



Preparation and characterization of hollow magnetic composite nanoparticles for immobilized pectinase



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ABSTRACT

The aims of the present work were to establish the magnetite core based nanometer microspheres of morphology concept of multifunctional core/shell nanometer microspheres which had the potential use as pectinase supporter. We had described the convenient method of synthesis and characterization of biodegradable/biocompatible Fe₃O₄/SiO₂@CMCS. The larger aspect ratios of hollow materials could be taken up by cells in larger amounts and have higher internalization rates, which was of great significance for the enhancement of chemotherapy effectiveness. We investigated the enzymatic activity of pectinase adsorbing on novel hollow magnetic composite microspheres. The immobilized enzyme retained more than 50% of its initial activity over 40 days, and the optimum temperature/pH also increased to the range of 50–60 °C/3.0–5.0. The immobilized enzyme exhibited great operational stability, and more than 75% residual activity was observed after 10 batch reactions. Moreover, the Fe₃O₄/SiO₂@Carboxymethyl chitosan (CMCS) support presented a very simple, mild and time-saving process for enzyme immobilization. This strategy of immobilizing pectinase made expensive enzymes economically viable, strengthening repeated usage of them because of their rapid and easy separation with a magnet.

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1. Introduction

As one of the important members of biocatalysts, pectinase exhibit a number of features that make them more advantageous than conventional biocatalysts [1,2]. Foremost the high level of catalytic efficiency and high degree of specificity allow them to discriminate themselves not only from reactions but also from substrate specificity with similar parts of molecules (regiospecificity) [3]. Pectinase is used for the fruit-processing industry to increase yields, and improve liquefaction, clarification and filterability of juices, maceration and extraction of plant tissues [4,5]. Despite excellent catalytic properties of pectinase, the native enzymes as biocatalysts always present some drawbacks, such as poor stability under operational conditions, difficulty of product recovery, and impossibility of multiple reuses in an industrial process [6]. To overcome these problems, enzyme immobilizations are involved to improve the catalytic features of enzymes against several forms of denaturation as well as to make expensive enzymes economically viable, strengthening repeated usage [7]. Immobilization is usually considered to be an important technique to enhance stability of enzymes. Recently, a large number of nano-scaled carriers have been applied in the enzyme immobilization [8], and enzyme catalytic biotechnology is

explored for its potential application. Immobilization of enzyme has been studied using various supports, such as inorganic materials [9], activated agar-gel [10] and polymer [11]. The essential requirement for any carrier is the need to have a large surface area. In this respect, porous polymeric materials, which have an obvious advantage of high internal surface areas, are being increasingly employed as the solid supports. Either the surface reaction or the diffusion of substrate and product in the pore may be the rate-controlling step in the immobilized enzyme system [12–14].

Magnetic polymer microsphere is a kind of hybrid microsphere with magnetic materials, which is composed of polymer and inorganic magnetic particles. Magnetic composite materials not only possess the unique characters of magnetic materials such as superparamagnetism but also the properties of the polymer. At the same time, the hollow structure makes them have large surface area, small density, large internal space and good surface permeability. Therefore, the materials have potential applications in biomedicine, catalysis, sewage treatment etc. Based on the research background and the development prospect, we modified the magnetic hollow particles, via coated carboxymethyl chitosan (CMCS) to develop the biocompatibility and the dispersibility in the aqueous solution.

CMCS is an amphoteric chitosan possessing many useful chemical and biological properties in addition to its low toxicity [15], excellent biocompatibility [16], and high solubility in water. Among many carriers

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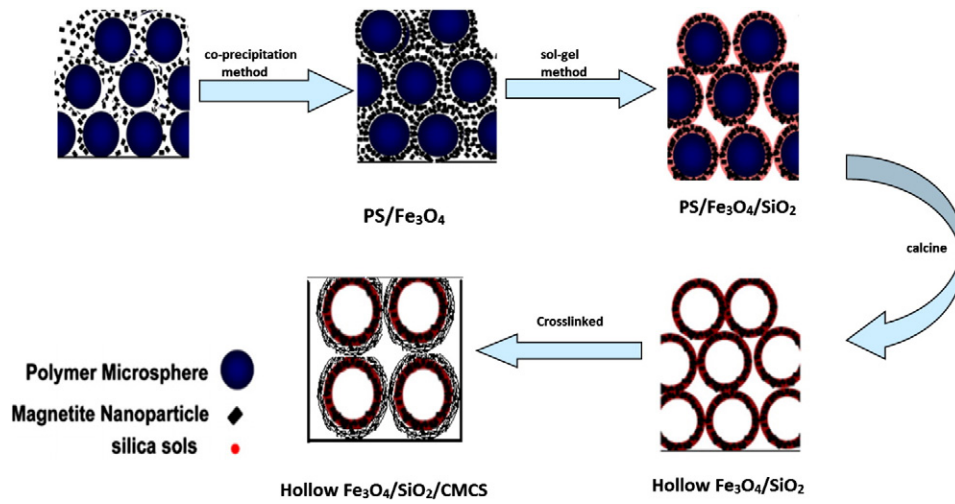


Fig. 1. Schematic showing the synthesis of hollow magnetic composite nanoparticles.

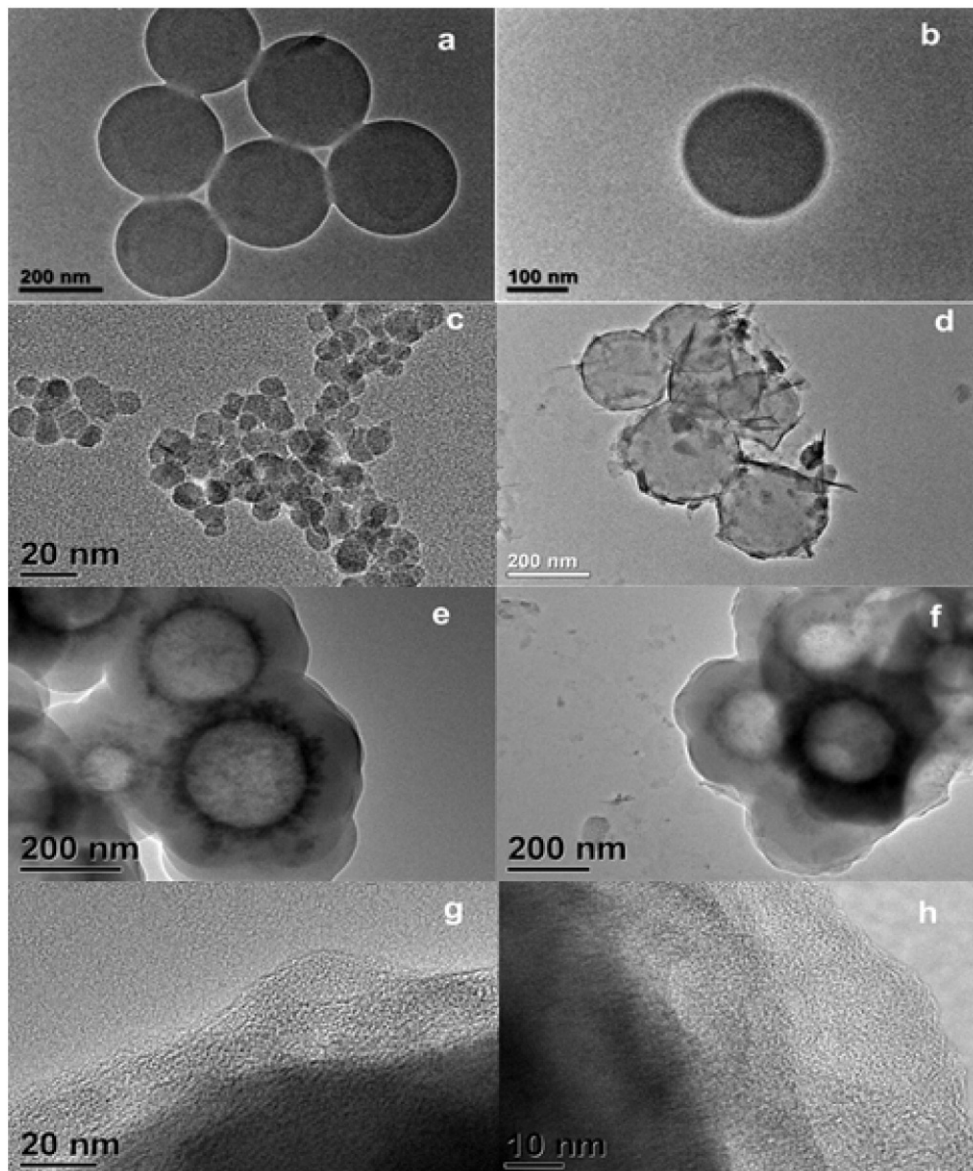


Fig. 2. TEM images of (a, b) PS, (c) Fe₃O₄, (d) Fe₃O₄/PS, (e) HMS, and (f, g, h) different multiples of CMCS-HMS.

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