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Effect of ionic strength and protein concentration on the transport of proteins through chitosan/polystyrene sulfonate multilayer membrane

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ABSTRACT

The influence of ionic strength and protein concentration on the transport of bovine serum albumin (BSA), ovalbumin and lysozyme through chitosan (CHI)/polystyrenesulfonate (PSS) multilayers on polyether sulfone supports are investigated under ultrafiltration conditions. The percentage transmission and flux of BSA, ovalbumin and lysozyme were found to increase with increase in salt concentration in the protein. The percentage transmission of BSA through 9 bilayer membrane was found to increase from 5.3 to 115.6 when the salt concentration was varied from 0 to 1 M. It was observed that 0.1 M NaCl in BSA solution is capable of permeating all the BSA. When the salt concentration in BSA was further increased, a negative solute rejection (solute enrichment in permeate) was found to take place. With 9 bilayer membrane, the percentage transmission of ovalbumin was found to increase from 23.3 to 125.8 when the salt concentration in protein was increased from 0 to 0.05 M. The effect of protein concentration on protein transport is studied taking BSA as a model protein. BSA was rejected by the multilayer membrane at all the studied concentrations (0.25, 0.5, 1 and 2 mg/ml). With increase in feed concentration, maximum rejection of protein occurred at higher number of CHI/PSS bilayers. BSA solution flux was found to decrease with an increase in BSA concentration. This study indicates that it is possible to fine tune the transport properties of proteins through multilayer membranes by varying the concentration and ionic strength of protein solutions.

1. Introduction

Surface modification using polyelectrolytes by the self-assembly method [1], which involves alternate dipping of the surface with polycations and polyanions, is a fast developing area and is widely accepted because of its ease of fabrication, fine tunability of thickness, porosity, etc. One of the striking features of polyelectrolyte multilayer (PEM) is its selective permeability for different species ranging from smaller ions to large molecules like proteins. The permeability of PEM depends on layer thickness, porosity, chemical composition of layers and size and charge of the permeating species. Quite a lot of work has been devoted to the fabrication and characterization of multilayers [2–8] but only a limited number of reports are concerned with permeation studies through PEM [9–14]. The transport studies through multilayers so far conducted mainly focuses on ion permeations. By the proper choice of polyelectrolytes and the dipping conditions, the porosity of the multilayer can be varied and the multilayer may be selectively permeable to different species varying from ions to larger molecules like proteins.

Protein transmission can be influenced by adjusting the salt concentration in protein solution. Few authors have discussed the influence of ionic strength and pH on protein transmission through charged ultrafiltration (UF) membranes [15–21]. Balakrishnan and Agarwal studied the effect of ionic strength on the flux and transmission of lysozyme and ovalbumin through hydrophilic polyacrylonitrile ultrafiltration membrane at pH 6.8 and they observed a dramatic change in protein transmission with ionic strength [15]. Increase in protein transmission with increase in ionic strength is observed on the surface modification of composite regenerated cellulose membranes by the attachment of quaternary amine functionality [16]. The sieving coefficient of bovine serum albumin (BSA) through a 100,000 MWCO polyether sulfone membrane is reported to be increased by nearly two orders of magnitude as the NaCl concentration was increased from 1.5 to 150 mM [17]. Similar results were obtained for the transmission of BSA and lysozyme through hydrophilic inorganic membranes [18]. In these cases, the increase in protein sieving coefficient with increasing ionic strength was consistent with the enhancement in electrostatic shielding of repulsive interaction at high salt concentration. The protein transmission through a hydrophilic membrane is

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governed by electrostatic phenomenon. Salt addition causes decrease of protein–membrane interaction, which may be quantitatively obtained from streaming potential data. It is reported that as the salt concentration in protein increases, streaming potential decreases to minimum value and no electrostatic repulsion or attraction is opposed to protein transfer across the membrane [19].

The protein transmission can also be affected by concentration variations in protein solution. The effect of protein concentration on protein transport through charged ultrafiltration membranes have been discussed in literature [15,22–25]. Most of the works attribute the change in the percentage transmission of proteins with protein concentration due to concentration polarization effect.

Recently, we reported the transport behavior of BSA, ovalbumin and lysozyme through a surface modified microfiltration membrane by self-assembly of PEMs [14]. The effects of pH at isoelectric point, above and below the isoelectric point of the proteins were discussed. chitosan (CHI)/polystyrenesulfonate (PSS) polyelectrolyte pair was used for the deposition. The supporting membrane was permeable to all the three proteins. Only a few bilayers (bls) were needed to sieve a protein as large as BSA from its solution. The transmission of proteins through these multilayers was very much dependent on the number of deposited layers and the solution pH. When the protein solution pH was 8.8, a 9 bl CHI/PSS multilayer system was capable of rejecting 94.7% BSA. A 5 bl CHI/PSS system was capable of rejecting 97.8% lysosyme. With 5 bl CHI/PSS system, ovalbumin transmission was found to exceed 100% (104.2%) due to negative solute rejection (solute enrichment in permeate). This result highlighted the potent application of this multilayer system on a polymeric support in the preparation of ovalbumin free lysozyme. Here we discuss the influence of ionic strength and protein concentration on the transport behavior of proteins (BSA, ovalbumin and lysozyme) through CHI/PSS multilayers on supor (polyethersulfone, 0.45 μm) under ultrafiltration conditions. Our main objective is to explore the permeation characteristics of proteins through multilayer membranes on varying salt concentration and to get an idea about the type of force which holds the proteins to the membrane. Polyelectrolyte multilayer can act as platforms for interaction with biochemical and biological compounds such as drugs, proteins, peptides, cells, etc. Surface modification of membranes by PEM can improve their sieving characteristics with respect to flux, separation and antifouling properties [26]. As the multilayer system may find application in protein separation [14], it is quite significant to investigate the influence of ionic strength and protein concentration in protein transport through the multilayer.

2. Experimental

2.1. Materials

The supporting membrane was Supor 450 (0.45 μm pore size, polyethersulfone, Pall Life Sciences). Chitosan (medium MW, 75–85% deacetylated, Aldrich), poly (styrene sulfonic acid) sodium salt (PSS MW 200,000, 30 wt% in water, Aldrich), BSA (MW 67,000, SRL, Mumbai), ovalbumin (MW 45,000, Aldrich), lysozyme (14,000, SRL, Mumbai), tris buffer (CDH) and sodium chloride (Merck) were used as received. Deionized double distilled water was used for membrane rinsing and for the preparation of polyelectrolyte solutions.

2.2. Film deposition

The polyether sulfone supporting membrane was rinsed with double distilled water and kept in water for 24 h. The clean membrane was dipped in alternate polycationic (CHI) and polyanionic (PSS) solutions. pH of the polyelectrolyte solutions was adjusted to 1.7 by HCl. The structure of the polyelectrolytes used for deposition is given in Fig. 1. Bare membrane (Supor) was first dipped in 0.01 M chitosan solution in water (molarities of polyelectrolytes were taken with respect to repeating unit) at pH 1.7. The dipping time was 15 min. After dipping, the membrane was rinsed with 50 ml distilled water for 1 min. Then the membrane was dipped in 0.01 M PSS solution prepared in 0.1 M NaCl at pH 1.7. The membrane was then rinsed with 50 ml-distilled water. The dipping process was repeated till required number of bilayers was formed. BSA solutions of different concentrations (0.25, 0.5, 1 and 2 mg/ml) at pH 8.8 were prepared in tris–HCl buffer. BSA (0.5 mg/ml), ovalbumin (0.5 mg/ml) and lysozyme (0.25 mg/ml) solutions of varying salt concentrations (0.01, 0.025, 0.05, 0.1, 0.5 and 1 M NaCl) were prepared by weighing required amounts of protein, dissolving in tris–HCl buffer (pH 8.8), and making up with NaCl solutions of required concentration.

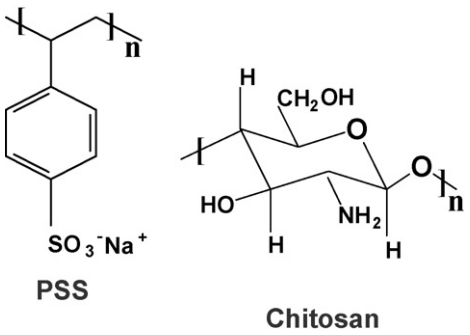

Fig. 1. Structure of polyelectrolytes for self-assembly.

2.3. Film characterization

FT-IR (Shimadzu 8400S) and SEM (JSM-840, Scanning Electron Microscope) were used for the characterization of the bare membrane and the multilayers deposited on the bare membrane.

2.4. Ultrafiltration

Ultrafiltration experiments were performed with amicon 8050-ultrafiltration cell (Millipore). All ultrafiltrations were carried out at 10 psi pressure at 400 rpm at room temperature (28–30 °C). We studied the transport of BSA (MW 67,000) of different concentrations (0.25, 0.5, 0.5 and 1 mg/ml) through different number of bilayers of the composite membrane, supor/CHI/PSS. In order to study the effect of added salt in protein solution on protein transmission, lysozyme (0.25 mg/ml) solutions of varying salt concentrations (0.01–1 M NaCl) were filtered through 5 bl membranes; ovalbumin (0.5 mg/ml) solutions of varying salt concentrations (0.01–1 M NaCl) through 5 bl and 9 bl membranes and BSA (0.5 mg/ml) solutions of varying salt concentrations (0.01–1 M NaCl) were filtered through 9 bl membranes. All experiments were conducted in triplicate and the results were nearly identical. The error limit is within 2%. It is observed for good reproducibility, the membranes must be prepared under exactly identical conditions. Protein concentration in permeate was determined spectrophotometrically at 280 nm with a Shimadzu UV visible spectrophotometer (UV-1700 Pharmaspec). The percentage transmission was calculated using the following relationship:

$$\%\,\text{rejection} = \frac{C_{\text{feed}} - C_{\text{permeate}}}{C_{\text{feed}}} 100$$

$$\%\,\text{transmission} = 100 - \%\,\text{rejection}$$