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Adsorption of protein onto double layer mixed matrix membranes

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ABSTRACT

This work proposed a novel approach for protein purification by using double layer mixed matrix membranes (MMMs). The double layer MMMs consisting of an active support and separating layer were prepared by co-casting two polymer solutions onto a glass plate. The active support layer consisted of nano hydroxyapatite (HAP) particles embedded in macroporous polyether sulfone (PES) and the separating layer was particle free PES membrane. The influence of separating layer with different PES content on membrane morphology was studied. The double layer MMMs were further characterized concerning permeability and adsorption capacity. The double layer MMMs showed purification of protein via diffusion as well as adsorption. The bovine serum albumin (BSA) was used as a model protein. The properties and structures of double layer MMMs prepared by immersion phase separation process were characterized by pure water flux, BSA adsorption and scanning electron microscopy (SEM).

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1. Introduction

In the recent years, there has been increasing interest in adsorptive membranes as carriers for macromolecules such as proteins [1], enzymes [2] and viruses [3]. Adsorptive membranes have shown great promise for bioseparations as an alternative to packed bed chromatography. Mixed matrix membranes (MMMs) as a kind of adsorptive membrane, a composition of polymers and active particles, combine the selectivity of the filler material with the low costs, manufacturing ease and flow behavior of polymer membranes [4,5]. The resultant MMMs have excellent separation performance and special properties from particles.

To improve protein purification, some researchers prepared MMMs embedding adsorptive particles. Recently some researches combined ion exchange resins and polymer together to make MMMs which were applied in protein capturing, purifying and polishing steps. The MMMs combined the principles of chromatography and membrane filtration in a single separation device. Avramescu prepared ethylene-vinyl alcohol (EVAL)/Lewatit ion-exchange resins MMMs to adsorb BSA [6] and separate BSA and bovine hemoglobin (Hb) [7], and prepared PES/Lewatit ion-exchange resins adsorber for lysozyme (LZ) separation [8]. Saiful [9] prepared EVAL/ion-exchange resins MMMs to capture LZ. Kopec [10] 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. Saufi prepared some MMMs embedding anion-exchange resins and cation-exchange resins. EVAL/Lewatie anion-exchange resins MMMs [11], EVAL/SP Sepharose^{TM} cation exchange resin MMMs [12] and a novel mixed mode interaction MMMs incorporating 42.5 wt% Lewatit MP500 anionic resin and 7.5 wt% SP Sepharose cationic resin into EVAL [13] were prepared for whey protein fractionation. The MMMs had good static binding capacities for β-lactoglobulin, α-lactalbumin, BSA and lactoferin in individual protein solutions.

Moreover different inorganic nanoparticles such as carbon nanotubes [14,15], titanium dioxide (TiO_2) [16] and silicon dioxide (SiO_2) [17] were dispersed in microporous and macroporous polymeric structures to prepare MMMs. Some inorganic nanoparticles such as copper sulfide nanoparticles [18], NiO nanoparticles [19], gold nanoparticles [20] and silver nanoparticles [21] process adsorption ability and were applied in the adsorptive field. Inorganic nanoparticles have some potential advantages over other polymeric nanoparticles because of their low susceptibility to immune response and low toxicity. Among the inorganic nanoparticles, hydroxyapatite (HAP, $Ca_{10}(PO_4)_6{\cdot}(OH)_2$) has attracted much attention as a carrier for biomolecules because of its excellent biocompatibility and bioactivity. It 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 [22,23]. HAP has been used in adsorption

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chromatography for many years, and widely applied for separating various proteins as a column in a high performance liquid chromatograph apparatus [24]. It was known that HAP has two different binding sites, the C and P sites on its surface respectively, which can provide proteins a multiple site binding opportunity [25]. The C sites are rich in calcium ions or positive charge and bind to acidic groups of proteins, and the P sites lack calcium ions or positive charge and attach to basic groups of proteins [26]. A lot of researches tried different ways to synthesis HAP with high affinity for protein. Mohandes synthesized nano HAP with controllable morphologies by the aid of various Schiff bases [27–29]. Liu [30] prepared calcium-deficient HAP for controlled drug delivery. Kandori [31] prepared positively charged calcium HAP which had high BSA adsorption. Dasgupta synthesized Zn and Mg doped HAP nanoparticles for controlled release of protein [32]. Moreover HAP has selective adsorption property to some protein by controlling preparation methods. Ozeki [33] prepared rod-shaped and plate-shaped HAP which selectively adsorbed BSA and lysozyme (LSZ), and BSA/LSZ adsorption ratio depended on the crystal shape. Kandori [34] found the sheet-like HAP particles could be applied to separate completely BSA from BSA/LSZ mixed solution. Fujii [35] prepared nano-crystalline Zn-containing HAP (ZnHAP) which had selective adsorption to pathogenic protein such as β_2-microglobulin (β_2-MG) in the BSA/β_2-MG mixed solution. Takemoto [36] synthesized hydroxyl-carbonate apatite which had higher selectivity for β_2-MG adsorption in the BSA/β_2-MG mixed solution. Some US patens reported HAP was used in polymer matrix. HAP was embedded in polytetrafluoroethylene (PTFE) matrix for absorption [37]. HAP/poly(etheretherketone) nanocomposites was prepared for a wide variety of applications, such as biological, medical, biochemical, biosensor, fuel cell, and aerospace applications [38]. HAP-targeting poly(ethylene glycol) having biologically active conjugates was provided [39]. The chitosan/graphene oxide/HAP nanocomposite with high bioactivity was prepared via a simple precipitation method with the aid of a new capping agent based on Schiff base compounds [40]. Previously, we prepared single layer PES/HAP MMMs which showed high BSA adsorption capacity and desorption rate [41].

In this study, the novel double layer MMMs combining diffusion and adsorption of BSA retention solutes in one step were prepared. The double layer MMMs consisting of an active support and separating layer were prepared by co-casting two polymer solutions onto a glass plate. The active support layer consists of HAP particles which are embedded in macroporous polyether sulfone (PES), and the separating layer is particle free PES membrane. The co-casting process opens the possibility to improve the mechanical stability and the biocompatibility of double layer MMMs while prevent particle loss during preparation and processing. Tijink prepared dual-layer MMMs embedded with activated carbon (AC) particles in flat membranes [42] and hollow fiber membranes [43]. The dual-layer MMMs had a higher clean water flux (350 l m^{-2} h^{-1} bar^{-1}) and higher creatinine adsorption (29 mg g^{-1} AC).

Compared to single layer MMMs, the double layer MMMs prevent HAP particles from releasing into the circulation. The top particle free layer is important for the application of MMMs, especially for the application in the field of blood purification [42,43]. In this work, double layer MMMs were prepared by an immersion phase separation process. PES was used for the preparation of porous membrane matrix and top particle free layer for double layer MMMs. HAP particles were used as adsorptive particles and were incorporated into a porous PES matrix with a high particle loading. Bovine serum albumin (BSA, molecular weight 67,000 Da, size 4 nm × 14 nm) was used as a model protein. This study investigated the combination of diffusion and adsorption in a single step, which probably leads to more efficient protein purification devices.

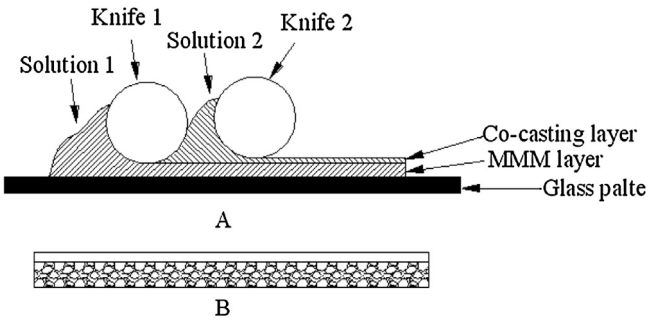

Fig. 1. (A) Schematic representation of the co-casting process. (B) Resulting membrane structure comprising a porous surface layer and a layer containing HAP particles.

2. Experiment

2.1. Materials

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

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 was 14% and 5%, respectively. The HAP particles were added into the casting solution and were dispersed in the casting solution for 24 h in order to make HAP particles have good dispersion in the mixed solution and improve membrane performance. The amount of HAP was 60 wt% in dry PES/HAP MMMs. A slit of 300 μm of casting knife for single layer MMMs was used. PES concentration for top layer of double layer MMMs was 5%, 10% and 15%, respectively. Fig. 1 shows schematic representation of the co-casting process. The heights of the slits of the first and second knife were 300 and 400 μm, respectively. The polymeric mixture incorporating HAP were cast on a glass plate and immersed into 60% DMAc aqueous solution. The single layer PES/HAP MMMs formed a few moments after immersion. The pure polymer dope and polymeric mixtures incorporating HAP were co-cast on a glass plate and immersed into 60% DMAc aqueous solution and double layer MMMs formed. The MMMs 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

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