Supporting information

Modified chitosan based pH-responsive membrane for protein separation

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Section S1. Instrumental analysis

FTIR spectra (4000–450 cm^{-1}) of dried membrane samples were recorded by Spectrum GX series 49 387 spectrometer. ^1H NMR spectra of *O*-CMCS was recorded by spectrometer (Bruker 500 MHz) in d_6-D_2O. Thermal degradation and stability of *O*-CMCS and *N*-CMCS membranes was investigated by thermogravimetric analyzer (TGA) (Mettler Toledo TGA/SDTA851 with Star software) under N_2 atmosphere at 10 ◦C/min. heating rate (0-700 ◦C). Differential scanning calorimetric (DSC) measurements were carried by Mettler Toledo DSC822e thermal analyzer with stare software, equipped with a TASC 414/2 thermal analysis controller under 50–300 ◦C temperature range. The 10 mg sample was loaded into aluminium pans, and heating rate was 10◦C/min. The empty aluminium pan was used as a reference.

Wide angle X-ray diffractograms (WXRDs) of the developed membranes were recorded using a Philips Xpert X-ray diffractometer with Cu-Ka (1.54056) radiation.

For scanning electron microscopy (SEM), gold sputter coatings were carried out on desired membrane samples at 1-0.1 Pa pressure. The sample was loaded in the machine, which was operated at 1022 to 1023 Pa with EHT 15.00 kV with 300 V collector bias using Leo microscope.

Section S2. Membrane thickness, water content, H^+/OH^- exchange capacities and surface charge concentration.

The thickness of the membranes was measured by a digital micrometer up to 0.10μm accuracy. The membrane water content was determined by the weight difference of membrane in wet and dry condition using the following Eq.:

$$Water\ content\ (\%) = \frac{W_w - W_d}{W_d} \times 100 \qquad (1).$$

Where W_w and W_d are the weight of the wet and dry membrane, respectively.

For determining ion-exchange capacities (IEC), membrane samples of known weight were equilibrated in 1.0 M HCl or in 1.0 M NaOH solutions for 24 h to ensure that allcharged sites of the membrane were either in H^+ or OH^- form. The membranes were then washed with distilled water free ofacid or base before equilibration in 0.10 M NaCl for 24 h. Ion exchange capacity was determined from the increase in acidity or basicity upon acid–base titration. The total molar number of H^+ or OH^- was obtained and IEC was calculated by dividing this number by the dry membrane weight.

Section S3. Water flux, pore radius, protein diffusion coefficient and flux measurement:

For determination of molecular cut-off, neutral organic molecule probes solutions (poly(ethylene glycol) and dextran). Customized total organic carbon (TOC) digestion method was used to quantitatively measure the concentration of neutral organic solutes in permeate and retentate. Calibration plots for all three types of analyses were run with standard feed solutions prior to the studies to ensure accuracy of the measurements. Solute rejection was estimated by following equations.

$$\text{Rejection, R (\%)} = \left[1 - \frac{Permeateconcentration}{feedconcentration}\right] \times 100 \qquad (1)$$

All experiments were carried thrice and average value was considered.

For the estimation of effective pore size of the membranes, Ferry equation (eq. 2) was used to correlate the rejection of protein by the membrane, considering uniform pore size distributions.

$$R = 100^{\times \left[1 - \left(1 - \frac{r}{a}\right)^2\right]^2} \qquad (2)$$

Where R is the rejection (%), r is the solute diameter, and a is the pore size (diameter) of the membrane (assuming a uniform pore size).

$r = 0.096\ Mw^{0.59} + 0.128 Mw^{0.5}$ (3)

M is molecular weight of solute (g/mol). Obtained rejection data was fitted in Ferry equation and molecular weight cut off rejection data for neutral probe pore diameter was estimated.

Table S1: Stability test in different solvents.

Solvent	**CS**	**N-CMCS**	**O-CMCS**
Isopropanol	N	N	N
Hexane	N	slightly affected	Slightly affected
Tetrahydrofuron	N	N	N
Chloroform	N	--	--
Water under boiling condition (pH- neutral)	N	N	N

N: Not affected (W_L value was less than 1%).
Slightly affected: W_L value was less than 3% but higher than 1%.

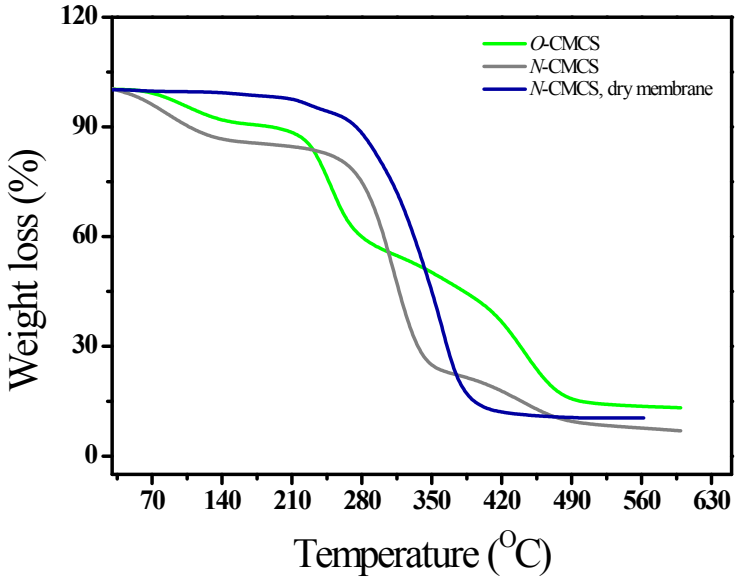

Figure S1. TGA curve for *N*-CMCS and *O*-CMCS membrane and N-CMCS dry membrane.

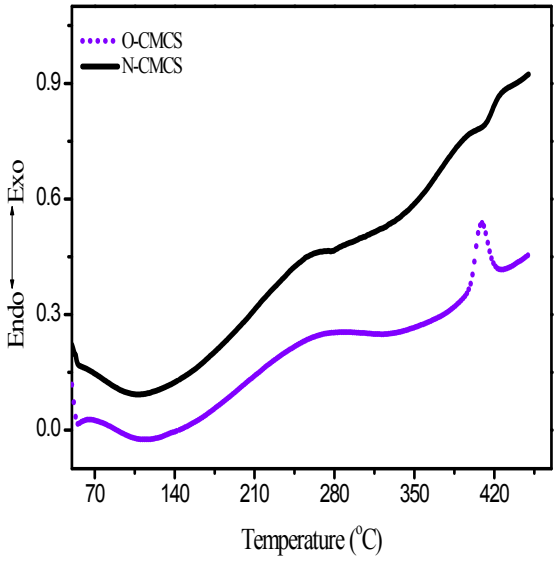

Figure S2. DSC thermograms for *O*-CMCS and *N*-CSMCS.

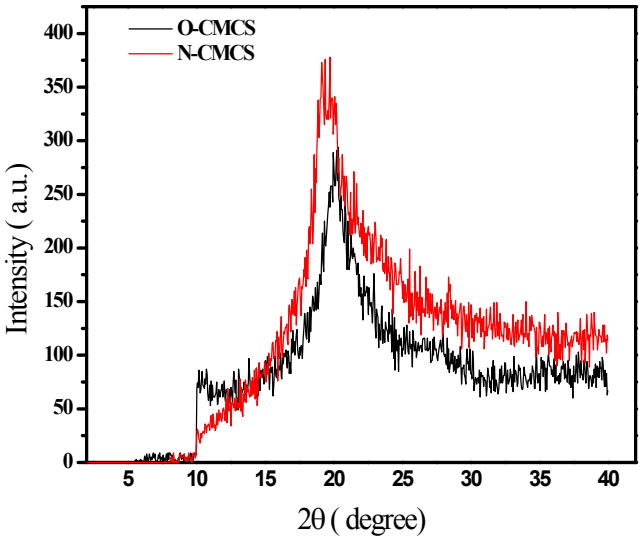

Figure S3.WXRD study for *N*-CMCS and *O*-CMCS membrane.