Duan, J. Mol. Catal. B 11 (2000) 45.
[7] A.S.M. Chong, X.S. Zhao, Catal. Today 93–95 (2004) 293.
[8] Y.J. Lü, G.Z. Lu, Y.Q. Wang, Y.L. Guo, Y. Guo, Z.G. Zhang, Y.S. Wang, X.H. Liu, Adv. Funct. Mater. 17 (2007) 2160.
[9] P. Xue, G.Z.Y. Lu, L. Guo, Y.S. Wang, Y. Guo, J. Mol. Catal. B 30 (2004) 75.
[10] Y.H. Lee, Y.K. Bae, E.J. Jeong, J. Microbiol. Biotechnol. 6 (1996) 432.
[11] M. Qhobosheane, S. Santra, P. Zhang, W. Tan, Analyst 126 (2001) 1274.
[12] W. Wang, L. Deng, Z.H. Peng, X. Xiao, Enzyme Microb. Technol. 40 (2007) 255.
[13] S.C. Tsang, C.H. Yu, X. Gao, K. Tam, J. Phys. Chem. B 110 (2006) 16914.
[14] R.H. Aimee, K. Byeong-Su, T. Andrew Taton, Bioconjug. Chem. 18 (2007) 183.
[15] J.D. Hong, X.I. Gong, J. Yu, H. Ma, S. Yao, Micropor. Mesopor. Mater. 109 (2008) 470.
[16] J. Kim, Y. Lee, T. Hyeon, Small 4 (2008) 143.
[17] (a) X. Xu, G. Friedman, K.D. Humfeld, S.A. Majetich, S.A. Asher, Adv. Mater. 13 (2001) 1681;
(b) Y. Lu, Y. Yin, B.T. Mayers, Y. Xia, Nano Lett. 2 (2002) 183.
[18] Y.H. Deng, D.W. Qi, C.H. Deng, X.M. Zhang, D.Y. Zhao, J. Am. Chem. Soc. 130 (2008) 28.

[19] X.Q. Xu, C.H. Deng, M.X. Gao, W.J. Yu, P.Y. Yang, X.M. Zhang, Adv. Mater. 18 (2006) 3289.
[20] Y. Lu, Y.D. Yin, B.T. Mayers, Y. Xia, Nano Lett. 2 (2002) 183.
[21] Y.H. Deng, C.C. Wang, J.H. Hu, W.L. Yang, S.K. Fu, Colloids Surf. A: Physicochem. Eng. Aspects 262 (2005) 87.
[22] B.F. Shi, Y.S. Wang, Y.L. Guo, Y.Q. Wang, Y. Wang, Y. Guo, Z.G. Zhang, X.H. Liu, G.Z. Lu, Catal. Today 148 (2009) 184.
[23] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[24] G. Alvaro, R. Fernandez-Lafuente, R.M. Blanco, J.M. Guisan, Appl. Biochem. Biotechnol. 26 (1991) 181.
[25] E. Emregul, S. Sungur, U. Akbulut, Food Chem. 97 (2006) 591.
[26] R. Reshmi, G. Sanjay, S. Sugunan, Catal. Commun. 7 (2006) 460.
[27] K. Leena, A. Rohini, S.B. Gadre, C. Rajan, S.P. Raman, J. Chem. Technol. Biotechnol. 49 (1990) 173.
[28] A. Arslan, S. Kiralp, L. Toppare, A. Bozkurt, Langmuir 22 (2006) 2912.