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Preparation of amine-functionalized mesoporous magnetic colloidal nanocrystal clusters for glucoamylase immobilization

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HIGHLIGHTS

- Mesoporous carboxyl-functionalized magnetic colloidal nanocrystal clusters (MCNCs) were synthesized.
- The mesoporous MCNCs showed large surface area and high magnetization.
- The sizes of the MCNCs could be easily tuned by varying the surfactant concentration.
- The amine-functionalized mesoporous MCNCs were utilized to immobilize enzyme.

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ABSTRACT

A facile one-pot synthesis of size-tunable mesoporous carboxyl-functionalized magnetic colloidal nanocrystal clusters (MCNCs) with high magnetization (82.0 emu/g), large surface area (95 m^2/g), and excellent colloidal stability has been developed. The mesostructured MCNCs were synthesized by a solvothermal approach with iron (III) chloride hexahydrate as a precursor, ethylene glycol as a reducing agent, ammonium acetate as a porogen, and ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) as a surface-modification agent. Glucoamylase was immobilized onto the mesoporous MCNCs via the different routes. These immobilized glucoamylase exhibited excellent thermal stability and reusability in comparison with the free enzyme. The residual activity of immobilized enzyme remained above 65% after 6 h, while free glucoamylase was only left over 45% of the initial activity. And the residual activity of the immobilized enzyme was about 60% of the initial activity after the 10th reuse.

1. Introduction

Magnetic colloidal nanocrystal clusters (MCNCs) have attracted significant interest because of the unique superparamagnetic properties, high magnetization, and high water dispersibility [1], which has been widely used in diverse areas of bioseparation [2,3], MRI contrast agents [4,5], heterogeneous catalysts [6,7] and drug delivery [8,9]. For bioapplication applications, magnetism makes possible heterogeneous catalysis by which fast separation of biocatalysts is made feasible, and the process can be carried out continuously [10–12]. In principle, the ideal MCNCs for bioapplication should possess suitable surface area, narrow size distribution, strong magnetic response, abundant functional groups and excellent biocompatibility. Therefore, the synthesis of monodisperse MCNCs with a hydrophilic functional surface, especially for the template-free synthesis of functional mesoporous MCNCs, is still an essential yet challenging step as they have much potential in biological and medical fields, such as the immobilization of proteins, peptides, and enzymes [13].

Over the past two decades, there have been various techniques for the preparation of these MCNCs, such as coprecipitation and microemulsion methods [14,15]. However, the relatively poor size uniformity and monodisperse of the nanoparticles obtained strongly affect their magnetic properties. Exciting progress has been made in synthesizing iron oxide magnetic nanocrystals with controlled size and shape by high-temperature solution-phase reaction of $Fe(acac)_3$ [16,17]. These small magnetic nanocrystals have specific surface area and uniformly size, but the weak magnetic response and high reaction temperature limits their technical use. Among the various synthetic methodologies, Li group [18] first described the one-pot solvothermal synthesis method that has

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attracted researchers' attention for preparing cluster-structure magnetic nanomaterials. This facile method evokes much interest, and is subjected to extensive studies for the preparation of MCNCs that satisfy all major requirements in biotechnology. Yin et al. [19] synthesized highly water-dispersed MCNCs using poly (acrylic acid) to the surface of MCNCs as a stabilizer. Cheng et al. [20] fabricated sodium citrate stabilized MCNCs to improve biocompatibility, and the tuning effect of sodium citrate on the magnetite nanocrystal clusters was well illustrated. Li and coworkers [21] also reported the synthesis of amine-functionalized MCNCs using 1,6-hexanediamine as precipitation agent and amine-functional agent. Unfortunately, saturation magnetization is improved by the sacrifice of the large surface area so as to restrict their application [22]. Therefore, it is a new challenge to fabrication of functional mesoporous MCNCs with high magnetization and large surface area that satisfy all major requirements in biotechnology.

Herein, we report, for the first time, the synthesis of the mesoporous carboxyl-functionalized MCNCs with tunable size and high magnetization. As depicted in Fig. 1, the nanocrystals were assembled into interior porous clusters by using iron (III) chloride hexahydrate as precursor, ethylene glycol as reducing agent, ammonium acetate as a porogen and EDTA-2Na as a surface modification agent. Moreover, the particle sizes can be simply controlled by varying the relative concentrations of EDTA-2Na. To estimate the applicability of the obtained MCNCs in biotechnology, the mesoporous carboxyl-functionalized MCNCs were used to immobilize glucoamylases by covalent bonding, and amine-functionalized mesoporous MCNCs were used to immobilize glucoamylases by electrostatic adsorption. The properties of the immobilized glucoamylases also were studied systematically.

2. Materials and methods

2.1. Enzymes and reagents

Glucoamylase (exo-1, 4-a-D-glucosidase, EC 3.2.1.3 from *Aspergillus niger* 10 U mg^{-1}) was purchased from Yixing Enzyme Preparation Company (China); Bovine serum albumin (BSA), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 99%), *N*-hydroxysuccinimide (NHS, 97%), and tris(2-aminoethyl)amine (TAEA) were purchased from Sigma Chemical Co.; Other chemicals and reagents were analytical grade, obtained from Tianjing Chemical Reagent Company (China).

2.2. Preparation of mesoporous carboxyl-functionalized MCNCs

The mesoporous carboxyl-functionalized MCNCs were prepared through a modified solvothermal reaction [23–25]. Typically, anhydrous NH_4OAc (2.0 g), $FeCl_3{\cdot}6H_2O$ (0.8 g) and EDTA-2Na (with various weight: 0, 0.1, 0.3, 0.5, and 1.0 g) were dissolved in ethylene glycol (40 mL) under vigorously stirring to give a homogeneous yellow solution. The solution was sealed in a Teflon-lined stainless-steel autoclave heated at 200 °C for 12 h, and the mixture was then cooled to ambient temperature. The resulting black magnetite particles were washed with deionized water and ethanol, and dried at 60 °C before characterization and application.

2.3. Amine functionalization of mesoporous MCNCs

The carboxyl-functionalized mesoporous MCNCs were first activated with EDC/NHS mixture [26]. 0.2 g of mesoporous MCNCs in

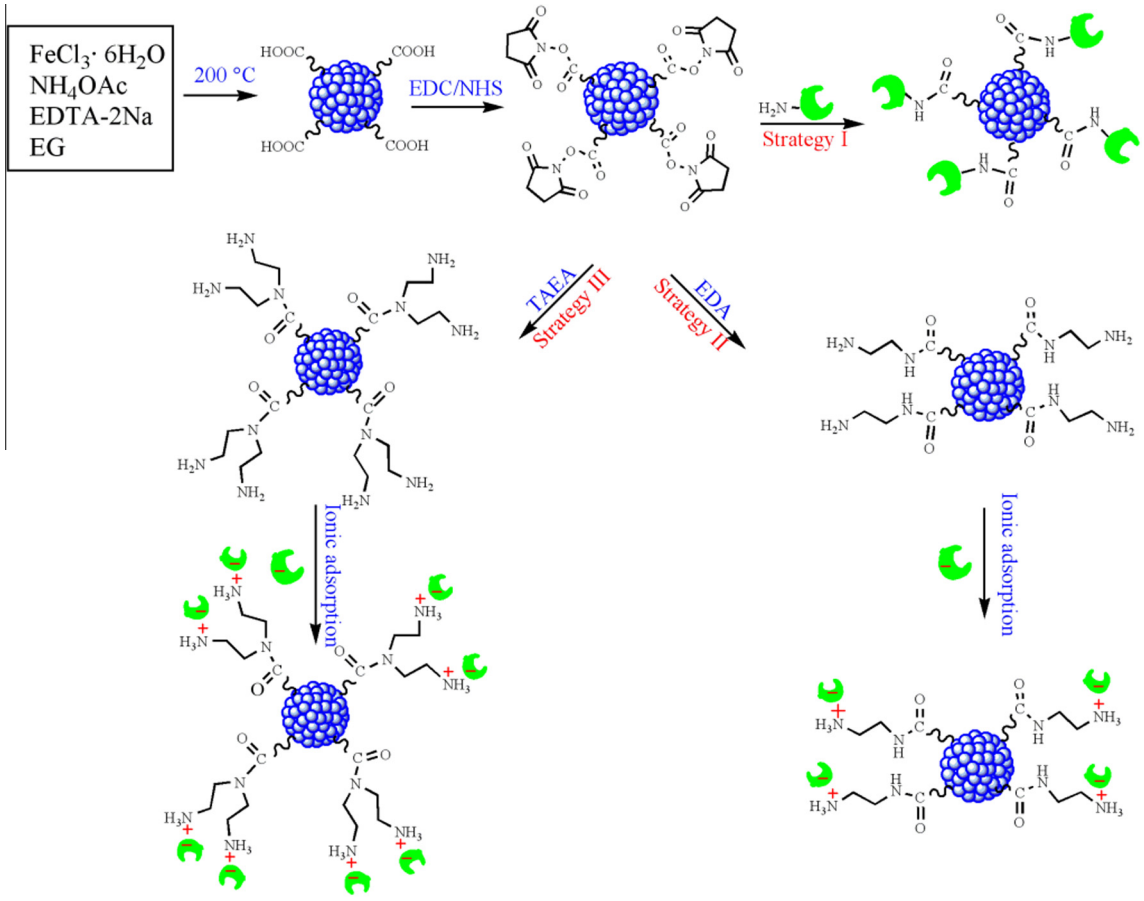

Fig. 1. Schematic representative for the preparation of the supports and enzyme immobilization.