Supplementary Information (SI) for Soft Matter.

Supporting Information

Preparation of Hybrid β-Chitosan – Squid Pen Protein Hydrogel Beads by Ionic Liquid Regeneration for Adsorption of Copper (II) and Zinc (II) from Wastewater

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Supplementary Text

S1. Determination of geometry

The geometry of the different hydrogel bead types was determined by visual inspection. A representative sample of each bead type was dispensed on a tray. The two principal dimensions, as observed from above, were compared. Beads with near identical principal dimensions were spherical, while those with notable elongation were ellipsoidal.

S2. Determination of size measurements

The size measurements for a randomly selected sample of 20 beads, from each bead type, were recorded using a digital calliper. The radius was measured for spherical beads, while both the length and width were recorded for ellipsoidal beads.

S3. Kinetic data analysis: Pseudo-first-order model

The pseudo-first-order (PFO) model was introduced by Lagergran to describe adsorption for which the rate-limiting step is diffusion through a boundary such as in physisorption processes.[1, 2] The PFO model assumes that the adsorbate is present in infinitely miniscule amounts compared to the solvent/water such that its concentration may be integrated into the rate constant. This simplification results in the rate law being expressed solely in terms of the saturation of adsorbent sites, as per the following equation:

$$\frac{dq_t}{dt} = K_1(q_e - q_t)$$

where q_t and q_e are the adsorbent's capacity for heavy metal ions, on a dry mass basis, after an arbitrary treatment time and at equilibrium, respectively, in mg g^{-1}. K_1 is the rate constant (min^{-1}) for PFO kinetics. For initial conditions of $q_t = 0$ at $t = 0$ using fresh adsorbent, a linearized form is obtained:

$$\log(q_e - q_t) = \log(q_e) - t\left(\frac{K_1}{2.303}\right)$$

for which K_1 is the gradient of a $\log(q_e - q_t)$ versus time plot. Meanwhile, q_e must be inputted based on data obtained experimentally but can be verified from the y-intercept to assess the suitability of the PFO model.

S4. Kinetic data analysis: Pseudo-second-order model

The pseudo-second-order (PSO) model was introduced by Ho and McKay for describing chemisorption.[2, 3] According to the PSO model, the rate-limiting step for adsorption is the chemical reaction between the adsorbate and adsorbent, as per the equation:

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2$$

where q_e and q_t are as previously defined, and K_2 is the rate constant (g mg^{-1} min^{-1}) for PSO kinetics. The equation can be linearized with initial conditions of $q_t = 0$ at $t = 0$ to obtain the following form:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e}$$

from which K_2 and q_e can be determined from the y-intercept and gradient of a plot of $^{t}/_{q_t}$ versus time, respectively.

S5. Equilibrium data analysis: Langmuir isotherm

The Langmuir isotherm describes adsorption onto a homogenous adsorbent with active sites of equivalent energy.[4] It supports adsorbate deposition by monolayer formation with no lateral interactions between adsorbed particles. The Langmuir isotherm is expressed as follows:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

where q_e has been defined, q_m is the adsorbent's maximum adsorption capacity (mg g^{-1}), C_e is the equilibrium solution concentration and K_L is the Langmuir constant (L mg^{-1}). The linearized form is given by the following equation:

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m}$$

from which q_m and K_L can be determined from the y-intercept and gradient respectively. The Langmuir isotherm can be used to assess the favourability of adsorption through the equilibrium parameter, R_L, as expressed by the following equation:

$$R_L = \frac{1}{1 + K_L C_i}$$

where C_i is the initial solution concentration (ppm). The value of the equilibrium parameter is indicative of irreversible ($R_L = 0$), favourable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavourable ($R_L > 1$) adsorption.[5]

S6. Equilibrium data analysis: Freundlich isotherm

The Freundlich isotherm governs a heterogeneous surface. It suggests that sorption energy decreases with the completion of active sites.[6] The Freundlich isotherm describes multilayer adsorption according to the following equation:

$$q_e = K_F C_e^{1/n}$$

where q_e and C_e are as previously defined while K_F and $^1/_n$ are the Freundlich constants describing the capacity and intensity of adsorption, respectively. The linearized form of the Freundlich isotherm is expressed by:

$$\ln(q_e) = \ln(K_F) + \frac{1}{n} \ln C_e$$

from which $^1/_n$ and K_F can be determined from the gradient and y-intercept of a plot of $\ln(q_e)$ versus $\ln(C_e)$ respectively. Lastly, $^1/_n$ is indicative of unfavourable ($^1/_n > 1$), linear ($^1/_n = 1$) or favourable ($0 < {}^1/_n < 1$) adsorption.[5]

S7. Determination of atom economy for the preparation of CH beads

The atom economy for the synthesis of CH beads was estimated assuming that only the glucosamine (GLcN) monomers on β-chitosan underwent glutaraldehyde crosslinking. Crosslinking was considered to occur by imine bond formation, involving condensation reactions. A conservative estimate for the atom economy was obtained considering the complete saturation of amine groups on adjacent β-chitosan chains.

The average molecular weight (MW) for a monomer on β-chitosan (Deacetylation: 91.7%) was calculated based on the MW of GLcN and acetylglucosamine (GLcNAc) monomers:

$$Avg.\,MW\ monomer = DA \times MW\ GLcN + (1 - DA) \times MW\ GLcNAc$$

$$Avg.\,MW\ monomer = 0.917 \times 161.2 + (1 - 0.917) \times 203.2$$

$$Avg.\,MW\ monomer = 164.69\ g\ mol^{-1}$$

The average number of monomers, GLcN and GLcNAc, was estimated based on the average MW for β-chitosan provided by the manufacturer:

$$Total\ Monomers = \frac{Avg.\,MW\ polymer}{Avg.\,MW\ monomer}$$

$$Total\ Monomers = \frac{580{,}000}{164.69}$$

$$Total\ Monomers = 3{,}522\ units$$

The number of GLcN units per polymer was estimated in order to determine the number of amine sites available for crosslinking:

$$GLcN\ Monomers = Monomers\ per\ polymer\ \times\ DA$$

$$GLcN\ Monomers = 3{,}522\ \times\ 0.917$$

$$GLcN\ Monomers = 3{,}230\ units$$

The MW of the reactants was calculated based on two adjacent β-chitosan chains and enough glutaraldehyde for saturation (i.e. same amount as GLcN monomers):

$$MW\ reactants = Avg.\ MW\ polymer\ \times 2 +\ glutaraldehyde\ molecules\ \times\ MW\ glutaraldehyde$$

$$MW\ reactants = 580{,}000\ \times 2 +\ 3230\ \times\ 100.12$$

$$MW\ reactants = 1{,}483{,}387.60\ g\ mol^{-1}$$

The MW of the desired products was calculated based on the formation of two water molecules as byproduct for every glutaraldehyde molecule involved in crosslinking:

$$MW\ products = MW\ reactants - \ 2 \times\ Glutaraldehyde\ molecules\ \times\ MW\ water$$

$$MW\ products = 1{,}483{,}387.60 -\ 2 \times\ 3230\ \times\ 18$$

$$MW\ products = 1{,}367{,}107.60\ g\ mol^{-1}$$

The atom economy was obtained from the MW of the reactants and desired products:

$$Atom\ economy = \frac{MW\ products}{MW\ reactants} \times 100$$

$$Atom\ economy = \frac{1{,}367{,}107.60}{1{,}483{,}387.60} \times 100$$

$$Atom\ economy = 92.2\%$$

S8. Determination of E-factor for the preparation of CH beads

The E-factor for the preparation of CH beads was estimated under the assumption that [BMIM][OAc], DMSO and absolute ethanol were fully recyclable. Furthermore, energy and water considerations were omitted. Lastly, all losses solely took into consideration spent glutaraldehyde solution. An optimal estimate for the E-factor was obtained for complete saturation of amine groups on β-chitosan, which would correspond to the maximum retention of glutaraldehyde in the desired product. A more conservative estimate was also calculated based on all the glutaraldehyde being discarded. (Some estimates from the previous section were utilized where appropriate)

The moles of β-chitosan present in a batch equivalent of casting solution was estimated based on the average MW for β-chitosan provided by the manufacturer:

$$Moles\ of\ \beta - chitosan\ = \frac{Mass\ of\ \beta - chitosan}{Avg.\ MW\ polymer}$$

$$Moles\ of\ \beta - chitosan = \frac{0.6}{580{,}000}$$

$$Moles\ of\ \beta - chitosan = 1.03\ \times\ 10^{-6}\, moles$$

The amount of GLcN monomers present in a batch equivalent of casting solution was estimated based on the ratio of glucosamine units per polymer:

$$Moles\ of\ GLcN\ = GLcN\ Monomers\ \times\ Moles\ of\ \beta - chitosan$$

$$Moles\ of\ GLcN = 3230 \times (1.03\ \times 10^{-6})$$

$$Moles\ of\ GLcN\ = 0.00334\, moles$$

The amount of glutaraldehyde necessary for complete saturation of amine groups was calculated based on a stoichiometric ratio of 1:2 with GLcN monomers:

$$Mass\ of\ glutaraldehyde\ at\ saturation = 0.5\ \times\ Moles\ of\ GLcN\ \times\ MW\ glutaraldehyde$$

$$Mass\ of\ glutaraldehyde\ at\ saturation = 0.5\ \times\ 0.00334 \times\ 100.12$$

$$Mass\ of\ glutaraldehyde\ at\ saturation = 0.1673\ g$$

The amount of glutaraldehyde used during the crosslinking procedure was calculated:

$$Mass\ of\ glutaraldehyde\ used = Volume\ (ml) \times\ Conc(v/v)\ \times\ Density\ (g\ ml^{-1})$$

$$Mass\ of\ glutaraldehyde\ used = 100 \times\ 0.0025\ \times\ 1.06$$

$$Mass\ of\ glutaraldehyde\ used = 0.265\ g$$

The optimum value for the E-factor was obtained as follows:

$$E - factor = \frac{Mass\ of\ glutaraldehyde\ used - Mass\ of\ glutaraldehyde\ at\ saturation}{Mass\ of\ glutaraldehyde\ at\ saturation + Mass\ of\ \ \beta - chitosan}$$

$$E - factor = \frac{0.265 - 0.1673}{0.1673 + 0.6}$$

$$E - factor =\ 0.127$$

The conservative value for the E-factor was obtained as follows:

$$E - factor = \frac{Mass\ of\ glutaraldehyde\ used}{Mass\ of\ \ \beta - chitosan}$$

$$E - factor = \frac{0.265}{0.6}$$

$$E - factor =\ 0.442$$

S9. Determination of degree of deacetylation of β-chitosan

The ^{1}H NMR spectrum of β-chitosan was obtained using a JNM-ECZL 400S spectrometer (JEOL). Sample preparation entailed dissolving approximately 20 mg of β-chitosan in 0.7 ml of 2 wt% deuterium chloride (DCl) in deuterium oxide (D_2O) through gentle heating at 40 ℃ for an overnight period.

The assignments and chemical shifts for the obtained ^1H NMR signals are detailed in the following analysis according to **Figure S3**. β-chitosan ^1H NMR (D_2O/DCl): $\delta = 4.68$ (H1 of GLcN), $\delta = 3.06 - 3.79$ (H2, H3, H4, H5, H6 of GLcNAc and GLcN) and $\delta = 4.68$ (H7 of HN−$COCH_3$).

The degree of deacetylation, DD, was calculated based on the integrals of the signal corresponding to H7 methyl protons of GLcNAc and sum of integrals of H2-H6 signals as proposed by Perez-Alvarez et. al.[7] The calculation was as follows:

$$DD = \left(1 - \frac{\frac{1}{3} \times I_{N-COCH_3}}{\frac{1}{6} \times I_{(H2-H6)}}\right) * 100$$

$$DD = \left(1 - \frac{\frac{1}{3} \times 1.00}{\frac{1}{6} \times 24.13}\right) * 100$$

$$DD = 91.7\%$$

Supplementary Tables

Table S 1. Rate constants and adsorption capacity at equilibrium for PFO and PSO models

Heavy metal ion	Experimental	PFO model			PSO model		
	q_e (mg g^{-1})	K_1 (min^{-1})	q_e (mg g^{-1})	R^2	K_2 (g mg^{-1} min^{-1})	q_e (mg g^{-1})	R^2
Cu^{2+}	40.1	0.00422	16.7	0.9709	0.00070	41.0	0.9997
Zn^{2+}	8.3	0.00943	7.1	0.9649	0.00235	8.61	0.9993

Table S 2. Langmuir and Freundlich parameters for the uptake of Cu^{2+} and Zn^{2+} by CH-SQ-25 beads

Heavy metal ion	Langmuir isotherm			Freundlich isotherm		
	K_L (L mg^{-1})	q_m (mg g^{-1})	R^2	K_F (mg g^{-1})	$^1/_n$	R^2
Cu^{2+}	0.0815	70.2	0.9938	15.22	0.315	0.9792
Zn^{2+}	0.0046	24.0	0.9980	0.308	0.689	0.9943

Table S 3. Langmuir equilibrium parameter, RL, for different initial heavy metal concentrations

Heavy metal ion	Initial concentration, C_i (ppm)				
	50	100	150	200	250
Cu^{2+}	0.195	0.108	0.074	0.059	0.046
Zn^{2+}	0.816	0.692	0.593	0.514	0.471

Supplementary Figures

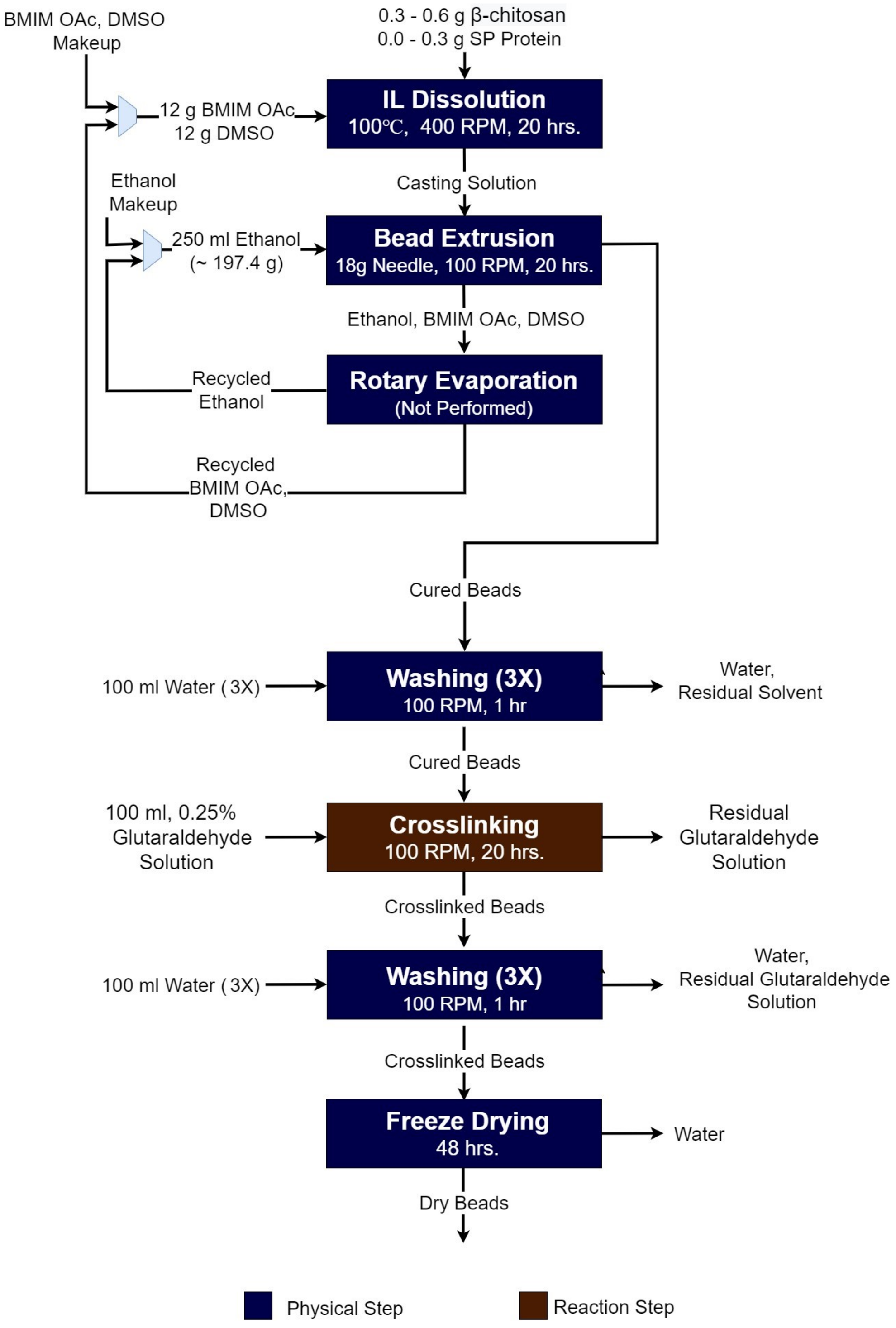

Figure S 1. Process diagram for β-chitosan – SP protein hydrogel bead synthesis by ionic liquid regeneration from [BMIM][OAc]

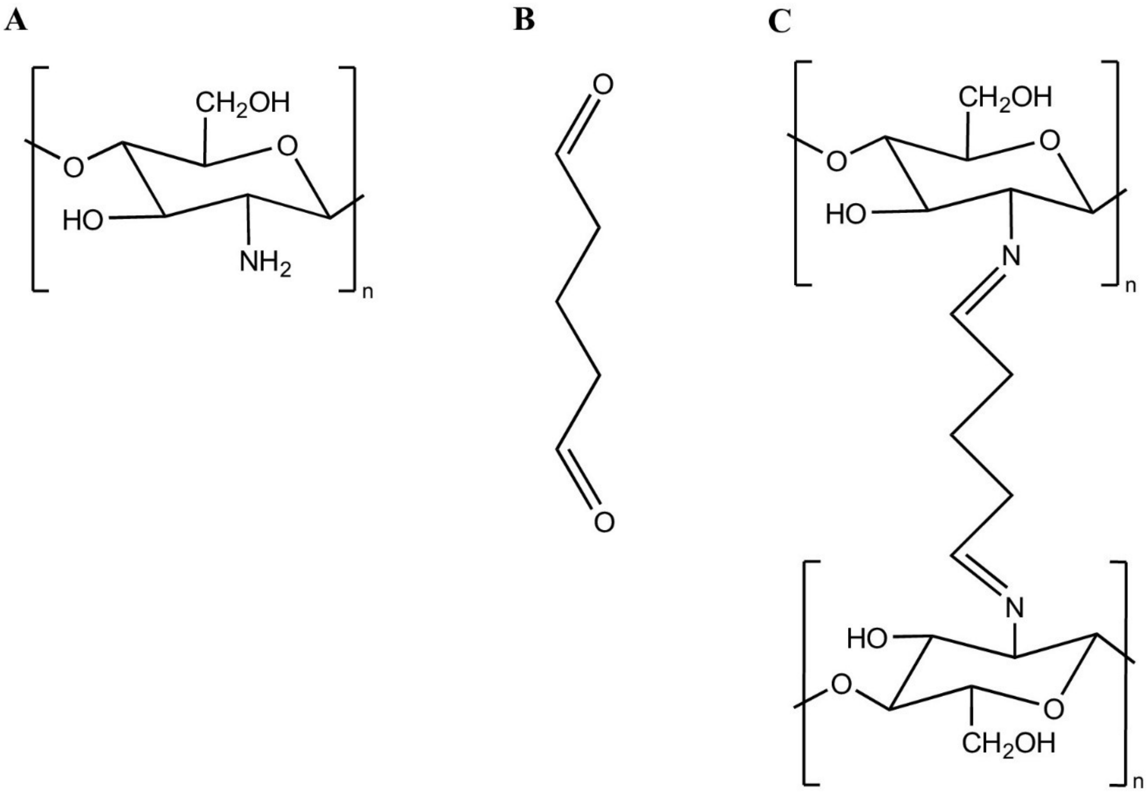

Figure S 2. Structure of (A) GLcN monomer on β-chitosan, (B) glutaraldehyde, (C) crosslinked GLcN monomers on adjacent β-chitosan chains

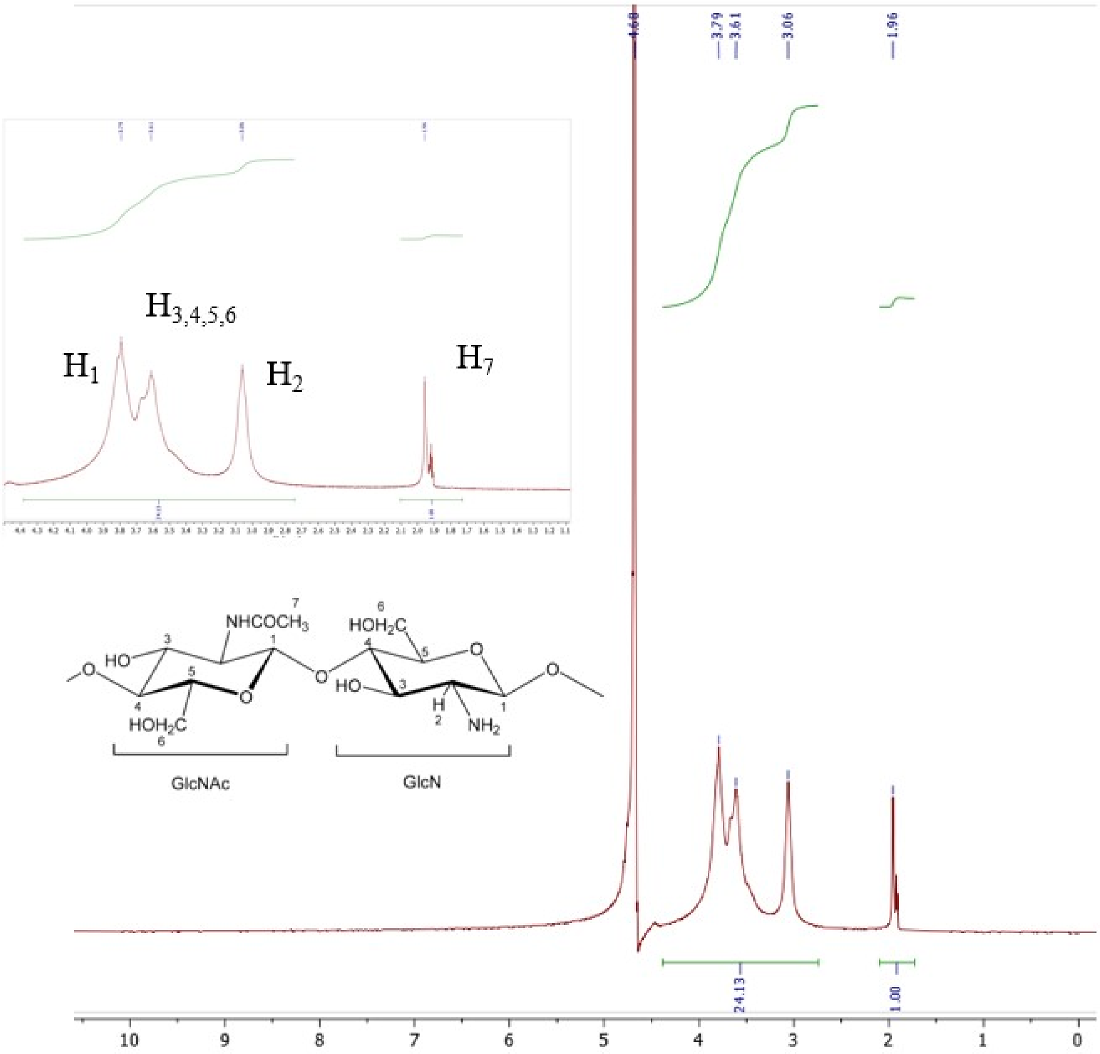

Figure S 3. NMR spectrum for β-chitosan in deuterium oxide and deuterium chloride

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