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Electrospun nanofibrous cellulose diacetate nitrate membrane for protein separation

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ABSTRACT

This contribution describes the preparation and characterization of cellulose diacetate nitrate (CDNA) nanofibrous membranes for bovine serum albumin (BSA) purification. CDNA was synthesized by nitration of cellulose diacetate (CDA), using HNO_3/CH_2Cl_2 as nitration agent. The chemical and morphological structures of CDNA were investigated by Fourier-transform infrared spectroscopy (FTIR), Elemental analysis, X-ray diffraction (XRD) and Scanning electron microscopy (SEM). Five solvents, that is, acetone, dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO), chloroform, and acetic acid were used to generate co-solvents for electrospinning CDNA. Long uniform CDNA nanofibers with an average diameter of 240 ± 80 nm were electrospun from a 15 wt% CDNA solution in DMSO/chloroform (2/1, v/v). The presence of nitrate groups on the surface of CDNA nanofiber membrane was confirmed by X-ray photoelectron spectroscopy (XPS). The prepared CDNA–DMSO/chloroform nanofibers were applied to adsorbing BSA, and the maximum equilibrium adsorption capacity (from Langmuir isotherm data) for BSA was 300.11 mg/g, which was higher than CDA nanofibers (18.63 mg/g). The membrane showed reusability after regeneration with elution buffer. In conclusion, the CDNA nanofibrous membrane is a promising material for protein purification.

1. Introduction

Electrospinning is a facile and continuous technique that has attracted much attention because of its capability and feasibility in the fabrication of large quantities of polymeric fibers on the sub-micrometer scale, ranging from approximately 50 nm to several micrometers [1,2]. The resulting electrospun nanofiber mats exhibit remarkable characteristics such as exceptionally high specific surface areas, porous structures in the nano-size range, high degrees of interconnections, and modifiable natures [3]. Because of these properties, electrospun fibers are ideal candidates in applications such as filtration [4], drug delivery [5], chemical and biological protection sensors [6], tissue engineering and tissue repair [7], and solar cells [8]. In particular, they have also been highly successful in the development of chromatographic columns [9–11]. Moreover, electrospun nanofibers facilitate chemical/physical functionalization that can lead to high separation efficiencies during purification processes [12–16]. Additionally, polymeric nanofibers have been gaining special attention in the recent years for unique properties that could potentially be applied in different types of separations such as protein adsorption [17–19], ion-exchange columns [20], and dialysis [21] due to their large surface area-to-volume ratios and their ease of handling as self-supporting membranes [22,23].

Cellulose diacetate (CDA, an acetate ester of cellulose) has been of increasing interest in the past decade in the area of nanofibers. It is useful for high-value applications such as chromatography [24], enzyme bioreactors [25], self-cleaning textiles [26], photochromic devices [27], and biosensors [28]. The CDA polymer is one of the most applicable polymers in the preparation of nanofibrous membranes due to its high hydrophilicity, good solubility in a wide variety of solvent systems, and low cost. However, the prepared mats have low oxidation and chemical affinity, which limit its application in the field of separation and purification. Grafting CDA with functional groups such as $–NO_2$, –COOH, and $–NH_3$ facilitates surface complexation with biomolecules (proteins, enzymes, amino acids). For instance, Yu et al. [29] prepared functional CDA fiber membranes by hydrolyzing the original cellulose triacetate semi-permeable membrane followed by carboxymethylation. Lu and Hsieh [30] prepared a highly efficient and versatile cellulose nanofibrous membrane by a nucleophilic reaction of the cellulose hydroxyl with the triazinyl chloride of the

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Cibacron Blue F3GA (CB) ligand. The resulting membrane had a facile lipase loading of approximately 150 mg/g. Huang et al. [31] prepared CDA nanofibers using hydrolysis, and subsequent oxidation by sodium periodate acted as a suitable immobilization platform for lipase. Ma et al. [10] prepared a protein A/G-functionalized electrospun regenerated cellulose nanofiber mat as an affinity membrane for immunoglobulin G purification. However, until now, few efforts have focused on electrospun cellulose diacetate nitrate (CDNA) nanofibrous membranes for the field of protein purification.

With the objective of exploring a novel nanofibrous membrane in the field of protein purification, cellulose diacetate nitrate mixed esters are first synthesized by nitration of hydroxyl groups in CDA. Fourier transform infrared (FTIR) and elemental analysis are used to characterize the CDNA structure. In the electrospinning process, a wider selection of mixed solvents that result in continuous electrospinning of CDNA fibers needs to be explored more extensively. Therefore, CDNA nanofibers are electrospun using three types of co-solvent systems [acetone/dimethylacetamide (DMAc), dimethyl sulfoxide (DMSO)/chloroform, acetic acid/acetone]. The morphology and structure of the prepared nanofiber membranes are investigated using scanning electron microscopy (SEM). The presence of nitrate groups on the surfaces of the CDNA nanofibrous membranes is confirmed by X-ray photoelectron spectroscopy (XPS). Bovine serum albumin (BSA, pI=4.7), which has structural homology with human serum albumin (HSA), is used as the model protein. BSA adsorption experiments are carried out to investigate the adsorption behaviors and adsorption mechanism of proteins on the CDNA nanofiber membranes.

2. Experimental

2.1. Materials

Cellulose diacetate (CDA, DS_{acetyl}=2.5, M_w=39,000 Da) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Dimethylacetamide (DMAc), acetic acid, acetone, chloroform, dimethylsulfoxide (DMSO), fuming nitric acid (HNO_3, 98%), dichloromethane (CH_2Cl_2, DCM) and other chemical reagents were commercial products of laboratory grade (Beijing Chemical Works, China) and used as received. Bovine serum albumin (BSA, M_w=68,000 Da, pI=4.7) was purchased from Newprobe Biopharmaceutical Technology Company, Ltd. (China). Water was doubly distilled immediately before used.

2.2. Synthesis of cellulose diacetate nitrate (CDNA)

The cellulose diacetate nitrate (CDNA) was synthesized for electrospun nanofiber membrane through hydrolysis followed by nitration as schematically shown in Fig. 1.

First, 15 g CDA powder was dissolved in 110 mL acetic acid and stirred for 6 h until a homogenous solution was obtained. Then H_2SO_4 (2.6 wt%) and acetic acid (38.9 wt%) were added to the CDA solution, and hydrolysis reaction was performed at 80 °C under vigorous stirring. The reaction was stopped by the addition of an amount of magnesium acetate (MgAc) under stirring for 15 min. After that, the hydrolyzed CDA particles were washed thoroughly with deionized water and oven dried at 50 °C for 24 h. In the second step, 10 g hydrolyzed CDA powder was dispersed in HNO_3/CH_2Cl_2 with 45/55, 55/45 and 65/35 (w/w) for 30 min. Ethanol was then added to the mixture under stirring to precipitate out the nitrated CDA. Finally, the precipitate was filtered, washed repeatedly with deionized water and dried in a vacuum oven at 50 °C for 24 h. The molecular weight of CDNA was investigated with gel permeation chromatography (GPC), which provided in the supplementary material (Table S3).

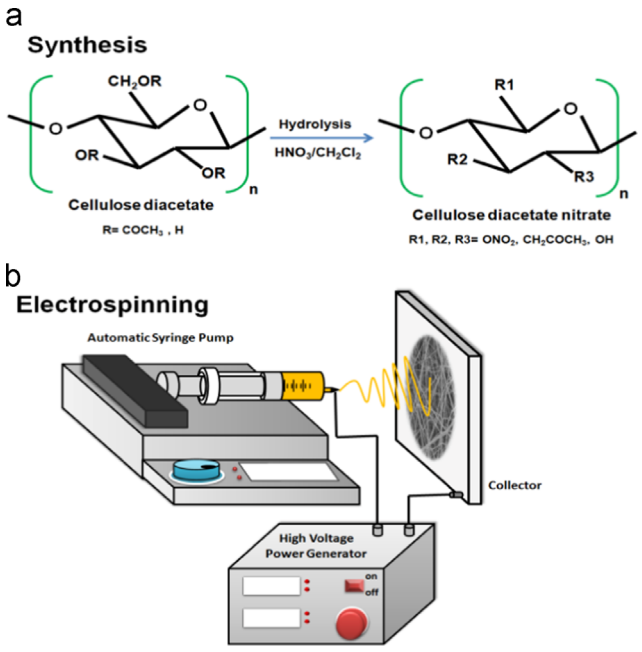

Fig. 1. The schematic diagram of (a) synthesis CDNA and (b) electrospinning of CDNA nanofibers.

2.3. Electrospinning

The experimental setup used for electrospinning process of this study is schematically shown in Fig. 1b. Solutions of CDNA were prepared in mixtures of acetone/DMAc 2/1(w/w), and DMSO/chloroform 2/1(v/v) and acetic acid/acetone 2/1(w/w). The concentration of CDNA solutions was varied between 10 wt% and 17 wt%. The electrospinning parameters were as follows: voltage potential, 15–20 kV; tip-to-collector distance, about 15 cm; flow rate of the solution through the syringe, 2–4 mL/h. The process was carried out in air at room temperature. The as-spun fiber was immersed in deionized water for 24 h to remove the co-solvent. The coagulated fiber was dried in an oven at 50 °C for 24 h.

2.4. Characterization

Fourier Transform Infrared (FTIR) spectra were measured from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1} by a Nicolet 6700 infrared spectrometer (USA). Elemental analyses of the prepared polymers were performed on an Elemantar Vario EL-III elemental analyzer (Germany). Morphologies of the electrospun nanofibrous membranes were examined using a field emission scanning electron microscope (HITACHI S-4800) operating at 5–15 kV. Prior to performing the FE-SEM analysis, the nanofiber samples were coated with gold up to 90 s in a turbo sputter coater. Diameters of the as-spun smooth or beaded fibers were measured manually from SEM images which were dealt by Image pro-plus analysis software, with an average value for each sample being calculated from at least 200 measurements. X-ray photoelectron spectroscopy (XPS) of the samples was performed on a Thermo Escalab 250Xi Photoelectron Spectrometer (Thermo Fisher Scientific, East Grinstead, USA) with an AlKα (1486.6 eV) X-ray source. Wide scan survey spectra were obtained using a binding energy (BE) step size of 1.0 eV. Subsequent high resolution scans over variable BE ranges with 0.05 eV steps were recorded for specific elements of interest. X-ray diffraction (XRD) measurements of CDNA nanofibers were