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Immobilization of carbonic anhydrase on epoxy-functionalized magnetic polymer microspheres for CO_2 capture

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ABSTRACT

Carbonic anhydrase (CA) is the most efficient catalyst for CO_2 hydration and dehydration. In the present work, magnetic polymer microspheres functionalized with epoxy group were prepared, and CA enzyme was immobilized on the carries by selective covalent binding. The parameters affecting CA immobilization, such as pH, temperature and enzyme dose were investigated. Based on the investigation and the characterization of the microspheres, the kinetic parameters of the immobilized and the free CA were also evaluated. The value of the Michaelis–Menten constant (K_m) and the maximum velocity (V_{max}) of the immobilized CA was 8.077 mmol/L (mM) and 0.027 μmol/(min mL), while that of the free CA was 6.091 mM and 0.091 μmol/(min mL), respectively. Moreover, the performance of the thermal stability, storage stability and reusability of the immobilized CA confirmed that CA immobilized on the epoxy-functionalized magnetic polymer microspheres owned a stable and efficient catalytic ability on CO_2 hydration, which seemed to be a suitable candidate for CO_2 capture.

1. Introduction

Carbon dioxide (CO_2) is one of the major contributors to the global climate change [1,2]. It is urgently needed to develop appropriate and efficient carbon capture technologies to reduce CO_2 emissions [3]. Various technologies, including absorption, adsorption, cryogenic, membrane, microbial/algal and geological methods have been proposed for capturing CO_2 [1]. Among them, only monoethanolamine (MEA)-based processes have been used in industry. Nevertheless, these processes still suffer some fatal drawbacks, such as amine degradation and high energy consumption [4,5].

Recently, an integrated vacuum carbonate absorption process (IVCAP) has attracted lots of attention, which employs the potassium carbonate (K_2CO_3) to absorb CO_2 [6]. Due to the weak affinity between CO_2 and K_2CO_3, CO_2 desorption from CO_2-saturated solution can be carried out at a relatively low temperature (50–70 °C) and 2–8 psia vacuum condition, which in turn significantly reduces the parasitic power loss [7,8]. However, the absorption rate of CO_2 in the K_2CO_3 system is much slower than that in the MEA-based system. To address this problem, the carbonic anhydrase (CA) is introduced as a biocatalyst to accelerate the absorption rate of CO_2 in the IVCAP system [9,10]. However, poor thermal and storage stability as well as the finite repeatable usage of the free CA would seriously limit their practical application. Whereafter, enzyme was immobilized onto a solid support to eliminate these disadvantages. A variety of support materials, such as controlled pore glass [5], mesoporous aluminosilicate [9], alginate [11], egg shell membrane [12], nanocomposite hydrogel [13,14], silica [15,16], polypyrrole film [17], and polyurethane foam [18] had been prepared for CA immobilization. However, a high mass transfer resistance and an inconvenient recovery of the immobilized enzyme from the reactor still limited their application.

In recent years, magnetic materials have been gradually applied to immobilize enzyme due to their low mass transfer resistance and easy operation [19–24]. In order to achieve perfect performance, some reactive functional groups, such as amine, hydroxyl, carboxyl and epoxy, were synthesized on the surfaces of the materials. Among them, the epoxy group on the supports can react with different nucleophilic groups of the protein, e.g., amino, hydroxy, or thiol moieties, to form extremely strong linkages [25,26]. Moreover, the conjugation of epoxy group and bio-molecules can be carried out at room temperature without adding activator or cross-linker [27]. Wang et al. [20] immobilized the laccase on the magnetic mesoporous silica nanoparticles in a magnetically stabilized fluidized bed. Their results showed that the immobilized laccase had a fast degradation rate of phenol and still retained 71.3% of its initial degradation ability after 10 batch treatments. Tural et al. [26] prepared an epoxy attached magnetic nanoparticle to immobilize benzoylformate decarboxylase (BFD) from *Pseudomonas putida*.

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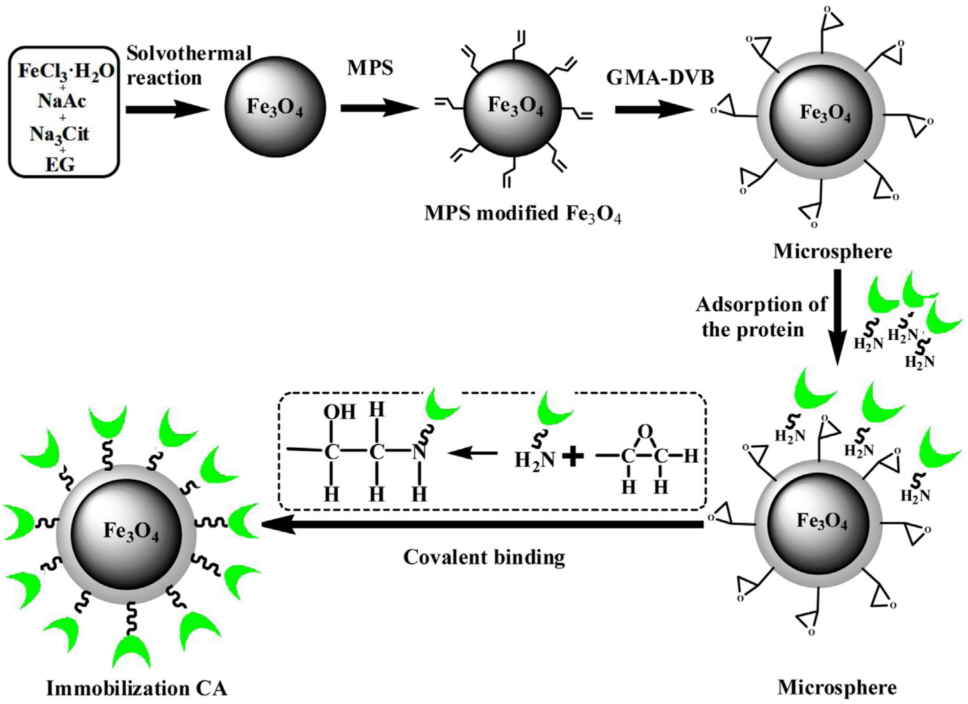

Fig. 1. Schematic diagram of the microspheres preparation and CA immobilization.

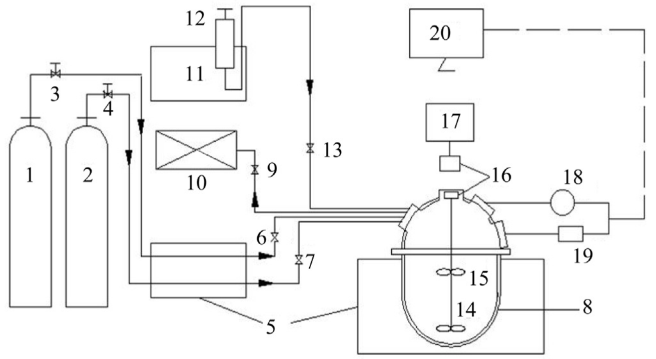

Fig. 2. Schematic diagram of the CO_2 hydration system.
(1) N_2 cylinder; (2) CO_2 cylinder; (3, 4, 6, 7, 9, 13) Valve; (5, 11) Water bath; (10) Vacuum pump; (12) Syringe; (14, 15) Impellers (16) Magnetic stirring rotor; (17) IKA electric machinery; (18) Precision pressure gauge; (19) Humidity and temperature meter; (20) Computer.

Chaleawlert-umpon and Pimpha [27] also successfully synthesized the epoxy functionalized magnetic polymer microspheres to immobilize antibody.

In the present work, a new epoxy functionalized magnetic poly(glycidyl methacrylate-divinyl benzene, GMA-DVB) microsphere was synthesized for CA immobilization by covalent binding method. The optimal conditions for CA immobilization were determined. The thermal stability, storage stability and reusability of the immobilized and the free CA were investigated. In addition, CO_2 hydration and some kinetic parameters of the immobilized CA were also compared with that of the free CA.

2. Materials and methods

2.1. Materials

CA from bovine erythrocytes were purchased from Worthington Biochemical Corporation, US. Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaAc), sodium hydroxide (NaOH), hydrochloric acid (HCl, 35%), trisodium citrate (Na_3Cit), potassium persulfate (KPS), *p*-nitrophenyl acetate (*p*-NPA) and *p*-nitrophenol (*p*-NP) were all purchased from Sinopharm Chemical Reagent Co., Ltd., China. Acetonitrile, anhydrous ethanol, ethylene glycol (EG) and aqueous ammonia solution ($NH_3{\cdot}H_2O$, 25%), were purchased from Xilong Chemical Co., Ltd., China. Glycidyl methacrylate (GMA, 98%), divinyl benzene (DVB, 50%) and 3-(trimethoxysilyl) propyl methacrylate (MPS, 98%) were purchased from Aladdin Chemistry Co., Ltd., China. All the other chemicals used in the study were of analytical grade. All solutions were prepared with ultrapure water.

2.2. Preparation of magnetic poly(GMA-DVB) microspheres

The magnetic poly(GMA-DVB) microspheres were synthesized according to a reported procedure with slight modification [28]. Briefly, 2.2 g of $FeCl_3{\cdot}6H_2O$, 2.6 g of NaAc, 0.47 g of Na_3Cit were dissolved in 40 mL of EG in a 50 mL three-necked flask under stirring

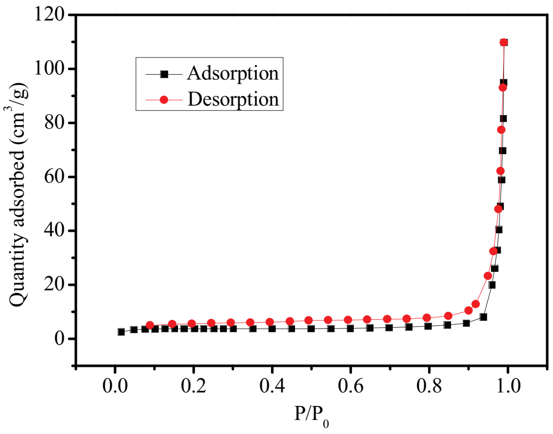

Fig. 3. N_2 adsorption isotherms (−196 °C) of the magnetic poly(GMA-DVB) microspheres.