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# 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*

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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).

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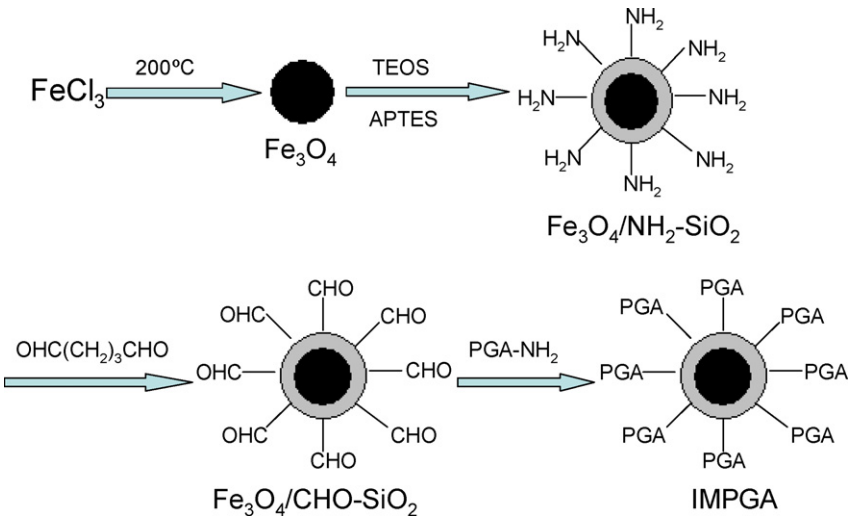


**Scheme 1.** The procedures for preparation of $Fe_3O_4/NH_2–SiO_2$ and immobilization of PGA.

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:

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