Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Supporting Information

## Modified chitosan emulsifiers: small compositional changes produce vastly different high internal phase emulsion types

Bernice H. L. Oh, Alexander Bismarck* and Mary B. Chan-Park*

# Table of Contents

## Experimental method

To synthesize CSNLYS, 1 g of chitosan was dissolved in 250 ml of 0.12% acetic acid and 10 g of NIPAM and 0.8 g of ceric ammonium nitrate (CAN) in 4 ml of 1 M nitric acid were added to the reaction solution. The reaction was maintained at 30°C and was allowed to proceed for 24 h in the dark while purging with nitrogen gas. The resulting solution was then dialyzed against deionized water and lyophilized. 0.5 g of CSN was then dissolved in 20 mM tetramethylethylenediamine/hydrochloric acid (TEMED/HCl, Sigma) pH 4.7 and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) (Sigma) and *N*-hydroxysuccinimide **(**NHS) (Sigma) and 0.06 mol, 0.03 mol or 0.01 mol of L-Lysine monohydrochloride, was added at room temperature and the reaction was allowed to continue for 2 days. At the end of the reaction, the solution was neutralized with 1 M sodium hydroxide solution (NaOH) and was clarified through filtration through 0.22 μm filter unit. The filtrate was then dialyzed against deionized water in a dialysis tubing (MWCO 12400) until the conductivity of the solution remained constant and the polymer solution lyophilized. The products were characterized by $^{1}$H NMR.

To measure ζ-potentials, the polymers were dissolved in deionized water at a concentration of 2.5% w/v and measured using ZetaPALS (Brookhaven Instruments). The reported values are mean values of 10 measurements.

Emulsions were prepared at room temperature by mixing 125 μl of either 20%w/v CSNLYS-L, CSNLYS-M or CSNLYS-H in the continuous water phase at pH 7 and 375 μl of *p*-xylene as internal oil phase to give an internal phase volume fraction of 0.75**.** The emulsions-templated porous polymers were formed by raising the temperature of the emulsions to 40°C, rapidly freezing them in liquid nitrogen and removing the entrapped solvents by freeze-drying. The foam morphology was studied using SEM images of platinum coated samples (JEOL JSM6701F, JEOL Ltd, Japan) and the pore and pore throat sizes were analyzed using (ImageJ, US National Institutes of Health, Bethesda, Maryland, USA). The surface area of the polyHIPEs

was determined from nitrogen adsorption isotherms at 77 K applying the Brunauer–Emmet–Teller (BET) model and the measurements made using a surface area analyzer (Autosorb1, Quantachrome Instruments). The porosity was determined by the Barrett, Joyner & Halenda (BJH) method. The surface and interfacial tension of the aqueous polymer solutions (1%w/v) and the oil phase was measured using a tensiometer (FTA32, First Ten Angstroms Inc.) using the pendant drop method. The envelope density of the foam, $\rho_e$, was determined by measuring the mass of cylindrical foams with radius of 25 mm and height of 40 mm and dividing by the cylinder volume. The true matrix density, $\rho_t$, of the macroporous polymers was determined by using a pycnometer (Ultrapycnometer 1000, Quantachrome Instruments). The porosity of the foam, P, was then calculated by $(1-\rho_e/\rho_t)$ X 100%.

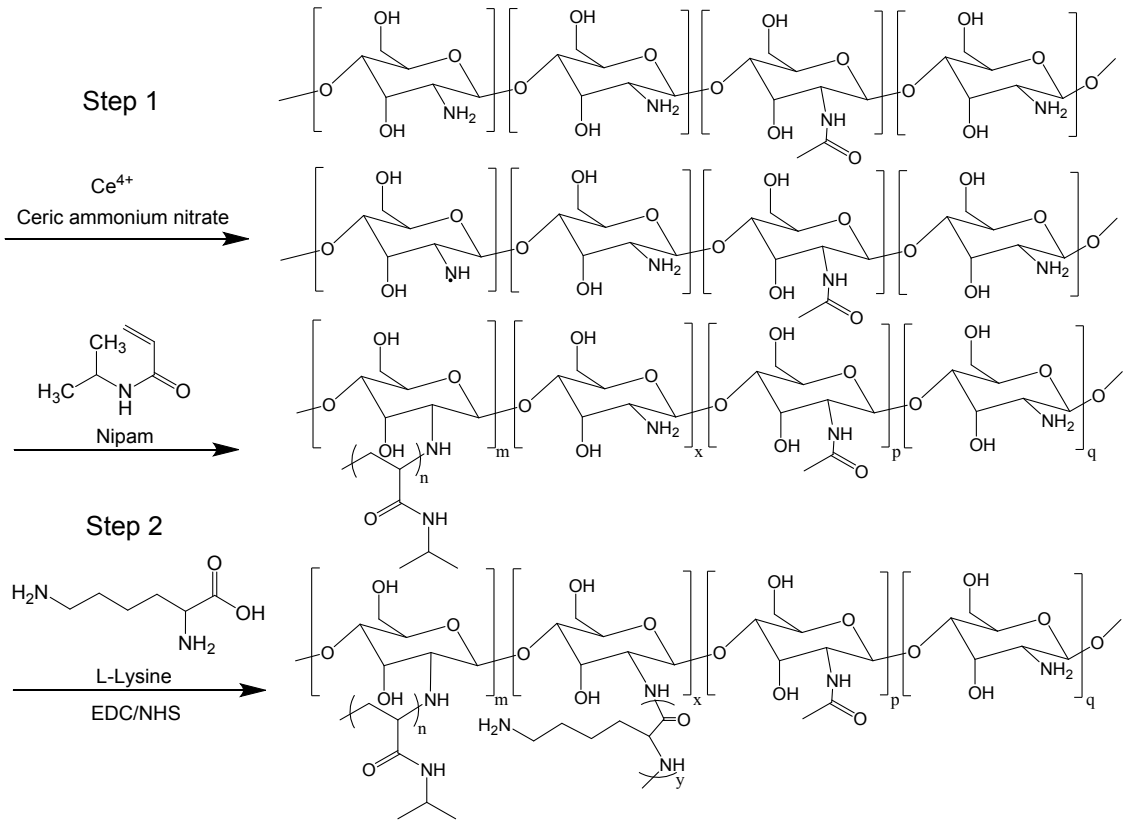


**Figure S1** Reaction scheme. Step 1: $Ce^{4+}$-initiated radical graft copolymerization of PNIPAM initiated from chitosan to form CSN. Step 2: Condensation of lysine monomers onto CSN to form CSNLYS.

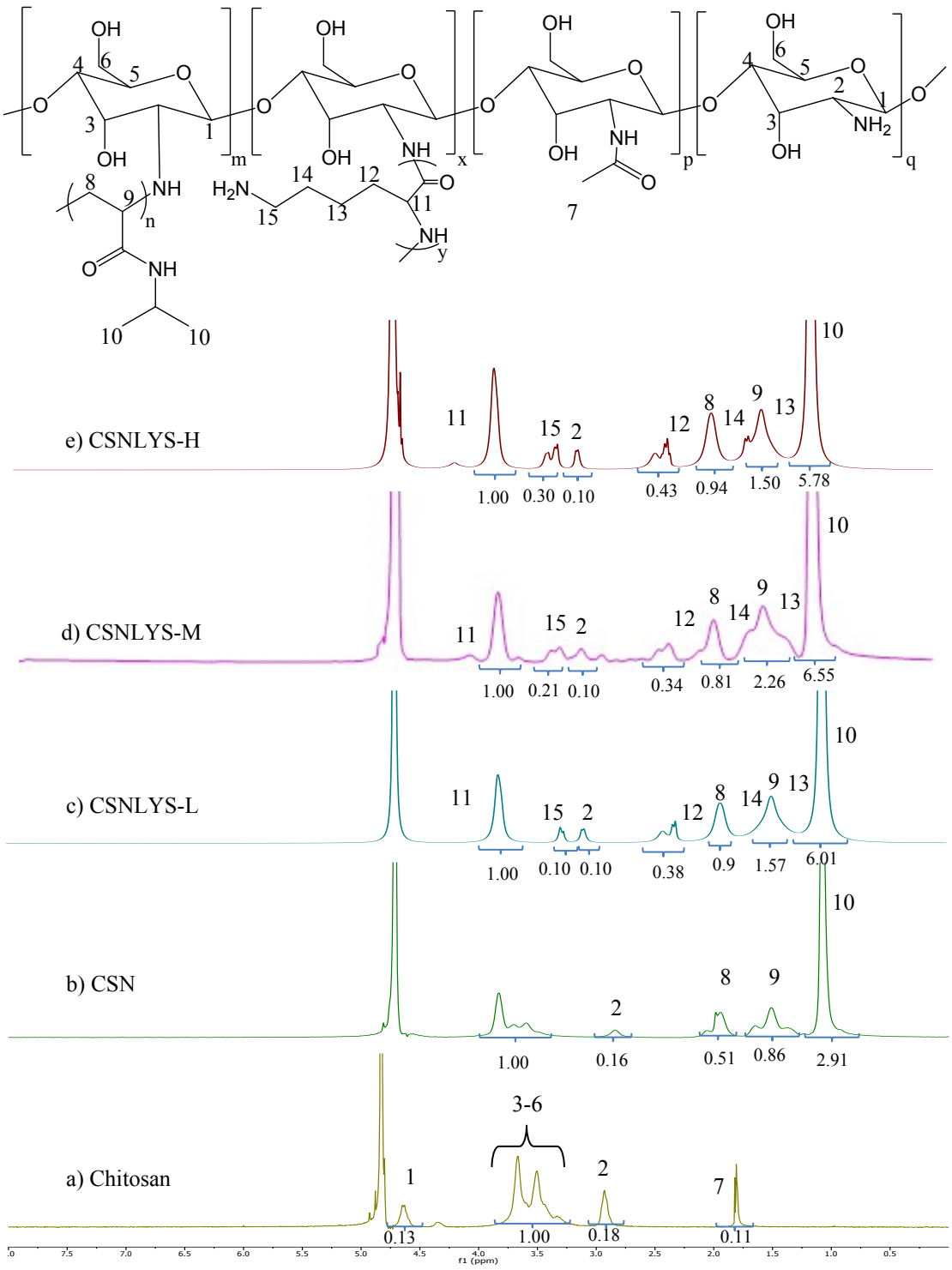


**Figure S2** $^{1}$H-NMR spectra of a) native chitosan in DCl solution 20% in $D_2O$, b) CSN in $D_2O$, c) CSNLYS-L, d) CSNLYS-M and e) CSNLYS-H) in $D_2O$. The degree of substitution (DS) and degree of polymerization (DP) were determined from the $^{1}$H-NMR integrals of chitosan and the grafted moieties.

| | Weight average molecular Weight (g/mol) | Polydispersity Index |
|---|---|---|
| Chitosan (CS) | 1.80E+06 | 1.03 |
| Chitosan-g-PNIPAM (CSN) | 2.25E+06 | 1.17 |
| CSNLYS-L | 2.32E+06 | 1.04 |
| CSNLYS-M | 2.34E+06 | 1.23 |
| CSNLYS-H | 2.35E+06 | 1.53 |

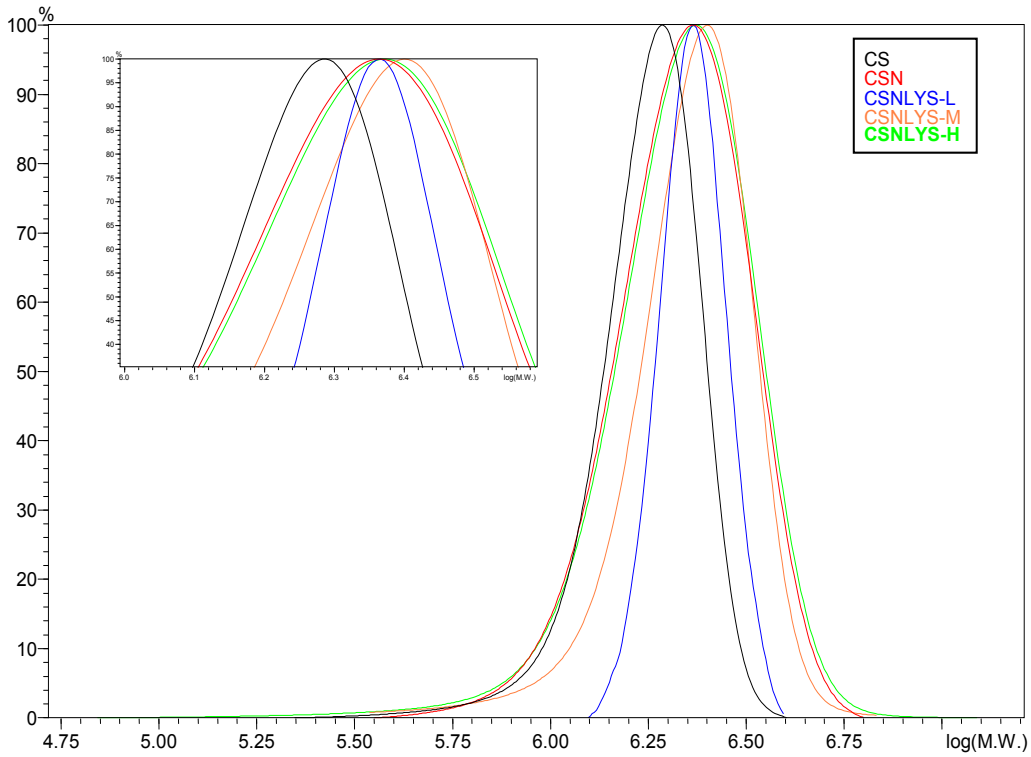


**Figure S3** GPC for weight average molecular weight determination.

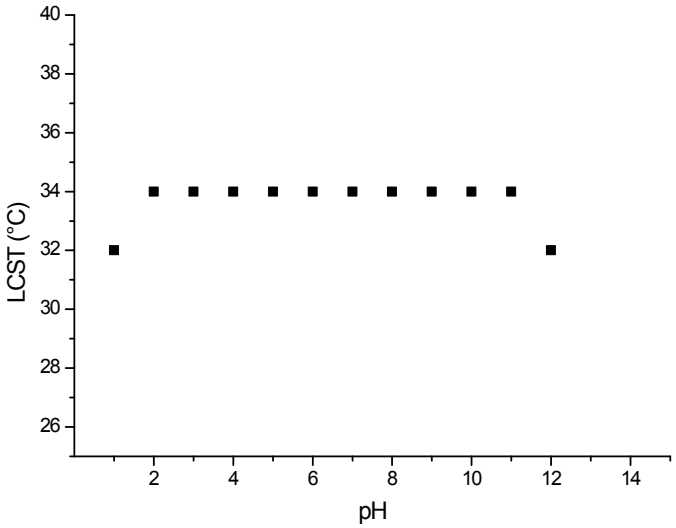


**Figure S4** Cloud points of the 2.5%w/v CSNLYS solution as function of pH 1-12 determined by cloud point measurements. CSNLYS was insoluble at pH 14.

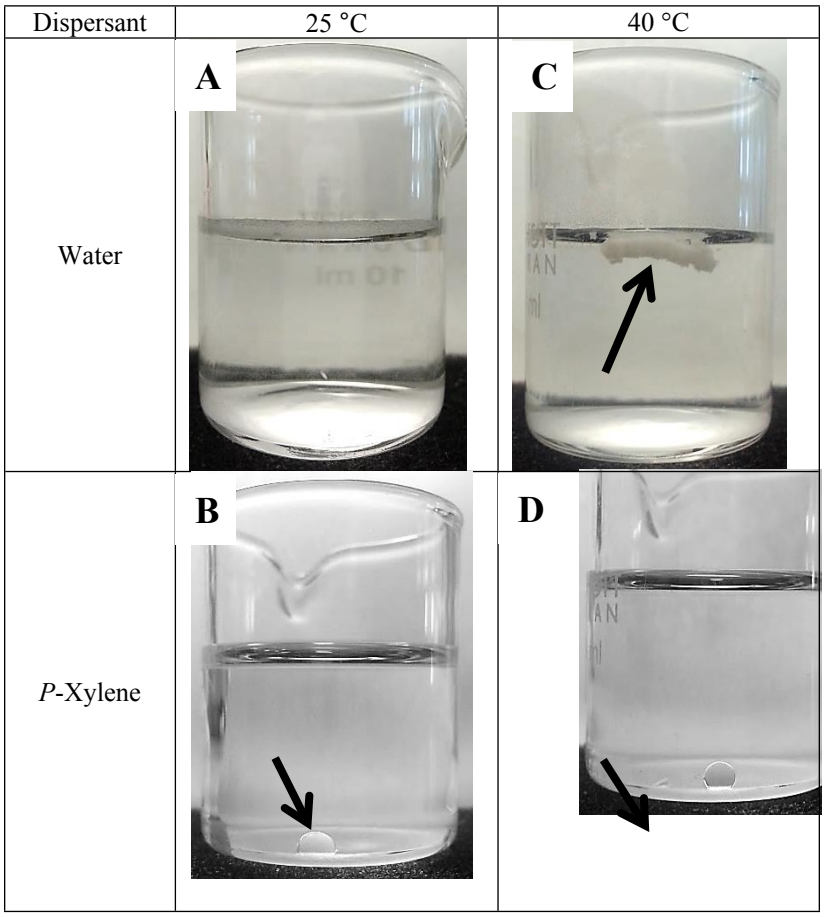


**Figure S5** Drop test to determine the emulsion type. The emulsion at 25°C (left column) and 40°C (right column) was dropped into water (top row) and *p*-xylene (bottom row) using a pipette. Inset arrows indicate the position of the emulsion droplet. When the emulsion is at 25°C, it A) dispersed completely in water while forming an oil layer at the surface, and B) remained as a droplet in *p*-xylene, indicating that it is an o/w emulsion. When the emulsion's temperature was increased to 40°C, it C) remained intact and floats in water, and D) sank to the bottom in *p*-xylene because of the higher density of the emulsion, containing 25 volume % water, indicating that it remained an o/w after solidification at 40°C.

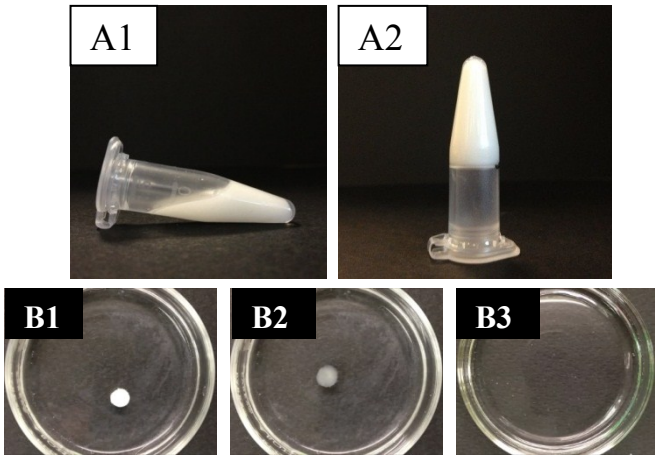


**Figure S6** Thermoresponsivity of the HIPE and polyHIPE. HIPE was seen to be A1) flowing at room temperature and A2) solidifying after incubating at 40°C. PolyHIPEs retained their thermoresponsive properties. B1) PolyHIPE was placed in water at 70°C. B2) As the temperature decreased to 40°C, it remained intact and only a slight swelling of the polyHIPE was observed becoming more translucent, indicating pore interconnectedness. B3) At room temperature which is below the cloud point (34°C), the polyHIPE was seen to dissolve completely in water.

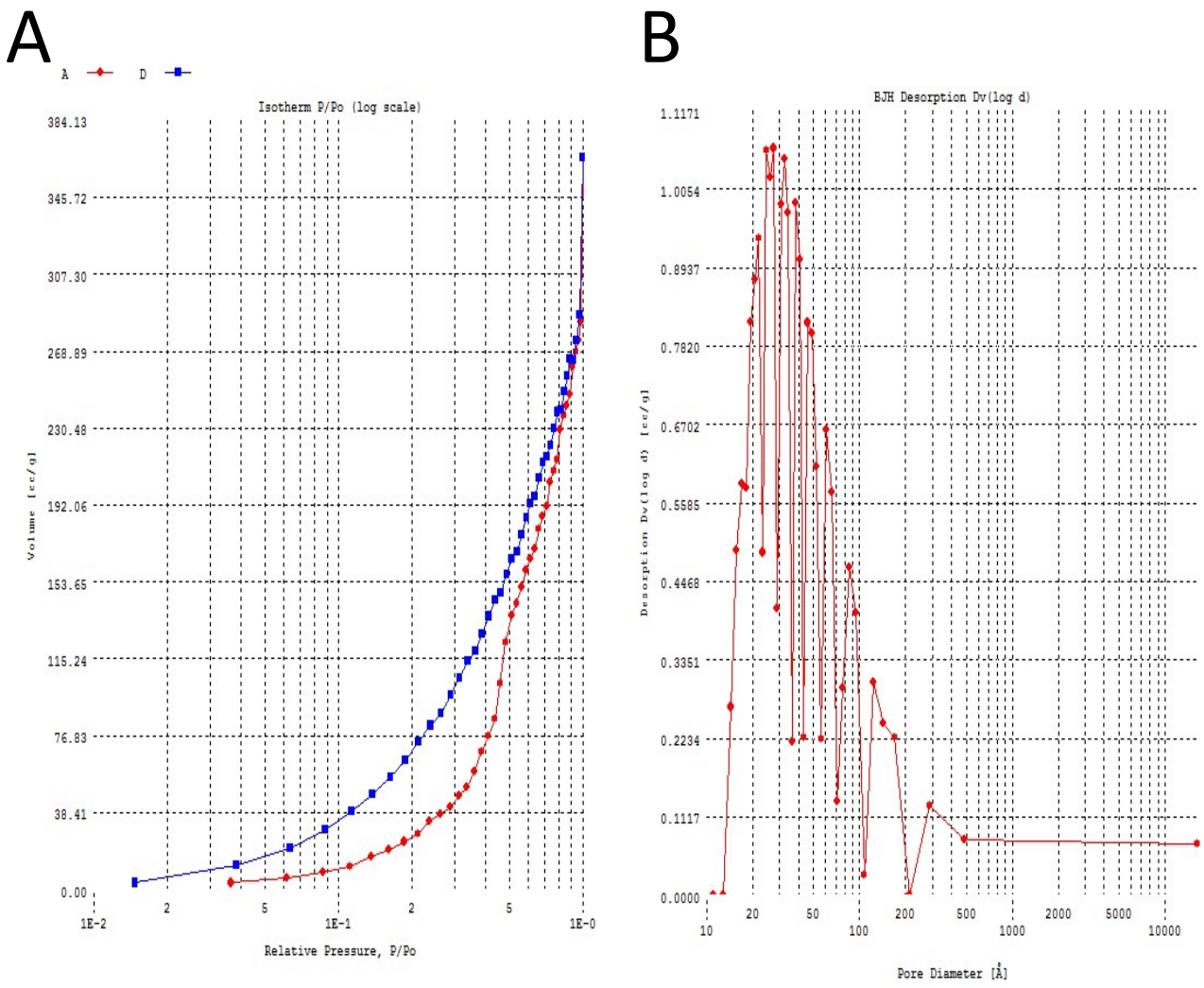


**Figure S7** A) Adsorption (red) and desorption (blue) isotherms and B) pore size distribution of CSNLYS-4.

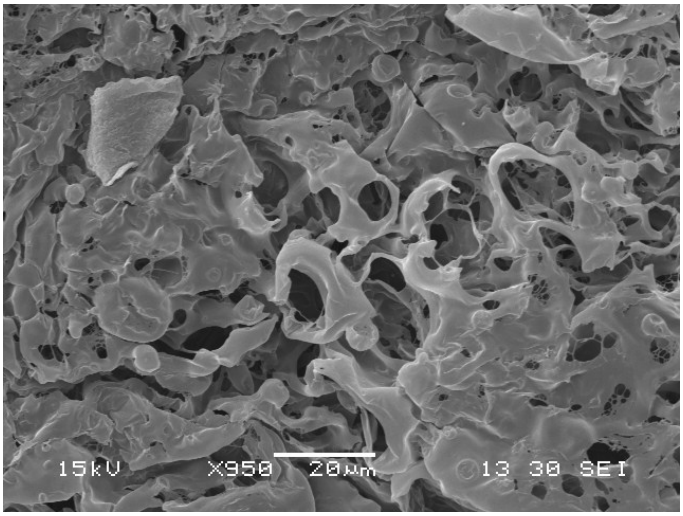


**Figure S8** Scanning electron micrograph of a foam formed by emulsion templating using 2%w/v CSN to stabilize the emulsion. This foam has an irregular pore structure and collapsed pores.

**Table S1** $^{1}H$ chemical shifts of chitosan, CSN and CSNLYS found in the NMR spectra.

| NMR | Protons | Shifts (ppm) |
|---|---|---|
| **Chitosan** | | |
| Proton on Anomeric carbon | H1 | 4.65 |
| $-CH_3$ of acetylated group | H7 | 1.81 |
| Proton on carbon bearing $NH_2$ | H2 | 2.84 |
| Protons on glucosidic ring | H3-6 | 3.67,3.50,3.33 |
| **PNIPAM** | | |
| $-CH-CH_2$ | H8,H9 | 1.91, 1.51 |
| $-CH_3$ | H10 | 1.08 |
| **Lysine** | | |
| $-NH_2$ | H15 | 3.02 |
| $-CH_2CH_2CH_2-$ | H12,H13,H14 | 2.0, 1.70, 1.45 |

# Appendix A

**Calculation of Degree of Substitution and Degree of Polymerization**

Degree of deacetylation (DD) of chitosan, degree of substitution (DS) and degree of polymerization (DP) of PNIPAM and lysine were determined by NMR[1].

**Degree of Deacetylation determined by NMR**

DDA of chitosan = [1-($I_{H7}$/3)/($I_{H2-6}$/6)] x100% =78%

**Degree of substitution of PNIPAM grafted**

Since the reaction occurs on the $NH_2$ of deacetylated groups, the reduction in $NH_2$ signal will represent the number of rings that have initiated the polymerization.

Taking the protons from the glucosidic ring as the base, $NH_2$ in chitosan= 0.18

$NH_2$ in CSN= 0.16

% of deacetylated ($NH_2$) rings grafted= ((0.18-0.16)/0.18)x100% =11.1%

Therefore, ratio of units grafted onto, m=0.11*78%=0.087

DP of PNIPAM per unit = [($I_{H10}$/6)/(($I_{H3-6}$/5)*0.087]] x100% = [(2.91/6)/((1/5)*0.087)] =21.4

**DS and DP of oligoLysine**

Ratio of $NH_2$ deacetylated rings used for conjugating lysine = [($I_{H3-H6}$)/5]*0.69-$I_{H2}$= (1/5)*0.69-0.10=0.039

DP of CSNLYS-H = ($I_{H15}$ /2)/0.039= 0.30/2/0.039= 3.89

DP of CSNLYS-M = ($I_{H15}$ /2)/0.039= 0.21/2/0.039= 2.69

DP of CSNLYS-L = ($I_{H15}$ /2)/0.039= 0.1/2/0.039= 1.29

$I_{H7}$, $I_{H2-6}$, $I_{H10}$ represents the integral signals of the –$CH_3$ protons on acetylated groups, anomeric protons on the glucosidic unit, -$CH_3$ protons on NIPAM units respectively.

## References

[1] M. Lavertu, Z. Xia, A. Serreqi, M. Berrada, A. Rodrigues, D. Wang, M. Buschmann, A. Gupta, *Journal of Pharmaceutical and Biomedical Analysis* **2003**, *32*, 1149-1158.