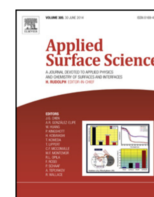
G Model
APSUSC-27723; No. of Pages 6
ARTICLE IN PRESS
Applied Surface Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc



Polyether sulfone/hydroxyapatite mixed matrix membranes for protein purification

Junfen Sun [a,*], Lishun Wu [b]

[a] State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Material Science and Engineering, Donghua University, North People Road 2999, Shanghai 201620, PR China
[b] Department of Chemistry and Chemical Engineering, Heze University, Daxue Road 2269, Heze, Shandong Province 274015, PR China

A R T I C L E I N F O

Article history:
Received 7 January 2014
Received in revised form 18 April 2014
Accepted 21 April 2014
Available online xxx

Keywords:
Polyether sulfone
Hydroxyapatite (HAP)
Mixed matrix membranes (MMMs)
BSA adsorption

A B S T R A C T

This work proposes a novel approach for protein purification from solution using mixed matrix membranes (MMMs) comprising of hydroxyapatite (HAP) inside polyether sulfone (PES) matrix. The influence of HAP particle loading on membrane morphology is studied. The MMMs are further characterized concerning permeability and adsorption capacity. The MMMs show purification of protein via both diffusion as well as adsorption, and show the potential of using MMMs for improvements in protein purification techniques. The bovine serum albumin (BSA) was used as a model protein. The properties and structures of MMMs prepared by immersion phase separation process were characterized by pure water flux, BSA adsorption and scanning electron microscopy (SEM).

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Mixed matrix membranes (MMMs), a composition of polymers and inorganic material, combine the selectivity of the filler material with the low costs, manufacturing ease and flow behavior of polymer membranes [1,2]. In recent years, an increasing number of people pay high attention to the research of MMMs. MMMs using different kinds of particles have a wide variety of applications, such as gas separation [1–4], waste water treatment [3], protein adsorption [4,7–9], etc. In membrane application, different adsorbents such as ion exchange particles [5,6] activated carbon particles [7], titanium dioxide (TiO_2) [8] and silicon dioxide (SiO_2) [9] were dispersed in microporous and macroporous polymeric structures. The resultant MMMs have excellent separation performance and special properties from particles. Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6{\cdot}(OH)_2$) has good adsorption to protein and is mainly used for repairing bone tissue and culturing sclerotin in the medical field because of its biocompatibility, bioactivity and osteoconductivity [10,11]. HAP has been used in adsorption chromatography for many years and widely applied for separating various proteins using as a column for a high performance liquid chromatograph apparatus [12]. It shows high affinity for proteins and much attention has been focused on HAP particles as sorbents to remove pathogenic proteins from blood in blood purification therapy [13], or to acts as a carrier for protein delivery [14]. Recently a lot of works focus on applying HAP for adsorbing proteins such as milk proteins [15], albumin [16] and lysozyme [17], etc. Moreover HAP has selective adsorption property to some protein by controlling crystal orientation and the shape of HAP [18,19], or substituting Ca^{2+} site in the HAP lattice with Zn^{2+} [20]. Iminodiacetic acid functionalized porous hydroxyapatite nanoparticles for capturing histidine-tagged proteins have high protein binding ability, and their specificity and affinity toward His-tagged proteins can remain after 5 times of recycling [21]. Hydroxyapatite-containing gelatin/chitosan microspheres for controlled release of lysozyme have high protein adsorption capability [22]. hydroxyapatite particles containing mercaptosuccinic acid (mercaptosuccinic acid (Mer), (Mer-HAp)) were synthesized and can load and release protein in a controllable way [23].

To improve protein purification, some researchers prepared MMMs embedding adsorptive particles. MMMs combine the selectivity of organic or inorganic particles with the high productivity of filtration membrane and have been applied for separation and recovery of proteins or enzymes. Protein separation in ion-exchange chromatography is mainly determined by the electrostatic interaction between the solute and the oppositely charged of the surface stationary phase [24]. Recently some researches combine ion exchange resins and polymer together to make MMMs which are applied in protein capturing, purifying and polishing steps. Avramescu prepared ethylene-vinyl alcohol (EVAL)/Lewatit

* Corresponding author. Tel.: +86 21 67792842; fax: +86 21 67792855.
E-mail addresses: junfensun@dhu.edu.cn, junfensun@sohu.com (J. Sun).

http://dx.doi.org/10.1016/j.apsusc.2014.04.123
0169-4332/© 2014 Elsevier B.V. All rights reserved.

Please cite this article in press as: J. Sun, L. Wu, Polyether sulfone/hydroxyapatite mixed matrix membranes for protein purification, Appl. Surf. Sci. (2014), http://dx.doi.org/10.1016/j.apsusc.2014.04.123

ion-exchange resins MMMs to adsorb BSA [6] and separate BSA and bovine hemoglobin (Hb) [25], and prepared PES/Lewatit ion-exchange resins adsorbers for lysozyme (LZ) separation [26]. Saiful prepared EVAL/ion-exchange resins MMMs to capture LZ [27] and EVAL/Lewatie anion-exchange resins MMMs to selectively bind β-Lactoglobulin from whey [28]. Saufi prepared PVDF/Phenyl Sepharose™ resin MMMs for binding whey protein [29]. Kopec [30] prepared solvent-resistant P84-based MMMs to adsorb BSA and LZ. BSA adsorption capacity is 77 mg/g membrane and LZ adsorption capacity is 85.1 mg/g membrane. In addition, activated carbon (AC) was used to combine polyether sulfone (PES) to prepare MMMs for removal of protein-bound toxins from human plasma [31]. Zirconium dioxide was used in MMMs to immobilize β-galactosidase, and the maximum β-galactosidase adsorbed on these membranes was 1.6 g/m^2 [32]. The mixed matrix polyvinylidene fluoride membranes with in-situ generated micro/nano polyethyleneimine particles was prepared to adsorb BSA, and BSA binding capacity is 105 mg of protein per ml of membrane [33]. A copper ion charged MMMs with highly interconnected pores and tightly incorporated beads can selectively adsorb hemoglobin (BHb) over BSA from a binary mixture [34]. Moreover some US patens reported HAP was used in polymer matrix. HAP was embedded in polytetrafluoroethylene (PTFE) matrix for absorption [35]. HAP/poly(etheretherketone) nanocomposites was prepared for a wide variety of applications, such as biological, medical, biochemical, biosensor, fuel cell, and aerospace applications [36]. HAP-targeting poly(ethylene glycol) having biologically active conjugates was provided [37].

In this study, a novel MMMs combining diffusion and adsorption of BSA retention solutes in one step were prepared. The adsorptive particles were incorporated in a porous membrane matrix. Compared to traditional columns, MMMs have low resistance, which allows the use of nano-particles, resulting in an improved adsorption capacity and adsorption kinetics [6]. MMMs are good choices to maintain the native conformation and the biological activity of proteins or enzymes during the isolation and purification process due to their low resistance and mild process conditions. The concept of MMMs has recently been recognized as a simple method to prepare adsorptive membranes. The MMMs were prepared by an immersion phase separation process in this work. PES was used for the preparation of the porous membrane matrix and HAP was used as adsorptive particle. Bovine serum albumin (BSA, molecular weight 67,000 Da, size $4 \times 14\,\mathrm{nm}^2$) was used as a model protein. This study investigates the combination of diffusion and adsorption in a single step, which probably leads to more efficient protein purification devices.

2. Experiment

2.1. Materials

Polyether sulfone (PES) (M_w = 58,000) and polyvinylpyrrolidone (PVP, K90) was produced by BASF Company. Hydroxyapatite (HAP, d = 40 nm) was purchased from Nanjing Emperor Nano Material Company. Dimethyl acetamide (DMAc, analysis grade) was used as solvent, which was produced by Sinopharm. DMAc and bovine serum albumin (BSA, M_w = 67,000) were supplied by China Medicine Chemical Reagent Company.

2.2. Membrane preparation

The membranes used in this study were prepared by the phase inversion method. PES, PVP and DMAc were mixed and heated until homogeneous mixed solutions with various compositions were obtained. The concentration of PES of PVP in DMAc is 14 wt% and 5 wt%, respectively. The HAP was added into the casting solution in order to improve membrane performance. The amount of HAP is 60 wt% in dry PES/HAP MMMs. The mixed matrix membranes were prepared by immersion precipitation. The polymeric mixture was cast on a glass plate and immersed into water bath and the membranes formed a few moments after immersion. The membranes were washed with tap water at room temperature to remove residual solvent.

2.3. Membrane characterization

2.3.1. Scanning electron microscopy

For scanning electron microscopy (SEM), membranes were dried in air at room temperature and cryogenically broken in liquid nitrogen. The obtained cross-sections were dried overnight under vacuum at 30 °C and gold coated. The cross sections, as well as the top and bottom surfaces of the membrane were characterized by scanning electron microscopy (SEM, JSM-5600LV, JEOL, Japan).

2.3.2. Pure water flux

The membranes were subjected to pure water flux estimation at a trans-membrane pressure of 0.1 MPa under cross-flow filtration. The permeability was measured under steady-state flow. Pure water flux was calculated as follows:

$$J_w = \frac{Q}{A \Delta t}$$

where Q is the quantity of permeate collected (in m^3), J_w is pure water flux (L m^{-2} h^{-1}), Δt is the sampling time (h), and A is membrane area (m^2).

2.3.3. BSA adsorption capacity

The bovine serum albumin (BSA) protein with molecular weight of 69 kDa and isoelectric point ~4.74 was used for the protein adsorption study. BSA adsorption was investigated within a range of pH from 5 (acetate buffer) and 6–9 (phosphate buffer).

The static protein adsorption capacity of membranes was determined with bovine serum albumin (BSA). The membranes were dried at 30° in a vacuum oven before examination. The samples containing 2 g/l BSA buffer solution were incubated with an exact amount of membranes in sealed containers under continuous shaking at 25°. The PES/HAP MMM adsorbed the BSA thereby reducing the BSA concentration in the bulk. The equilibrium BSA concentration after 24 h was monitored in time with a UV-1800 spectrophotometer which was produced by SHIMADZU Company. The BSA depletion was measured at 280 nm with 5 mm quartz cuvettes.

In order to determine the adsorption isotherm batch adsorption experiments were carried out with different initial protein concentration. The same amount of membrane was used in the experiments to reach different equilibrium concentrations. It is known that the Langmuir adsorption model can be used for protein adsorption from literature. The Langmuir equation can be rewritten as:

$$\frac{1}{q_{\mathrm{eq}}} = \frac{1}{q_{\mathrm{m}}} + \frac{K_{\mathrm{d}}}{q_{\mathrm{m}}} \times \frac{1}{C_{\mathrm{eq}}}$$

Since the parameters q_{eq} (adsorbed BSA concentration into the membrane adsorbers) and C_{eq} (the equilibrium BSA concentration in the bulk solution) are experimental data. A plot of $1/C_{\mathrm{eq}}$ versus $1/q_{\mathrm{eq}}$ allows to determine the maximum adsorption capacity (q_{m}) and the dissociation constant (K_{d}) by using a linear curve fitting.

2.3.4. BSA desorption capacity

After an adsorption and washing step the MMMs are transferred into the desorption buffer. Desorption was accomplished in static

Download English Version:

https://daneshyari.com/en/article/5350066

Download Persian Version:

https://daneshyari.com/article/5350066

Daneshyari.com