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Protein adsorption using novel carboxymethyl-curdlan microspheres

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ABSTRACT

Carboxymethyl-curdlan as a water soluble curdlan derivative, was synthesized in an aqueous alkaline medium using monochloroacetic acid. Novel carboxymethyl-curdlan (CC) microspheres were prepared by the method of W/O/W emulsion. The chemical and morphological structures of CC microspheres were investigated by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and particle size analysis. The CC microspheres were spherical, free flowing, non-aggregated and uniform mono-disperse with diameter of 260 μm. The prepared CC microspheres were applied to adsorbing Bovine serum albumin (BSA) as model protein. Factors influencing the adsorption of BSA such as solution pH, temperature, initial BSA concentration and ionic strength were examined by batch experiments. The maximum adsorption capacity was calculated as 168 mg/g under optimal conditions including BSA initial concentration (4 mg/mL), pH (4.7), adsorption time (9 h) and temperature (35 °C). The adsorption isotherm followed the Langmuir model and the adsorption kinetics fitted the pseudo-second-order model. In addition, the CC microspheres can be also regenerated and re-used.

1. Introduction

Proteins are relatively large biomolecules and have a tendency to accumulate at the interface between solutions and solid surfaces [1]. Purification of proteins is the first step and essential for not only disease and pharmaceutical research but also improvement of the significant applications such as bioreactors, biosensors and biofuel cells [2]. Adsorption of proteins to surfaces has been extensively studied because of its frequent occurrence in nature and in biomedical applications [3].

Curdlan is a bacterial unbranched polysaccharide composed of repeating units of β-D-glucose [4]. The unique rheological and thermal properties of curdlan make it a good gelling material for the improvement of the textural quality, water-holding capacity and thermal stability of various food products [5]. Curdlan is biodegradable, nontoxic toward humans and the environment, edible and it has growing capacity in the pharmaceutical industry because of its potent biological activities [5,6]. Curdlan and its derivatives have also been used for drug delivery [7], protein drug delivery [8], immune stimulating for the activation of immune responses [9]. Curdlan is insoluble in water, which limits its biological applications[4]. Carboxymethyl substitution is considered as another method to improve the functional properties for many polysaccharides [10]. In addition, the hydrophilicity of curdlan is increased by modifying the backbone with carboxymethyl groups that carboxymethyl-curdlan has good water solubility [10,11].

Polysaccharide microspheres have found wide applications in separation and purification processes [12]. Because of their low toxicity, good biocompatibility and biodegradability, they have shown great potential for use as a biocatalyst support [13], carrier for drug delivery [14], support for protein immobilization [15], protein adsorption and desorption [16]. The magnetic gelatin-starch microspheres were prepared by modified emulsion cross-linking method for BSA adsorption with a maximum adsorption capacity of 120 mg/g [17]. The maximum BSA adsorption was reported 110 mg/g onto the magnetic chitosan nanoparticles prepared by a W/O micro-emulsion system [18]. In addition, Various applications of polysaccharides have been reported in the literatures: removal of chromium(VI) from aqueous solution using guar gum-nano zinc oxide biocomposite adsorbent [19] and also using chitosan-citric acid nanoparticles [20] and removal of a model acid dye bromoth-

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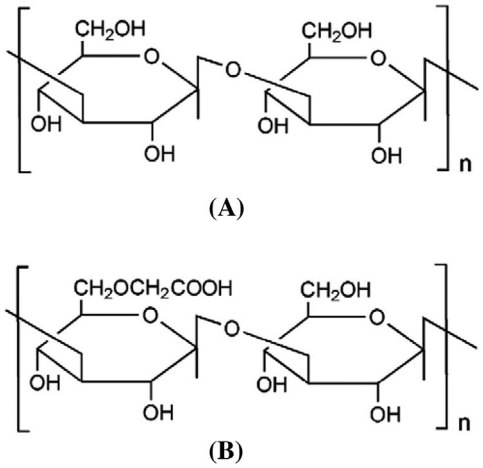

Fig. 1. Chemical structures: A) curdlan, B) carboxymethyl-curdlan.

ymol blue from aqueous solution using magnetic chitosan-bamboo sawdust composite [21].

In this work, carboxymethyl-curdlan was first synthesized and then, carboxymethyl-curdlan microspheres were prepared by water-in-oil-in-water (W/O/W) emulsion technique for protein adsorption. The structure, morphologies and particle size of the developed microspheres were characterized by Fourier transform infrared spectra (FTIR), Scanning electron microscopy (SEM) and particle size analysis. The adsorption of bovine serum albumin (BSA) as a model protein was achieved by using the microspheres. On the BSA adsorption, the effects of pH, temperature, initial protein concentration and ionic strength were investigated. The adsorption rates were determined quantitatively and simulated by the pseudo-first-order and pseudo-second-order kinetic models. The Langmuir and Freundlich isotherms were used to evaluate the equilibrium adsorption data.

2. Material and methods

2.1. Chemicals

Curdlan from *Alcaligenes faecalis* (C7821) and Bovine serum albumin (BSA) (A3059, fraction V) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade, and were used as received.

2.2. Carboxymethylation of curdlan

Carboxymethyl-curdlan was synthesized according to the previous method [11] with some modifications. A suspension of 2.0 g of curdlan in 80 mL isopropyl alcohol was stirred for 30 min at room temperature. Then, Four milliliter of sodium hydroxide solution (10 M) was added to the solution by stirring at room temperature for 60 min. Next, 3.2 g monochloroacetic acid was added over three separate intervals of 10 min each and the mixture was stirred at 55 °C for 3 h. The suspended product was recovered by filtration and washed successively with methanol–acetic acid (5:3 v/v), methanol–water (3:1 v/v), methanol and acetone. The precipitate was dissolved in water and dialyzed against distilled water at 4 °C for 2 days. The retentate was frozen and lyophilized to give the final product.

2.3. Preparation of microspheres

Two-step procedure was used for the sample preparation. The first step consisted of the preparation of the primary emulsion and the second step entailed dispersing a given amount of primary emulsion in the external phase.

The primary W/O emulsions were prepared as follows: Carboxymethyl-curdlan solution (1%w/v) was dissolved thoroughly into an aqueous solution of acetic acid (2% v/v) under stirring at 12,000 rpm until a complete homogenization. 2 g Tween 80 was added into 100 mL castor oil to form 2% solution (w/v). Then 200 mL carboxymethyl-curdlan solution was added dropwise (about 1 mL/min) into the liquid paraffin under magnetic stirring to form water-in-oil (w/o) emulsion.

In the second step, the primary emulsion added (40 wt.%) slowly in aqueous phase containing distilled water and the hydrophilic emulsifiers: Polysorbate 80, steareth-20 (1%) + PEG-40/50 stearate (1.2%), while the system was stirred at 500 rpm at room temperature for 30 min. The micro-particle suspension was subsequently centrifuged at 4000 rpm for 25 min and the supernatant was discarded. The pellet was collected and washed in turn with 5 mL toluene, acetone and distilled water, respectively. This washing procedure was repeated triplicate and the dried microspheres were stored at 4 °C in Petri dishes.

2.4. Characterization experiments of the microspheres

2.4.1. Fourier transform infrared spectroscopy

FT-IR spectra of curdlan and carboxymethyl-curdlan (CC) microspheres were recorded on a Shimadzu spectrometer (8400, Japan). The samples were diluted in solid KBr before the spectrum

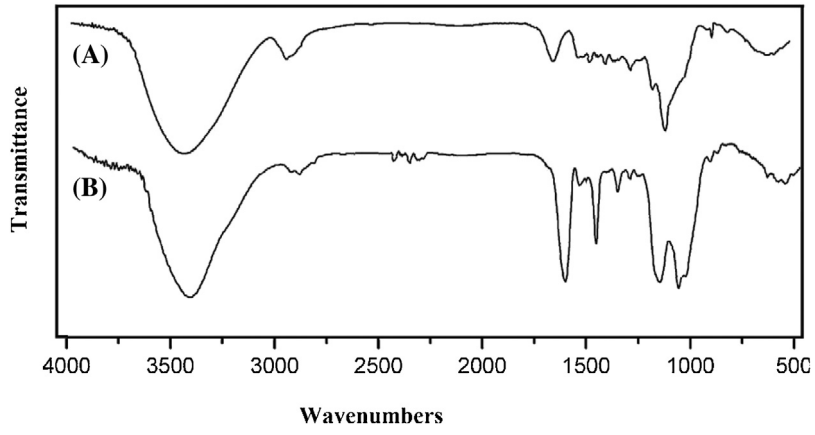

Fig. 2. Fourier transformed infrared (FTIR) spectra for (A) cudlan, (B) CC microspheres.