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Supplementary Information

Access to cross-linked chitosans by exploiting CO_2 and the double solvent-catalytic effect of ionic liquids

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Experimental Section

Materials. *N*-Methyl-2-pyrrolidone [NMP] (99%), ethanol (99,5%), chitosan MW and chitosan 85% desac. were purchased from Sigma-Aldrich. All chitosan samples were dried at 80 °C for 24 h before use. CO_2 with a purity of 99.5% was supplied by Rivoira Gas Srl, Milano (Italy). All reagents and solvents above mentioned were of analytical grade and were used without further purification. 1-butyl-3-methyl imidazolium acetate [bmim][OAc] was purchased from io-li-tech and 1-butyl-3-methyl imidazolium chloride [bmim]Cl has been synthesized using well-known procedures.

Chitosan cross-linking. All the cross-linking reactions were carried out in a EYELA PROCESS STATION PPV-4060 reactor equipped with four autoclaves HIP-60, a glass liner of 150 mL, and a magnetic stirrer.
In a standard experiment, 500 mg of chitosan (MW or 85%) and 8,00 g of [bmim][OAc] were firstly added into the glass tube. The reactor was then deoxygenated by purging the reaction mixture three times with CO_2 (10 bar). The reactor was then pressurized with CO_2 (35 bar) and heated to 170 °C. During the reaction, CO_2 pressure (60 bar) inside the reactor was maintained by using a gas reservoir equipped with a back-pressure regulator and a pressure transducer. After the desired reaction time (4h), the autoclave was allowed to cool to room temperature and then depressurized.
All the autoclave components, which were in contact with the reaction mixture, were washed thoroughly with water in order to fully recover the reaction products. The resulting suspension was centrifuged to separate the solid product, which was further washed three times with hot water (3 x 200 mL) and three times with hot ethanol (3 x 200 mL). The recovered solid was dried in an oven (60 °C, 12h) and characterized by ATR-FTIR.
The same procedure was followed for all the cross-linking test. In one case [bmim][OAc] was replaced with *N*-methyl-2-pyrrolidone (NMP) as a solvent and the starting chitosan was recovered at the end of the reaction.

Chitosan dissolution and regeneration. Typically, 1,80 g of chitosan (MW or 85%) were dissolved in 28,00 g of [bmim]Cl by stirring at 100 °C for 2 h. To the resulting solution, 100 mL of water were added to obtain a swelled chitosan precipitate. The precipitate was washed three time with hot water (2 x 200 mL) and one time with ethanol (100 mL) to remove the confined [bmim]Cl. The regenerated chitosan obtained was used for crosslinking experiments.

Determination of Cross-linking degree. The cross-linking degree, determined by ninhydrin assay, was determined as the percentage of free amino groups in the tested chitosan.[1] 1.5 mg lyophilized chitosan sample was heated with 1 mL ninhydrin solution in oil bath at 100 °C for 20 min. The obtained solution was cooled down to room temperature and diluted with 5 mL of a 1:1 isopropanol:water solution. The optical absorbance of the solution was read with a spectrophotometer (UV, Cary 300 UV-Vis) at 570 nm. The concentration of free NH_2 groups in the sample is determined by using glycine as calibration standard. The amount of free amino groups in the sample, after heating with ninhydrin, is proportional to the optical

absorbance of the solution. The concentration measured is divided by the sample weight, and multiplied by the sample molecular weight to obtain the mole NH_2/mole sample. The degree of cross-linking of the sample is then calculated following the equation:

$$Cross - linking\ degree = \left(\frac{(n_{NH2})_{pristine} - (n_{NH2})_{Cross-linked}}{(n_{NH2})_{pristine}}\right) \times 100$$

The n_{NH2} is the concentration of NH_2 in the sample in *mole NH_2/mole sample*. The determination of cross linking degree was evaluated in triplicate.

Ninhydrin solution. Solution A) 1.05 g citric acid, 10 mL (1.0 M) NaOH and 0.04 g $SnCl_2{\cdot}2H_2O$ were mixed, then deionized H_2O was added until 25 mL; Solution B) 1 g ninhydrin was added to 1,2-propylene glycol monomethyl ether (25 mL). The two solutions A and B were combined and stirred for 45 min, then stored in a dark bottle.

Solubility tests. *Acid water.* 100 mg of sample (either cross-linked chitosan or pristine chitosan) were suspended in 10 mL of 5 wt% HCl solution and left under stirring overnight.

[bmim][OAc]. 100 mg of sample (either cross-linked chitosan or pristine chitosan) were added to 1,60 g of [bmim][OAc] and the mixture was heated to 100 °C for 2h.

Characterization. Infrared spectra were registered using an ATR-FTIR Agilent 660 (Agilent Technologies, Santa Clara, CA, USA). The IR imaging were recorded using micro ATR-FTIR microscopy spectrometer Cary 620 FTIR coupled with Cary 660 FTIR, and FPA detector. The detector had an array size of 64 x 64 pixels, with a pixel resolution of about 1,4 μm, which in FTIR microscopy operation corresponds to an imaged area of about 90 x 90 μm. Thermal gravimetric analysis (TGA) was conducted with a TA Q500 (TA instruments, New Castle, DE, USA) TGA instrument at a heating rate of 10 °C min^{-1} from 30 °C to 600 °C under a nitrogen atmosphere.

FTIR analysis

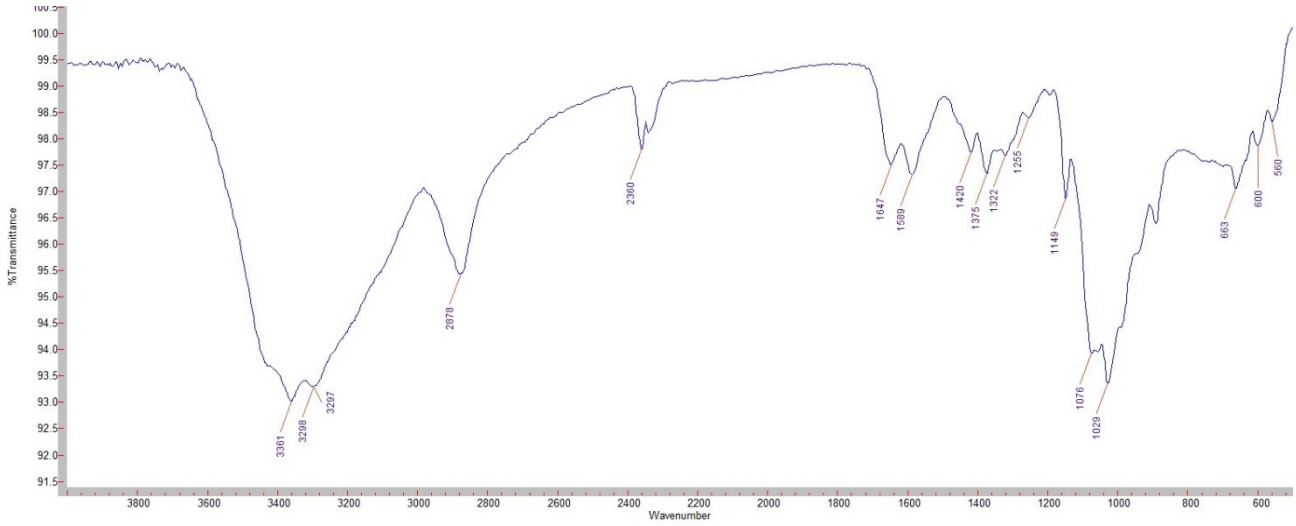


Figure S1. FTIR spectrum of pristine chitosan MW

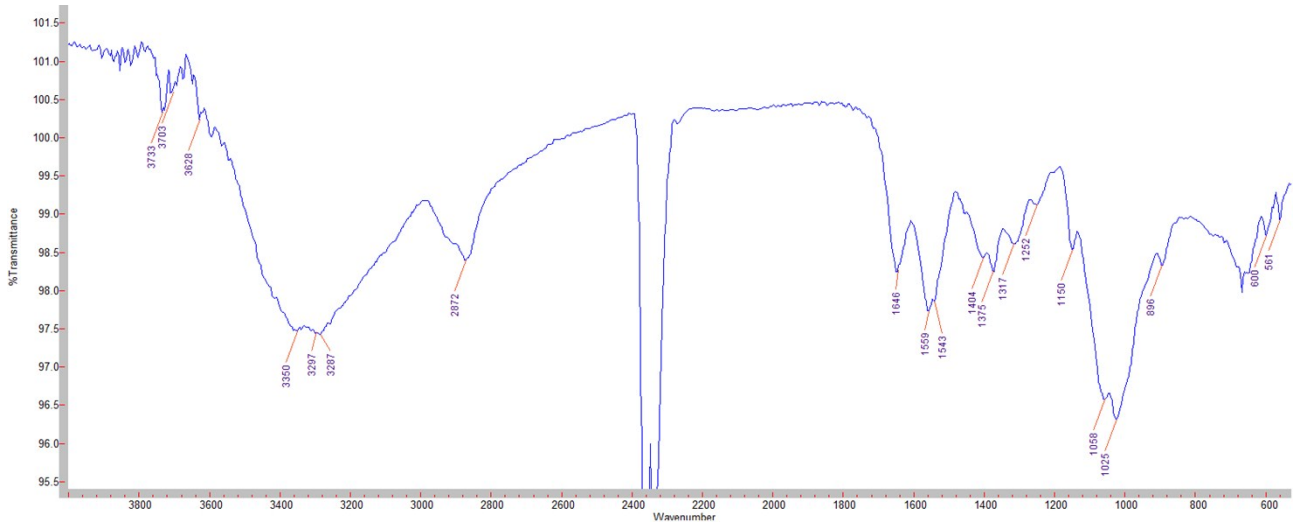


Figure S2. FTIR spectrum of cross-linked chitosan MW

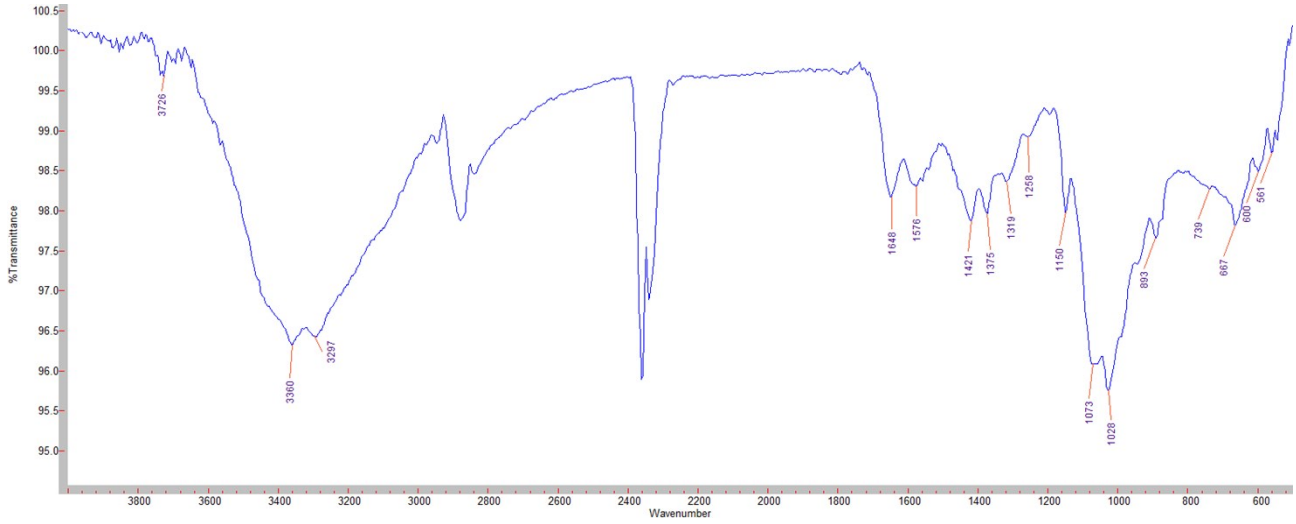


Figure S3. FTIR spectrum of pristine chitosan 85%

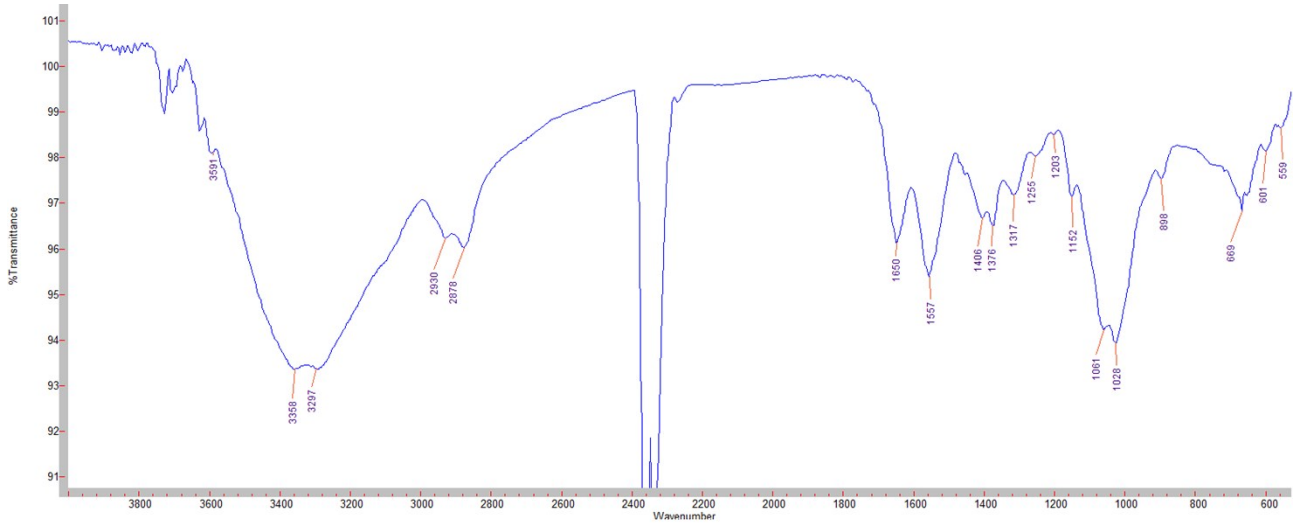


Figure S4. FTIR spectrum of cross-linked chitosan 85%

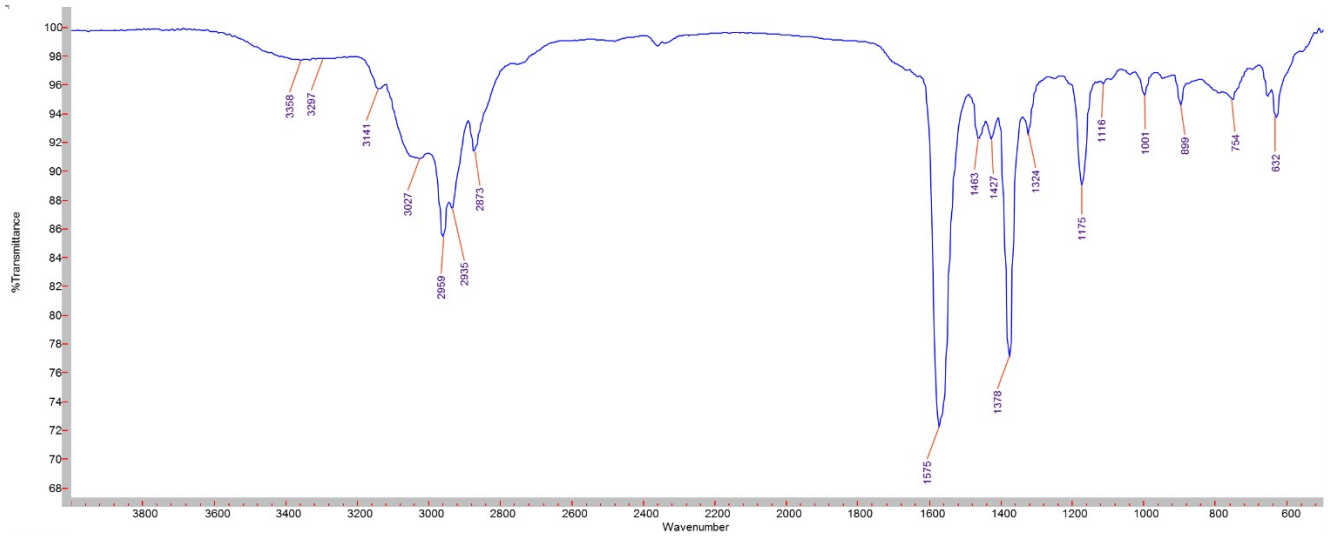


Figure S5. FTIR spectrum of [bmim][OAc]

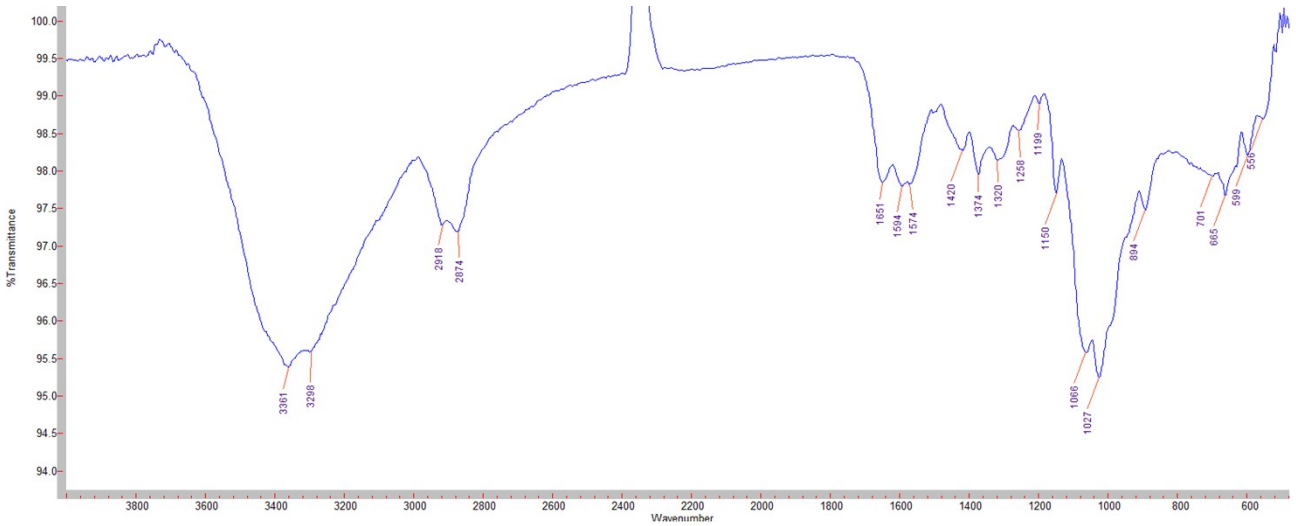


Figure S6. FTIR spectrum of chitosan obtained from the reaction performed with NMP as solvent.

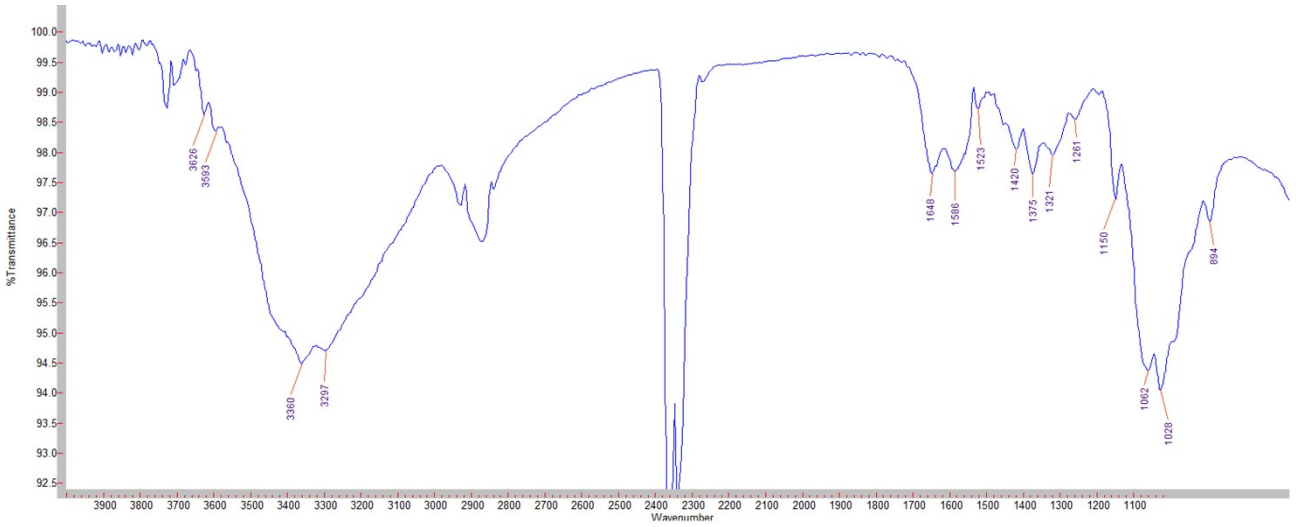


Figure S7. FTIR spectrum of chitosan MW regenerated from [bmim]Cl.

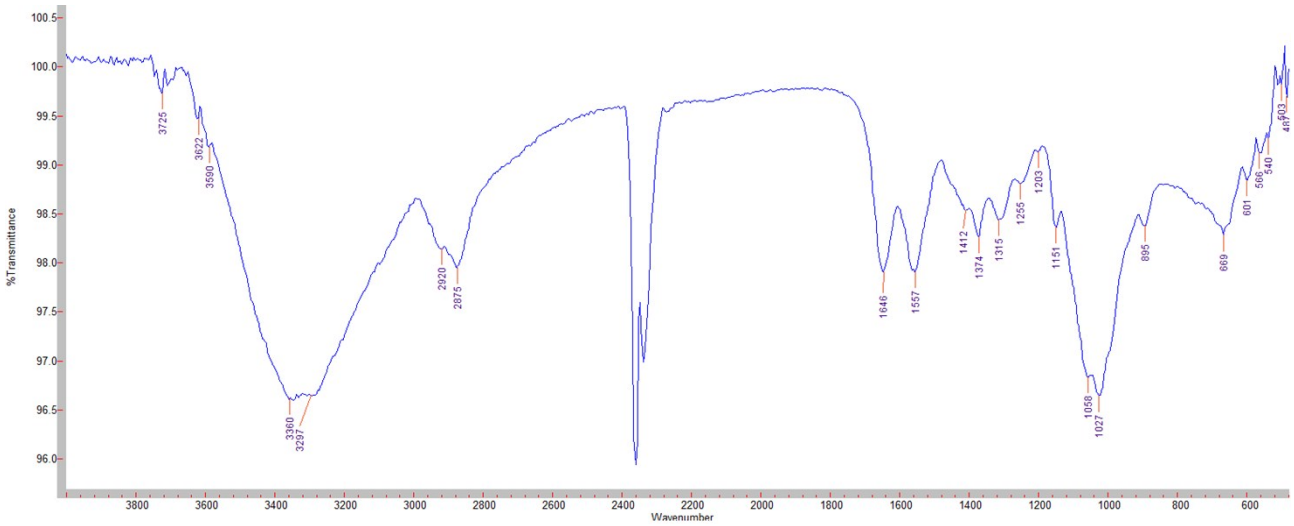


Figure S8. FTIR spectrum of cross-linked chitosan MW regenerated from [bmim]Cl.

Thermal gravimetric analysis TGA

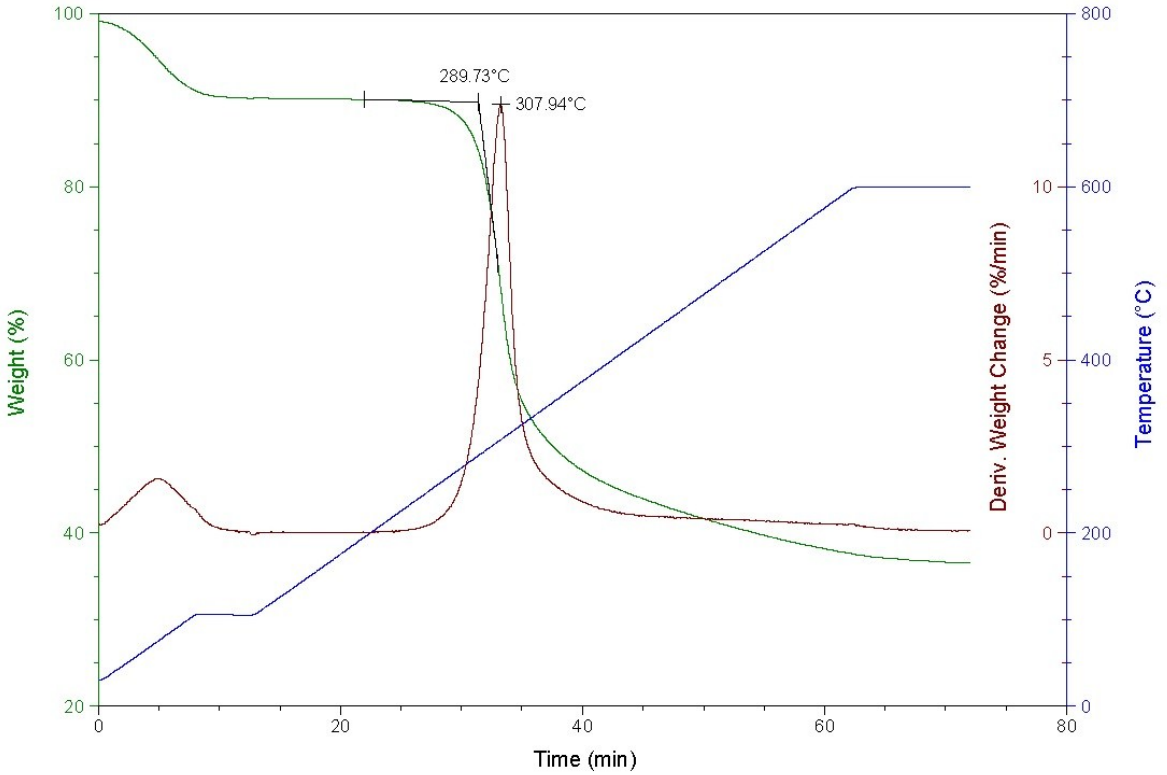


Figure S9. Thermal gravimetric analysis (TGA) of pristine chitosan MW.

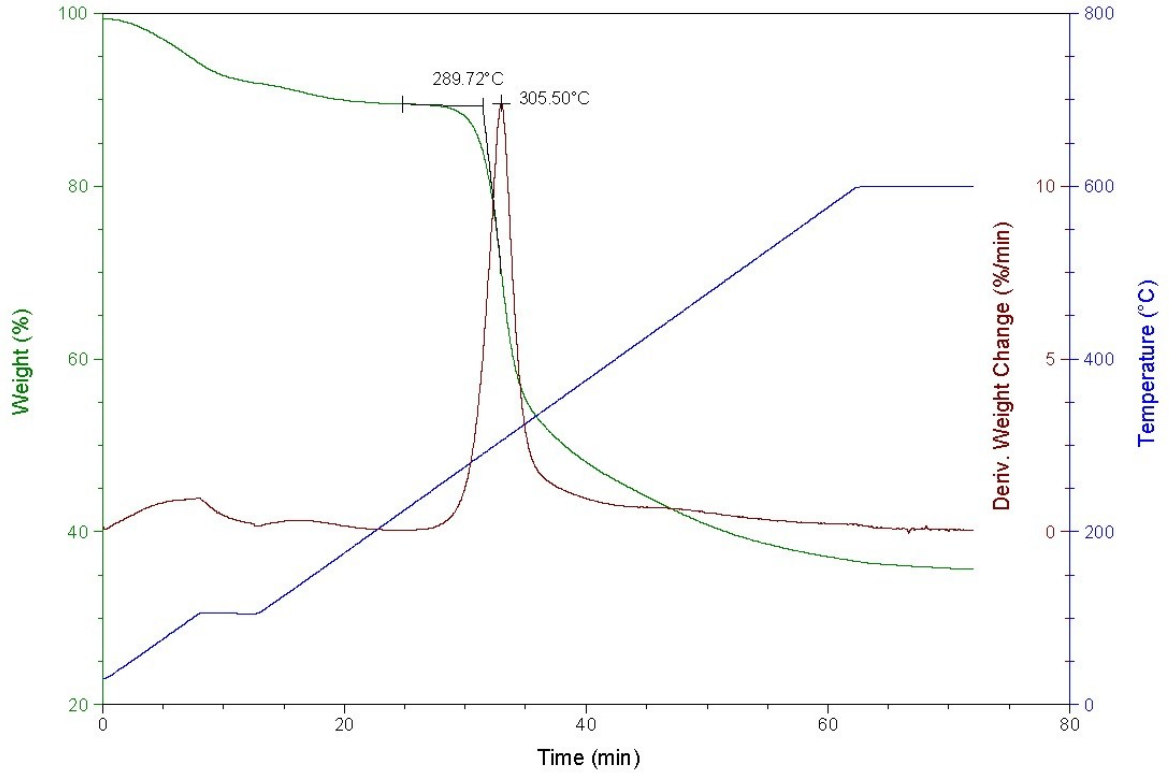


Figure S10. Thermal gravimetric analysis (TGA) of pristine chitosan 85%.

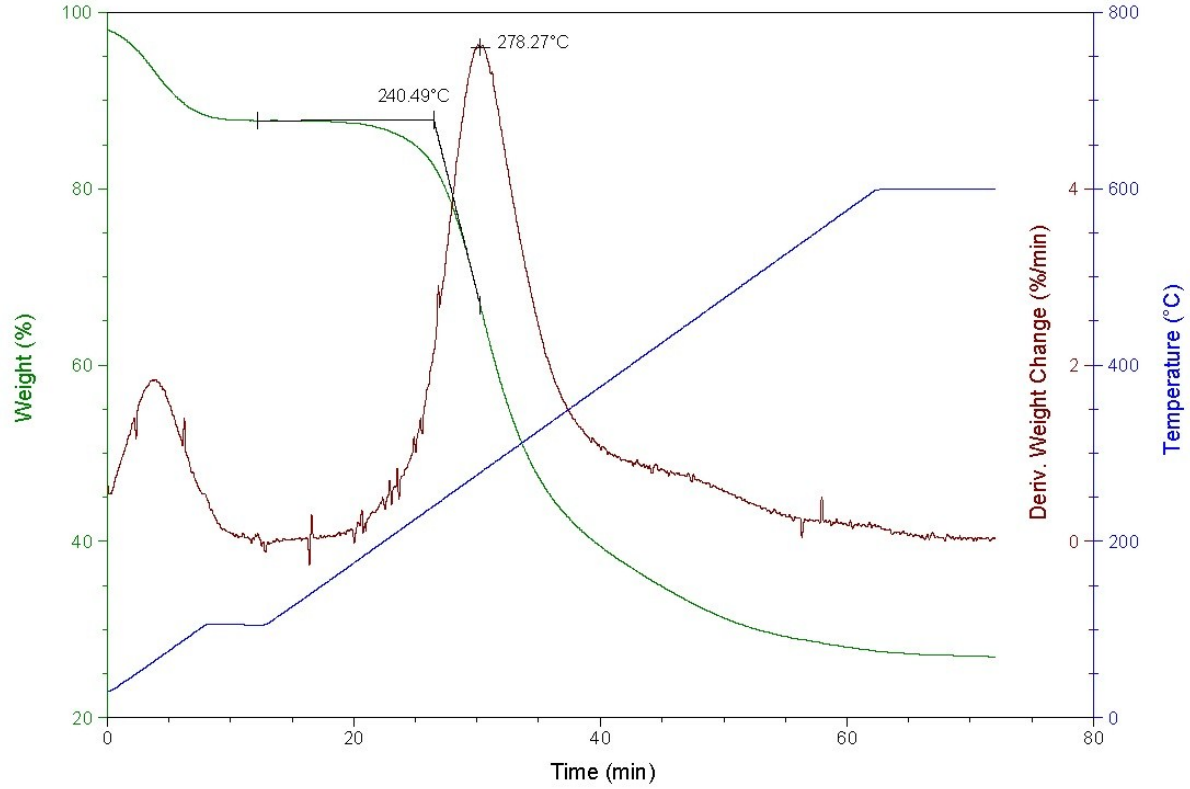


Figure S11. Thermal gravimetric analysis (TGA) of cross-linked chitosan MW.

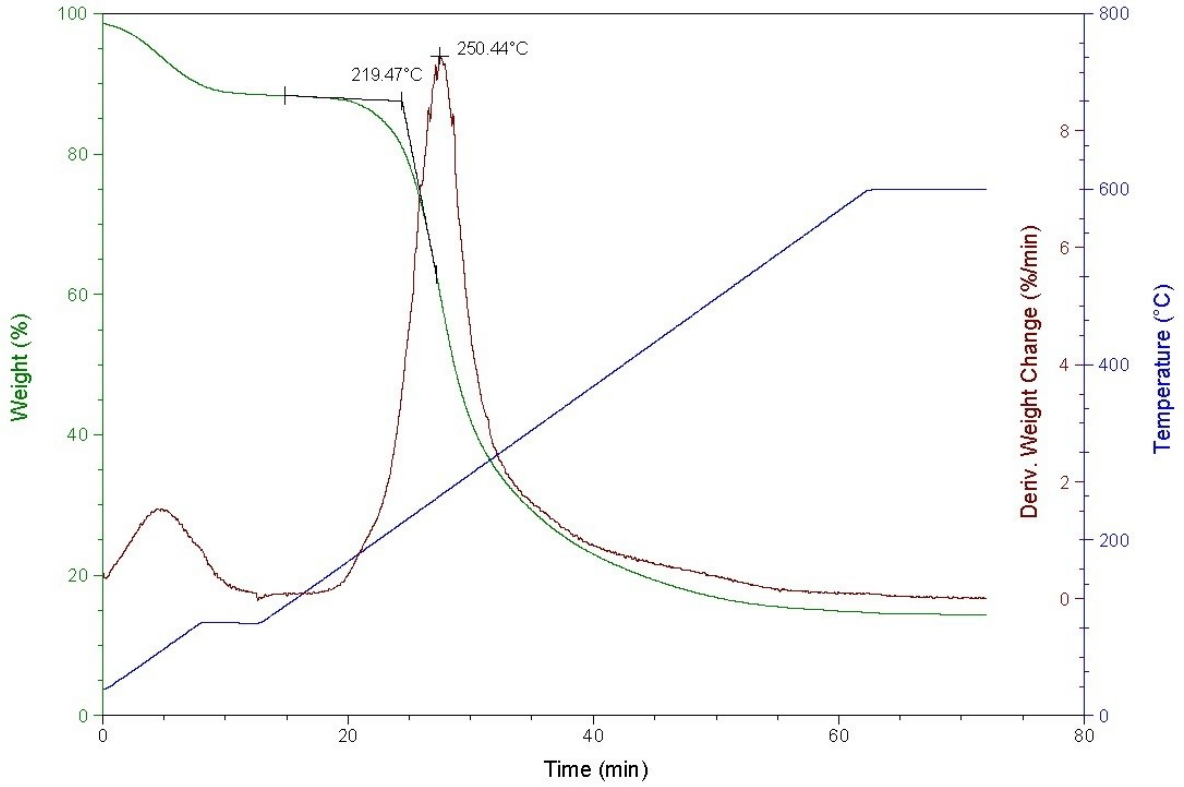


Figure S12. Thermal gravimetric analysis (TGA) of cross-linked chitosan 85%.

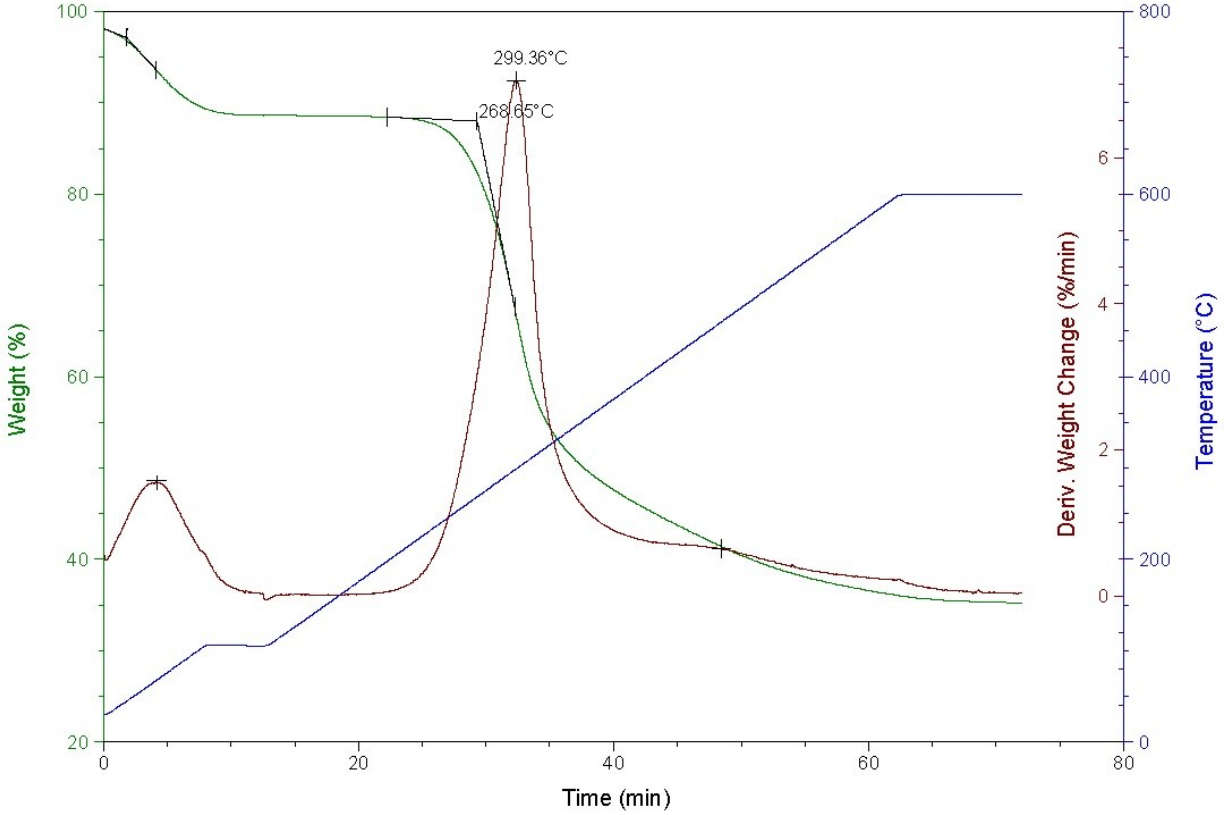


Figure S13. Thermal gravimetric analysis (TGA) of chitosan MW regenerated from [bmim]Cl.

FTIR Imaging

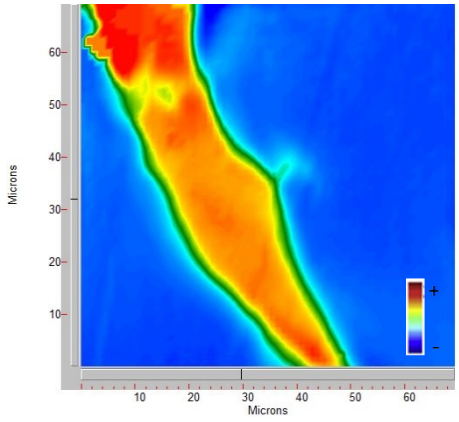
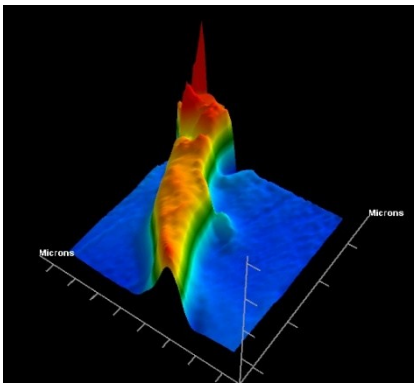
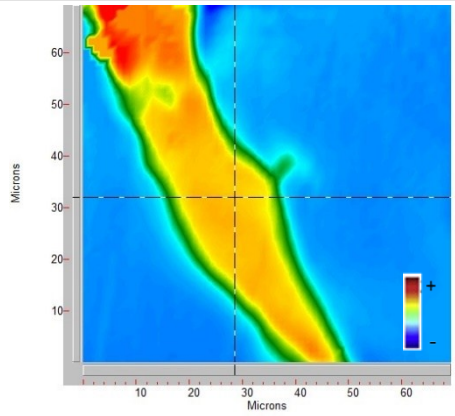
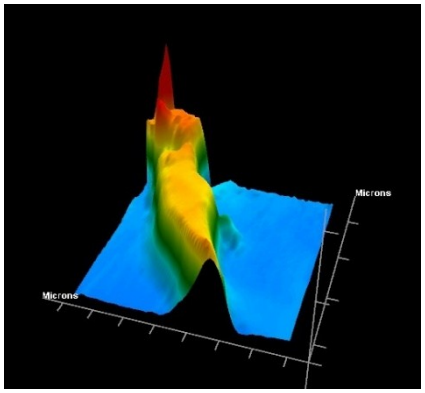
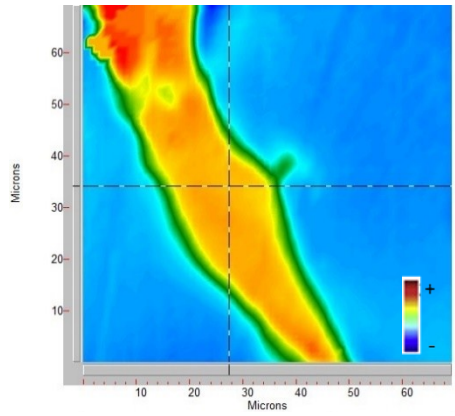
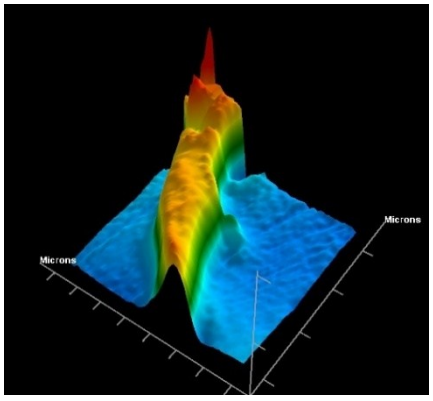
	2D	*3D*
1129 cm^{-1}		
1557 cm^{-1}		
1650 cm^{-1}		

Figure S14. Micro ATR FTIR imaging (2D and 3D) with FPA detector at different wavenumbers of cross-linked chitosan MW, which was regenerated from [bmim]Cl before performing the reaction.

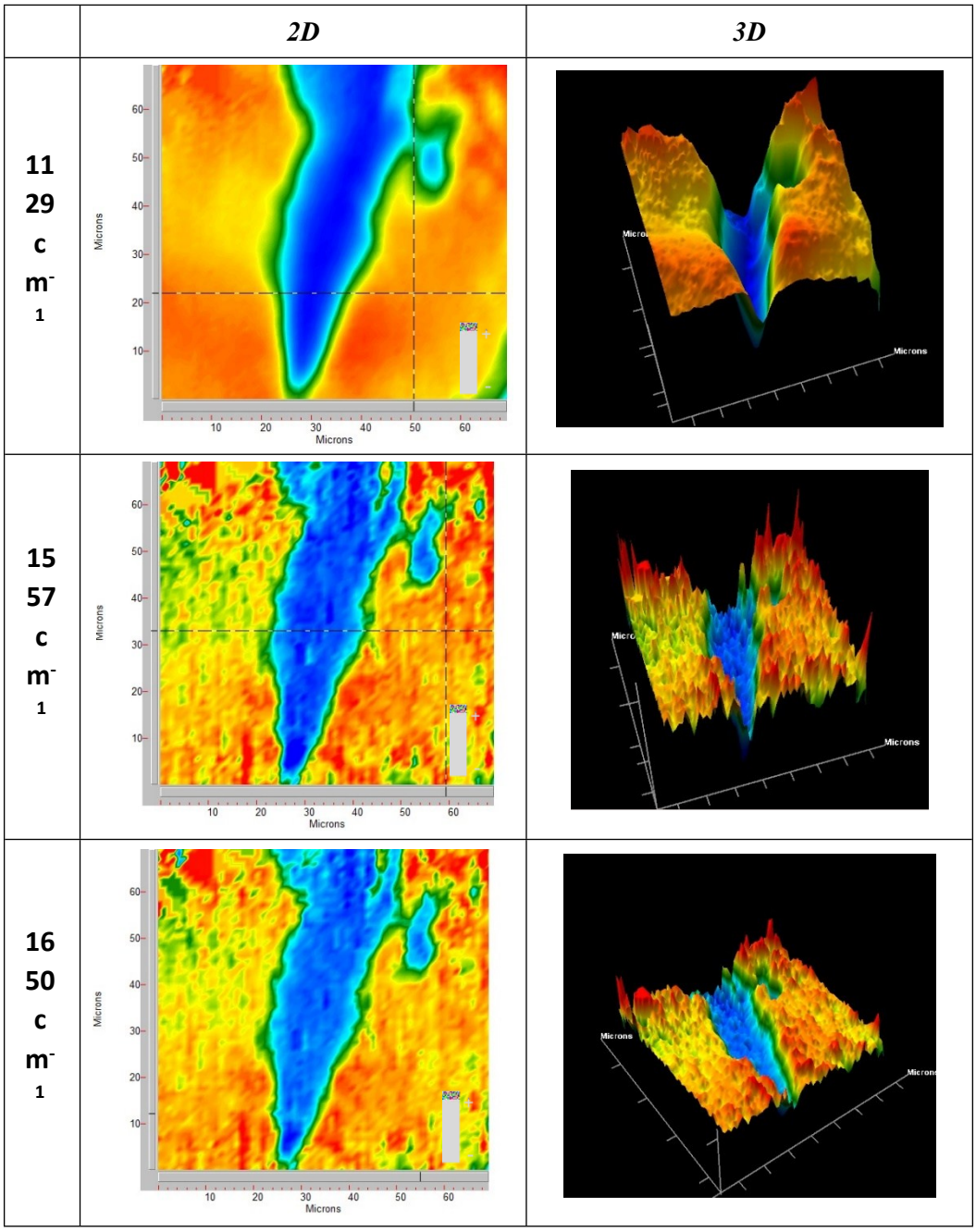


Figure S15. Micro ATR FTIR imaging (2D and 3D) with FPA detector at different wavenumbers of cross-linked chitosan MW.

References

1. Y. Yuan, B. M. Chesnutt, G. Utturkar, W. O. Haggard, Y. Yang, J. L. Ong, J. D. Bumgardner, *Carbohydr. Polym.*, 2007, **68**, 561-567.