Supporting Information

Functionalized chitosan derived novel positively charged organic-inorganic hybrid ultrafiltration membranes for protein separation

Mahendra Kumar, Declan McGlade and Jenny Lawler*

Membrane Technology Laboratory, School of Biotechnology, Dublin City University, Dublin 9, Ireland.

* E-mail: jenny.lawler@dcu.ie, Tel: +353 1 700 5394; Fax: +353 1 700 5412.

Experimental section

S1. Determination of membrane water uptake and porosity

The membranes were kept in DI water at RT for 24 h and then taken out from DI water. The surface water was wiped off with tissue paper and the mass of the wet membranes was immediately measured using an analytical balance. The wet membranes were dried in a vacuum oven at 60°C for 8 h and the masses of the membranes were again recorded. The water uptake (φ, in %), and the porosity (ε, in %) of the membranes were calculated using Eq's. (1) and (2): [12,21]

$$\phi = \frac{(W_w - W_d)}{W_d} \times 100 \quad (1)$$

and

$$\varepsilon = \frac{(W_w - W_d)}{A \times L \times \rho} \times 100 \quad (2)$$

where W_w and W_d are the mass (g) of membrane in wet and dry state, A is the membrane area (cm^2), L is the membrane thickness (cm) and ρ is the density of pure water (1 g cm^{-3}).

S2. Determination of ion-exchange capacity and fixed ion concentration

The ion-exchange capacity of membranes was estimated using a back titration method.[12,21] The membrane samples were placed into conical flasks containing 50 ml of 0.1 M HCl solution for 24 h and then samples were taken out. Afterwards the ion-exchanged solutions were titrated with 0.01 M NaOH solution using phenolphthalein as an indicator. The wet membranes were dried in vacuum oven at 60^{o}C for 6 h and the weights of membranes were recorded. Three replicates were performed for each membrane to ensure the reproducibility of data. The ion-exchange capacity (IEC; meqiv.g^{-1}) of membranes was calculated using Eq. (3) :[12,21]

$$IEC\left(meqiv.g^{-1}\right) = \frac{V_{O,NaOH} C_{NaOH} - V_{x,NaOH} C_{NaOH}}{W_d} \times 1000 \qquad (3)$$

where $V_{0,NaOH}$ and $V_{x,NaOH}$ are the consumed volume of the NaOH (ml) in titration without and with membranes. C_{NaOH} is the concentration of NaOH solution (mol ml^{-1}). The calculated values of IEC were used to determine the fixed ion concentration (A_f) of the membranes. The fixed ion concentration (A_f; meqiv.g^{-1} H_2O) of the membranes was calculated using Eq. (4):[24]

$$A_f = \frac{IEC}{\phi} \qquad (4)$$

S3. Procedure to determine tensile strength of the membranes

Uniaxial tensile tests were conducted on a Zwick Z005 displacement controlled tensile testing machine to determine the tensile strength and the elongation values at break point of the membranes. All of the dried membrane samples were cut out in a dog-bone shape (78 mm × 12 mm) and the fixed grips were mounted onto the tensile testing machine. Abrasive paper was placed between the membrane sample and the grip surface to prevent the slippage of the membrane samples during experiments. The stress and strain values for the membranes were recorded at a crosshead speed of 2 mm/min. Three samples of each membrane were tested to

ensure the reproducibility of the data. Furthermore, the elongation values at break point were calculated from the initial slope of the stress vs strain curve using the instrument handling software.

S4. Determination of OVA and LYZ concentration in mixture model solutions by SDS-PAGE analysis

The concentration of OVA and LYZ in binary mixture model solutions was determined by SDS-PAGE and fluorescence laser densitometry.[11,32] All materials used for SDS-PAGE analysis were obtained from Sigma-Aldrich. 1.8 ml of Milli-Q water, 1.88 ml of 1.5 M Tris-HCl (pH 8.8), 0.075 ml of 10% (w/v) SDS, 3.2 ml of 30% acrylamide, 0.038 ml of 10% ammonium persulfate and 0.0038 ml N,N,N′,N′-tetramethylenediamine (TEMED) were mixed to prepare 12.5% SDS-PAGE separation gel. On the other hand, 1.54 ml of Milli-Q water, 0.625 ml of 0.5 M Tris-HCl (pH 6.8), 0.325 ml of 30% acrylamide, 0.0125 ml of 10% ammonium persulfate and 0.0025 ml TEMED were mixed to prepare the stacking gel. The separation gel mixture solution was poured into the tight glass plates and the separating gel mixture was allowed to polymerize at RT for 60 min. Then 2 ml of stacking gel mixture solution was poured and subsequently, a 1-mm-thick 10-tooth well former comb was placed onto the top of the separating gel and the stacking gel mixture was allowed to polymerize at RT for 60 min. The well former comb was then gently removed from the stacking gel with care. The well was flooded with DI water to avoid any alternation in the wells of SDS-PAGE gel before sample loading. The gel plate was further activated in 1X tris/glycine/SDS buffer solution by applying 125 V potential across the Bio-Rad Protean II system for 30 min. 15 µl of denatured proteins mixture solution with dye was loaded into the wells of the gel. The electrophoresis (Bio-Rad Protean II system, USA) experiment was performed at 125 V until the spots of dye had run away from the gel and entered into 1X tris/glycine/SDS buffer solution. Thereafter, the gel was gently removed from the glass plates and placed into the

Coomassie stain (i.e. Coomassie Brilliant Blue R250 in 40% methanol: 10% acetic acid) for 24 h and subsequently, the gel was destained in destain solution containing 40% methanol and 10% acetic acid. The destained gel (cf. Fig. 10 (A) was washed with DI water and scanned on a Typhoon-Fla 9000 laser densitometer (GE Healthcare) in fluorescence mode. Bands were quantified on the Image Quant TL software to determine the concentration of OVA and LYZ in binary mixture model solutions.

Results

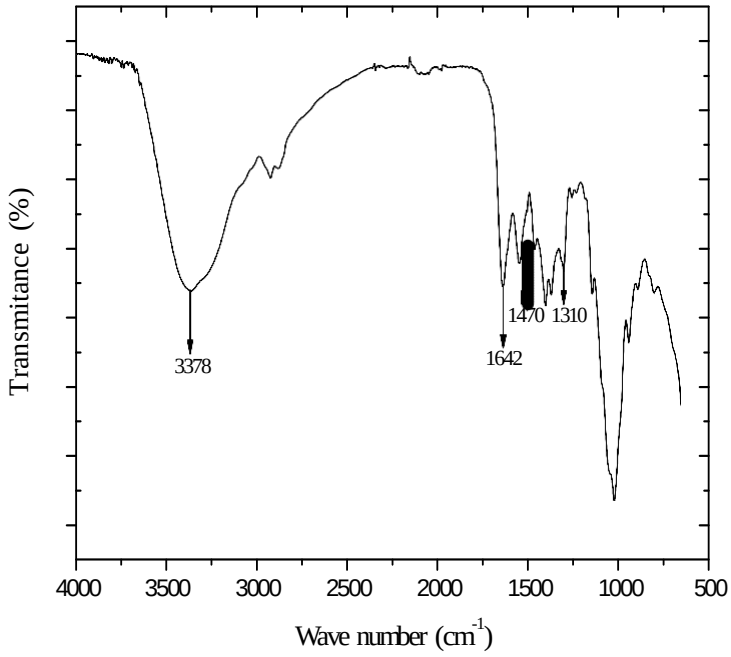

Fig. S1. FTIR spectrum for methylated N-(4-N,N-dimethylaminobenzyl) chitosan.

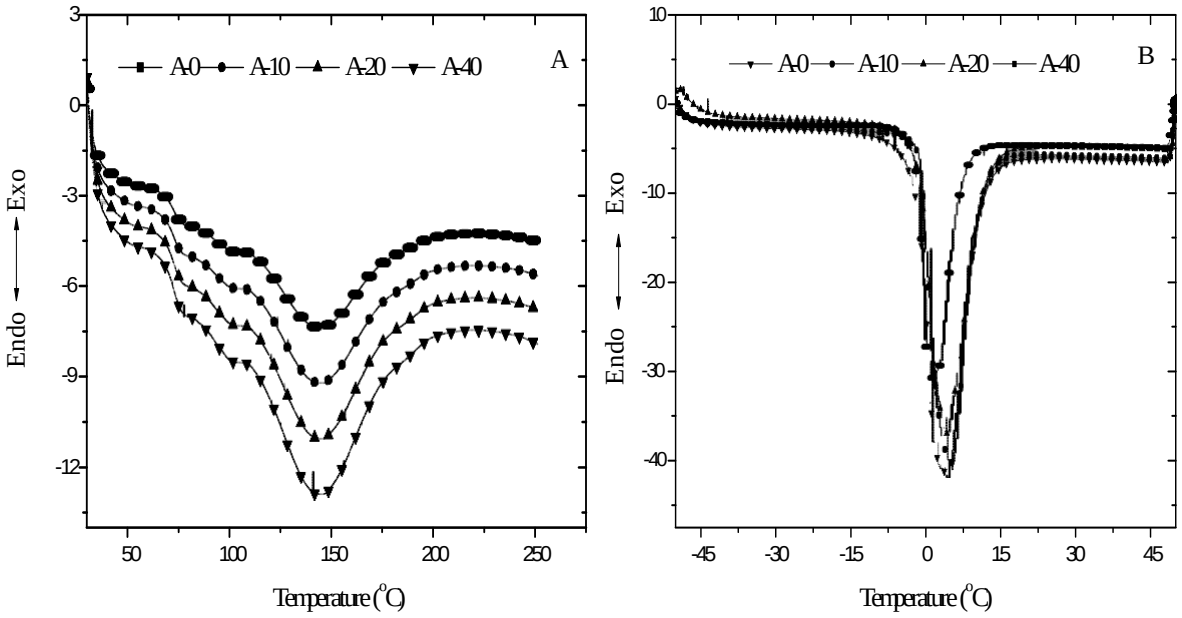

Fig. S2. DSC thermograms for the membranes in dry (A) and swollen (B) states with varied fraction of TMBC (%).

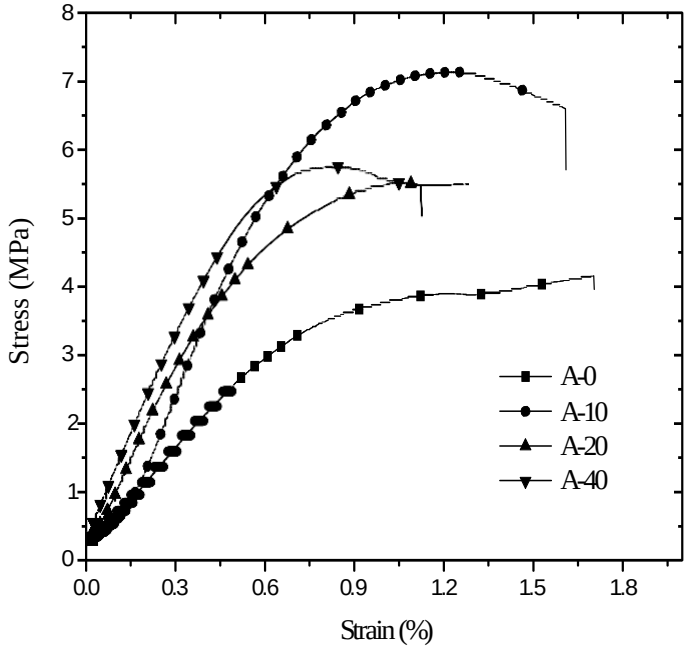

Fig. S3. Stress-strain curves for the membranes with varied fraction of TMBC (%).