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Letter

One-step synthesis of aldehyde-functionalized magnetic nanoparticles as adsorbent for fast and effective adsorption of proteins

Ruixia Gao [a,*], Yi Hao [a,b], Xihui Cui [a,b], Lili Zhang [a,b], Dechun Liu [c], Yuhai Tang [a,b,*]

[a] Institute of Analytical Science, School of Science, Xi'an Jiaotong University, Xi'an 710049, China
[b] School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China
[c] Department of Hepatobiliary Surgery, First Hospital of Xi'an Jiaotong University, China

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ABSTRACT

Novel directly aldehyde-functionalized magnetic nanoparticles were synthesized using a facile one-pot solvothermal method with glutaraldehyde as modification ligand. The as-synthesized nanoparticles exhibited a narrow size distribution, highly crystalline, and superparamagnetic properties. The obtained products possessed high saturation magnetization of 32.84 emu g^{-1}, which made them to be easily separated from solution by means of an external magnetic field. Moreover, the resultant functionalized magnetic nanomaterials were successfully applied for protein adsorption with high amount through reversible imine bond.

1. Introduction

Superparamagnetic iron oxide nanoparticles (NPs), due to their magnetic susceptibility, low cytotoxicity, good biocompatibility, and stability under physiological environment, have attracted considerable attention in a wealth of biological applications, including magnetic resonance imaging (MRI) [1], protein separation [2], carrier for aimed drug delivery [3], biomolecule immobilization [4], and therapy agents [5]. The majority of these applications are realized through conjugating biomolecules on the surface of magnetic NPs.

Generally, magnetic NPs need to be modified with active group which is able to interact with bioactive substances. Yan et al. [6] prepared Ti^{4+}-modified magnetic NPs for selective enrichment of phosphopeptides. Lee et al. [7] proposed a facile approach to prepare specific functionalized silica modified Fe_3O_4 NPs with diverse functional groups, such as thiol (–SH), disulfide (–S–S–), carbon chain ($–C_{18}$), carboxyl (–COOH), amino ($–NH_2$), and aldehyde (–CHO) for efficient separation of protein. Lu et al. [8] synthesized magnetic dialdehyde starch Fe_3O_4 NPs and applied as drug carriers to immobilize BSA. Basically, these modified magnetic NPs can be employed to conjugate biomolecules through two types of interactions. One is non-covalent interaction, such as hydrogen bond, metal coordination, and hydrophobic effect. The other is covalent linkage, such as aldehyde, epoxy, and cyanogen bromide, which is more stable considering possible dissociation in complex biological media. In this regard, magnetic NPs modified with active groups that could generate covalent interaction with bioactive substance, are more promising for conjugation of biomolecules. Currently, aldehyde modified magnetic nanoparticles generally need two [8] or three steps [9–11] to prepare, which are time-consuming and tedious.

To date, the solvothermal method has been proved as an efficient approach for the preparation of magnetic NPs or functionalized magnetic NPs with monodispersity and high crystallinity in a one-pot manner [12,13]. Herein, we develop a simple solvothermal route to obtain aldehyde-functionalized magnetic NPs adopting $FeCl_3{\cdot}6H_2O$ as a single iron source and glutaraldehyde as modification ligand through one step for the first time. As expected, the as-synthesized nanomaterials had high protein density attached stably on its surface, exhibiting potential value for adsorption of proteins.

2. Experiments

2.1. Raw materials

Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), anhydrous sodium acetate (NaOAc), ethylene glycol (EG), glutaraldehyde (GA), sodium dihydrogen phosphate (NaH_2PO_4), and disodium hydrogen phosphate (Na_2HPO_4) were provided by Xi'an

* Corresponding authors at: Institute of Analytical Science, School of Science, Xi'an Jiaotong University, Xi'an 710049, China (Y. Tang). Tel./fax: +86 29 82655399.
E-mail addresses: ruixiagao@mail.xjtu.edu.cn (R. Gao), tyh57@mail.xjtu.edu.cn (Y. Tang).

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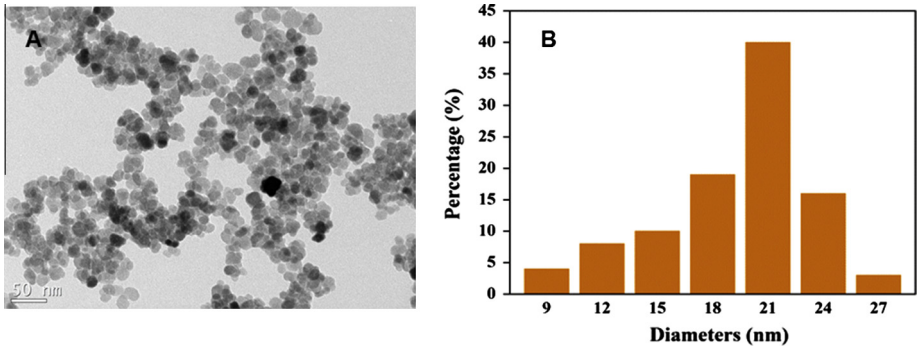

Fig. 1. TEM image (A) and the size distribution histogram (B) of γ-Fe_2O_3@CHO.

Chemicals Ltd. Bovine hemoglobin (BHb; p*I* = 6.9, *M*w = 64.0 kDa), bovine serum albumin (BSA; p*I* = 4.9, *M*w = 66.0 kDa), and lysozyme (Lyz; p*I* = 11.2, *M*w = 13.4 kDa) were obtained from Sigma. The highly purified water (18.0 MΩ cm^{-1}) was prepared with a WaterPro water system (Axlwater Corporation, TY10AXLC1805-2, China) and used throughout the experiments. All reagents were of at least analytical grade and used without further treatment.

2.2. Preparation of γ-Fe_2O_3@CHO

The aldehyde-functionalized magnetic nanoparticles (designated as γ-Fe_2O_3@CHO) were prepared through a one-pot solvothermal method by using $FeCl_3{\cdot}6H_2O$ as a single iron source and GA as modification ligand. In a typical reaction procedure, 0.5 g of $FeCl_3{\cdot}6H_2O$ and 1.8 g of NaOAc were dissolved in 15 mL of EG under stirring, and then 3.5 mL of GA was added into the mixture and merged evenly with assistance of sonication. The obtained homogeneous yellow solution was transferred to a Teflon-lined stainless-steel autoclave and sealed to heat at 200 °C. After reaction for 8 h, the autoclave was cooled to ambient temperature. The composite products were washed several times with highly purified water and ethanol, and then collected with a magnet and dried for further use.

2.3. Adsorption performance of γ-Fe_2O_3@CHO

The adsorption of protein by the obtained magnetic nanomaterials was investigated in phosphate buffer (0.1 M), selecting BSA (p*I* = 4.9), BHb (p*I* = 6.9), and Lyz (p*I* = 11.2) as model protein, respectively. In general, 20 mg of magnetic nanomaterials was added to 10 mL of protein solution at a concentration of 0.30 mg mL^{-1}, the mixture was shaken on a controlled shaker at 200 rpm for 30 min. Afterward, protein-laden magnetic nanomaterials were removed magnetically from solution, and the amount of protein adsorbed on the γ-Fe_2O_3@CHO was calculated through comparing the initial and final concentration of protein.

Factors affecting the adsorption performance of proposed method such as amount of γ-Fe_2O_3@CHO (10–50 mg), adsorption time (5–40 min), and pH (3–8) conditions were investigated and optimized. In these experiments, protein solution (0.30 mg mL^{-1}) was used to study the adsorption performance under different experimental conditions. The amount of protein adsorbed on the γ-Fe_2O_3@CHO was used to evaluate the influence of the factors on the adsorption performance. All the experiments were performed in quintuplicate, and the means of the results were used.

2.4. Characterization

The morphology of the as-synthesized sample was portrayed with Tecnai G2 T2 S-TWIN transmission electron microscope (TEM). Fourier transform infrared (FT-IR) spectrum was obtained on a Nicolet AVATAR 330 FT-IR spectrophotometer using KBr pressing method. KBr pellet was prepared with 1:200 mass ratio of material and KBr. The FT-IR was recorded using transmissive mode and the spectrum was collected at room temperature in the 4000–400 cm^{-1} region with a resolution of 4 cm^{-1} using 32 scans. Crystal structure information was recorded using a Rigaku D/max/2500v/pc X-ray diffractometer with Cu Kα radiation. The magnetization was measured using a vibrating sample magnetometer (VSM, LDJ 9600-1). Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface area by nitrogen adsorption/desorption isotherm on a Micromeritics TriStar 3000 apparatus. The pore volume and pore diameter distribution were derived from the adsorption branches of isotherms using the Barrett–Joyner–Halenda (BJH) model. The Zeta potential of γ-Fe_2O_3@CHO was measured using a Malvern Zetasizer Nano ZS90 pH automatic analysis system. Sample was prepared by mixing 0.25 g solid sample in 50 mL of electrolyte (NaCl, 0.01 M) in an Erlenmeyer flask and pH was adjusted with 0.01 M HCl or NaOH. Then the sample was stirred for 1 h at room temperature. Subsequently, the sample was allowed to stand for 15 min to let larger particles settle and the colloidal suspension was collected for analysis.

3. Results and discussion

3.1. Characterization of γ-Fe_2O_3@CHO

Fig. 1A presents the TEM image for γ-Fe_2O_3@CHO. The obtained nanoparticles are nearly spherical and dispersive with a diameter ranging from 15 to 24 nm. A particle size distribution histogram, based on the size of 100 particles measured from several TEM images, is shown in Fig. 1B. The average particle size is 19.3 nm with the standard deviation of 1.8 nm.

The nitrogen adsorption–desorption isotherm and pore-size distribution curve (inset) of the γ-Fe_2O_3@CHO were investigated. Generally, pores are assorted into three categories based on diameter as micropores (d < 2 nm), mesopores (2 nm < d < 50 nm), and macropores (d > 50 nm). As shown in Fig. 2, the isotherm belongs to type IV N_2 adsorption isotherm with an evident hysteresis, suggesting the presence of mesopores. Furthermore, the hysteresis loop shifts approach relative pressure (p/p_0) = 1, indicating the existence of macropores. It is in agreement with the result of pore diameter distribution that includes two main peaks with one at 2.1 nm and the other at approximately 60 nm. According to the IUPAC recommendations [14], hysteresis loops are classified into four types. Isotherms with type H3 loops that do not level off at relative pressures close to the saturation vapor pressure were reported for materials comprised of aggregates (loose assemblages) of plate like particles forming slit like pores. According to the standard Brunauer Emmett Teller (BET) method, the specific surface area of the γ-Fe_2O_3@CHO is calculated to be 31.5 m^2 g^{-1}, which is quite small. The reason may be attributed to the aggregation of nanoparticles as reflected by TEM. Meanwhile, the existence of pores in resultant nanomaterials may be the interspaces of

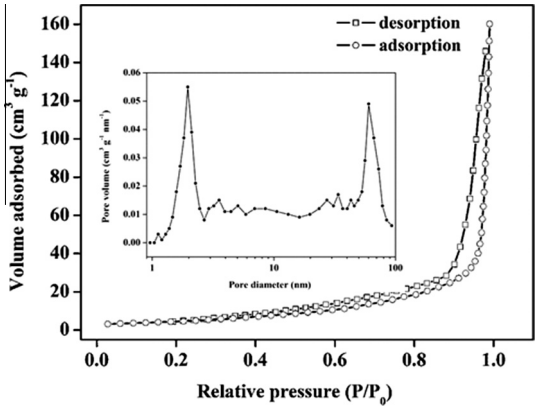

Fig. 2. Nitrogen adsorption–desorption isotherm and the pore diameter distribution (inset) of γ-Fe_2O_3@CHO.