Morphological Characterizations of Collagen-Modified Alumina Membranes

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Introduction:

Membranes can be engineered to separate materials based on size, concentration, charge, temperature or other characteristic, which makes them versatile enough to be employed in multiple industries such as environmental and chemical engineering, waste-water applications, food processing, biotechnology, desalination, and medical. In biopharmaceutical and biomedical applications, membrane techniques replace other conventional separation methods such as ELISA, western blotting, and polymerase chain reaction (PCR). Recently the protein collagen has been identified for coatings, films and membranes and found to be controllable in growth and morphology [1]. Those surface modified membranes need to be well characterized in order to be applied for a specific application [2].

The molecular weight cut off (MWCO) of a membrane gives insight about the pore size, the function of the membrane, selectivity, and its ability to separate a particular molecule. The aim of this project was to evaluate large pore modified alumina membranes coated with layers of collagen with the goal of developing a membrane with 90% solute rejection for biomolecules of different size fractions. The pore size of the modified membranes was determined by measuring the MWCO, which is the smallest possible molecular weight of a solute, in this case dextran, with at least 90% rejection [3]. Surface morphology was examined by scanning electron microscopy (SEM) imaging to further confirm the magnitude of modification in addition to its effect on membrane pore size.

Experimental Procedure:

The membranes used for the study were 20 nm pore size anodized alumina membranes etched with concentrated sulfuric acid, and spin-coated with 1, 3, 6 and 9 layers of dermal collagen.

Morphology. Freeze-fractured modified membranes were coated with a thin layer (5 nm) of copper metal and scanned under a JEOL SEM at an applied voltage of 5 kV. The membrane surfaces were viewed with a magnification of 30K-50K times and the images captured.

MWCO. Dextran (a polysaccharide) with molecular weights such as 25, 50, 80, 150, 270 kDa, was prepared at a concentration of 20 ppmC as the feed for the filtration experiments in an aqueous medium. The membrane was loaded in a stirred batch filtration cell and connected to a feed tank that was pressurized with ultrapure nitrogen gas at 10 psi. Filtration of each of the molecular weight dextran for each modified membrane (1, 3, 6 and 9 layered) was carried out and the permeate sample collected. An UV-Persulfate TOC analyzer (Teledyne-Tekhmar, Phoenix 8000) equipped with a NDIR detector was used to determine the unknown concentration of the dextran in the permeate in terms of the total organic carbon content [4]. Initial standard calibration standards were run for each molecular weight dextran. From the concentration values obtained from the analysis, the percentage rejection of each dextran for each modified membrane was calculated using the formula, % rejection = $(1 - (C_p/C_f)*100$, where C_p is the concentration of the permeate and C_f the concentration of feed.

Results and Discussion:

Figure 1 confirms that the 9-layer spun-collagen was in the form of long extended fibrils completely covering the

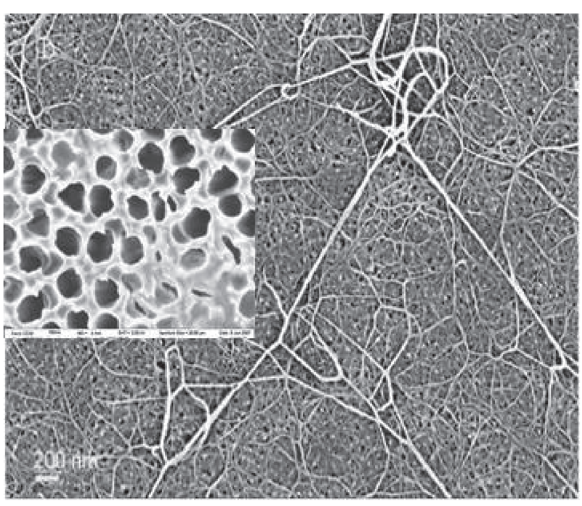

Figure 1: Nine-layer collagen modified membrane.

surface of the porous sulfuric-acid-etched alumina (refer to insert). Small monomeric collagen molecules were seen on three layers, and a mixture of monomers and short fibrils on six layers were observed (images not shown).

Figure 2 shows the percent rejections of various dextran by various modified-membranes. Generally, for each collagen-layered membrane, a trend of increasing percent rejections with increasing molecular weight of dextran was observed. For an unmodified membrane, the rejection for even the highest molecular weight (270 kDa) was negligibly small. However with three layers, the rejection significantly increased for all the dextrans, with 270 kDa nearing 90%. With six layers, except for the 25 kDa, all the other dextrans reached the 90% mark, where the MWCO of a membrane was determined. With further increase to nine layers, the rejection of 25 kDa also reached 90%. From the rejection values, the MWCO of the membranes were found to be > 270, 270, 50-80 and 25-50 kDa respectively for 0, 3, 6 and 9 layer modified membranes. Knowing the MWCO of these membranes, molecules of a given size can be specifically removed from a mixture of other molecules of varying size fraction.

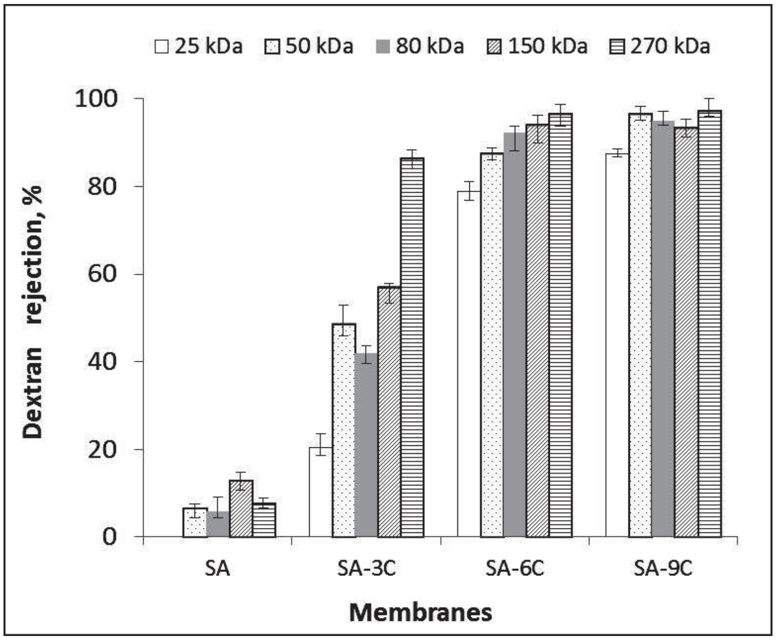

Figure 2: Dextran rejections of each membrane.

In future work, these membranes can be potentially applied for separating biomolecules such as DNA, Heme or proteins of a given size.

Conclusion:

Increasing the number of collagen layers significantly decreased the molecular weight cut-off of the membranes. The number of layers can be tailored for a specific separation. Reproducibility of the MWCO of the membrane confirms the stability of the collagen layers on the surface.

References:

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