



Preparation and characterization of negatively charged organic–inorganic hybrid ultrafiltration membranes for protein separation



Mahendra Kumar, Jenny Lawler*

School of Biotechnology, Dublin City University, Dublin 9, Ireland

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ABSTRACT

Negatively charged organic–inorganic hybrid ultrafiltration membranes with adjustable charge density were fabricated from blends of water soluble poly vinyl alcohol and N-O-sulfonic acid benzyl chitosan (NSBC) in combination with tetraethyl orthosilicate (TEOS) silica precursor by the sol–gel method and precipitation in a mixture of methanol and water (80 wt%:20 wt%) solvent. The porous hybrid membrane morphologies, structures and surface properties were characterized comprehensively using scanning electron microscopy, Fourier transform infrared spectroscopy in the attenuated total reflection mode, as well as contact angle measurements. The results confirmed that the fabricated membranes were porous, hydrophilic and mildly charged in nature. The water flux and flux recovery ratio (i.e. protein fouling resistance) of the membranes were highly dependent on the fraction of NSBC. The protein transmission as a function of *pH* and the fraction of NSBC was studied for two model proteins (bovine serum albumin; BSA and lysozyme; LYZ) and found to be controlled by size exclusion and the fraction of NSBC, and therefore the membrane charge density. The highest transmission of proteins at their isoelectric points was obtained for the membrane containing 40 wt% of NSBC. The best separation of LYZ from BSA in the model mixture solution was obtained at *pH* = 11 when membrane C-40 was used in ultrafiltration of protein solution.

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1. Introduction

Proteins are the building blocks of human life and take part in every process that occurs within cells. Proteins are widely used in the pharmaceutical, food, biotechnology and chemical industries [1–3]. Methods for protein concentration and separation are in high demand due to the rigorous requirements for high-purity protein derived drug products [1,3]. Several separation methods such as ultrafiltration (UF) [4,5], tangential flow filtration [6], adsorption [7], and electrophoretic membrane contactors [8] have been applied in the concentration and separation of proteins from their mixtures, either in model solutions or in fermentation broths. Among these methods, UF has been extensively adopted in the isolation and purification of proteins because it does not require large quantities of salts and buffers, offers continuous operation and eliminates some of the troublesome aspects related to chromatography techniques [3,5,8]. Polymeric UF membranes are the key component for more efficient use of UF processes in purification

and separation of proteins. Most of the commercial UF membranes are fabricated from hydrophobic polymers such as polyethersulfone (PES), polysulfone (PS), polypropylene (PP) and polyvinylidene fluoride (PVDF), by the phase inversion method [9]. These membranes are prone to a high degree of fouling due to the deposition of proteins on the membrane surface or adsorption into the membrane pores [9,10].

Membrane fouling is a major obstacle to the practical application of hydrophobic membranes in the UF of protein mixture solutions because the flux of the membranes is reduced with operating time and consequently, the energy consumption is increased. Chemical cleaning procedures which are used to recover and maintain the flux are responsible for an additional cost, also reducing the membrane lifespan [9–11]. There is an urgent need to find alternative ways to reduce the membrane fouling for practical applications of membranes in UF of protein solutions at a specific *pH*. To reduce membrane fouling, post-manufacturing modification by surface-initiated grafting; i.e. covalent attachment of functional hydrophilic monomers to the surface of membranes by polymerization using plasma, ultra-violet light or γ -radiation excitation or by atom transfer radical polymerization have been successfully

* Corresponding author. Tel.: +353 1 700 5394; fax: +353 1 700 5412.

E-mail address: jenny.lawler@dcu.ie (J. Lawler).

applied [12–15]. Surface modification methods may lead to variations in the barrier layer pore structure of the membranes, which can reduce their permeability [14,15].

To avoid this problem, several researchers have been reported that the hydrophilicity and selectivity of the hydrophobic membranes can be improved by anchoring anionic or cationic groups in the barrier layer of the membranes [7,16–18]. The anionic or cationic groups in the barrier layer of the membranes are anchored by blending hydrophobic polymers with various fractions (%) of hydrophilic functionalized polymers, [7,19,20] or by conducting the sol–gel reaction in acidic condition using water soluble unfunctionalized polymers (e.g. PVA) and functionalized polymers (e.g. phosphonated or quaternized chitosan) to make organic–inorganic hybrid materials [16].

Organic–inorganic hybrid materials with tunable properties and well-defined multi-dimensional architectures have attracted considerable interest due to their potential applications in catalysis, biology, optoelectronics, ion-conduction, and membranes [21,22]. In particular, much interest has been recently dedicated to design charged organic–inorganic hybrid membranes for electro-membrane separation processes [21], fuel cell applications [23], diffusion dialysis [24], pervaporation [25] and ultrafiltration [16]. The organic or polymeric component provides flexibility, toughness, functionality, and electrochemical properties, whereas the inorganic component contributes to the mechanical, physical, and thermal stability of the composite material [16,21,22]. Amongst the various membrane preparation methods, the sol–gel method is frequently employed to prepare organic–inorganic membranes in which the hybrid structure at the molecular level exhibits entirely different properties due to the hydrolysis/condensation of the silica precursors. The process makes it possible to obtain a relatively easy molecular-level incorporation of an inorganic component into an organic matrix at room temperature [21,22].

Saxena et al. developed negatively and positively charged organic–inorganic hybrid UF membranes for the separation of binary mixture of proteins (BSA and LYZ) solutions. The best separation of BSA or LYZ from binary mixture model solutions was obtained at their isoelectric point because the charged BSA or LYZ were retained by either the negatively charged or positively charged membranes. The membranes were thermally and mechanically stable [16]. Kumar et al. prepared negatively charged blended UF membranes from N-propyl phosphonic chitosan with a hydrophobic PSf polymer solution by the phase inversion method. The selectivity and antifouling capacity of the membranes were enhanced after the addition of the chitosan derivative due to the substantial increase in hydrophilicity and the charge density in the barrier layer of the membranes [20].

Recently, Kumar and Ulbricht have reported negatively charged UF membranes with adjustable charge density derived from sulfonated PAES/PAES block copolymer blends. The selectivity of the membranes towards LYZ versus BSA was tunable with increasing charge density of sulfonic acid groups in the barrier layers [5]. The separation performance of the membranes was dependent on both the size-exclusion and electrostatic interaction.

The combination of size- and charge-based selectivity enhances the separation performance of UF membranes. The transport of proteins across the charged UF membranes is also dependent on the type and strength of electrostatic interactions between the membrane and the protein at a specific solution *pH* [5,14,16,26]. Thus, selective separation and purification of protein mixtures using charged UF membranes is, in principle, possible at a controlled *pH* and applied transmembrane pressure. In this study, negatively charged organic–inorganic hybrid UF membranes from N-O-sulfonic acid benzyl chitosan, TEOS and PVA have been fabricated by the sol–gel and precipitation method followed by chemical cross-linking. Sulfonic acid benzyl chitosan was used to

anchor the negatively charged sulfonic acid groups in the barrier layer of the membranes. PVA was used in fabrication of the membranes due to its high solubility in deionized water, biodegradability, mechanical stability and non-toxic properties. In addition, PVA has an excellent film forming ability, hydrophilic properties, and a high density of reactive chemical functions that are favorable for cross-linking by irradiation, chemical, or thermal treatments [27]. To the best of our knowledge, N-O-sulfonic acid benzyl chitosan derived negatively charged organic–inorganic hybrid UF membranes with improved antifouling capacity for protein separation applications have not been reported previously. The fabricated membranes have been characterized comprehensively and then used in the UF studies with the model proteins (BSA and LYZ) solution at varied *pH* and constant applied transmembrane pressure.

2. Experimental section

2.1. Materials

Chitosan ($M_w \sim 200,000$; N-deacetylation degree 75–85%), poly vinyl alcohol (PVA; $M_w \sim 61,000$), 2-formylbenzenesulfonic acid sodium salt ($\geq 95.0\%$), tetraethyl orthosilane (TEOS), phenolphthalein and acetic acid were purchased from Sigma–Aldrich Chemicals, Ireland. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich and lysozyme (LYZ) was obtained from Fluka Chemicals. Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide and sodium sulfate decahydrate ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$) were procured from Merck Chemicals. Formaldehyde, acetone and methanol were purchased from VWR Chemicals, Ireland. Other reagents and chemicals were commercial grade and used as received. Distilled water (DW) and water purified with a Milli-Q system (Millipore) were used in this study.

2.2. Synthesis of N-O-sulfonic acid benzyl chitosan

N-O-sulfonic acid benzyl chitosan was synthesized by Schiff-base formation reaction (cf. Scheme 1). In a typical synthetic procedure, 1 g chitosan was dissolved in 0.7 wt% aqueous acetic acid solution and subsequently, the *pH* of the solution was adjusted to 3 using 1 M HCl solution. Thereafter, 3 equivalent of sodium 2-formyl benzenesulfonate to the mole of $-\text{NH}_2$ groups was added. The resulting mixture was then refluxed at 60 °C with constant stirring for 6 h. The temperature was lowered to room temperature (RT) and the *pH* of the mixture solution was increased to 5 by adding 1 M NaOH solution. Thereafter, an aqueous solution of sodium borohydride (2.5% w/v) was added drop-wise for the reduction of imine linkage in the modified chitosan (cf. Scheme 1). The reduced chitosan derivative was precipitated in acetone; the resulting precipitate was collected by vacuum filtration on filter paper and dried in a vacuum oven at 40 °C for 12 h. Finally, a white colored powder was obtained. The FTIR spectrum of NSBC was recorded (Supporting Information, Fig. S1). The broad band at 3335 cm^{-1} is attributed to the stretching vibration of $-\text{OH}$ groups in NSBC [5,28]. The absorption bands at 1255 and 1083 cm^{-1} are due to symmetric and asymmetric stretching vibration for the SO_3^- groups in modified chitosan [5,29]. These results confirm the successful grafting of sulfonic acid groups onto the chitosan by Schiff-base formation reaction followed by reduction.

2.3. Membrane preparation and characterization

N-O-sulfonic acid benzyl chitosan, tetraethyl orthosilane and poly vinyl alcohol derived hybrid membranes were prepared in three steps: (i) sol–gel method, (ii) precipitation and (iii) chemical

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