

## Access to new cross-linked chitosans by exploiting CO<sub>2</sub> and the double solvent-catalytic effect of ionic liquids

Received 00th January 20xx,  
Accepted 00th January 20xx

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DOI: 10.1039/x0xx00000x

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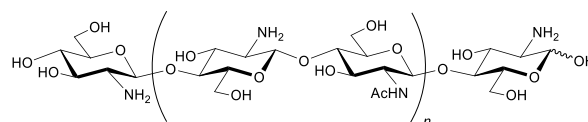
**Unreported ureido cross-linked chitosans were obtained employing the CO<sub>2</sub>-chitosan-Ionic Liquid three component system. In the conditions used, the greenhouse gas functions as a safe cross-linking agent of the biopolymer thanks to the solvent-catalytic effect of [bmim][OAc].**

Global warming deriving from the greenhouse gases, and the related climate changes and environmental impacts, are nowadays a real threat more than a simple concern. Atmospheric concentration of CO<sub>2</sub> is one of the main factors responsible for the observed temperature rise,<sup>1</sup> and at the moment its capture and storage (for example, in saline aquifers or deep ocean) is considered the best strategy to combat the problem.<sup>2</sup> However, CO<sub>2</sub> could be seen under a completely different light, namely as an attractive option in the preparation of new added-value products, an approach which is at present still underexploited. In fact, from a synthetic perspective, CO<sub>2</sub> is a non-toxic, safe, and economical C1 synthon and some methodologies to employ it in the preparation of valuable chemicals have been developed.<sup>3</sup> In particular, syntheses of urea derivatives (linear,<sup>4-6</sup> cyclic,<sup>7</sup> and polyureas<sup>8,9</sup>), a class of compounds which find application in a variety of areas ranging from pesticides,<sup>10</sup> to anti-cancer studies,<sup>11</sup> were optimized. Interestingly enough, a positive effect on the yield of isolated urea derivatives and/or on reaction times were observed when catalytic amounts of ionic liquids, either supported<sup>6</sup> or not,<sup>4,9</sup> were present as additives in the reaction mixtures.

Ionic Liquids (ILs) are organic salts which have attracted exponential interest in the last two decades due to their unique physicochemical properties (low vapour pressure, wide liquid range, good electrolytic properties with large electrochemical window, tunable polarity) especially as safe and greener substitutes of classical organic solvents.<sup>12,13</sup> In particular, the possibility to select cationic and anionic constituents of ILs allows for the tailoring of their structure depending on (as a function of) the problem tackled

each time, which is why they are often referred to as designer solvents. One new field of the ILs research began in 2002 when Rogers *et al.*<sup>14</sup> reported for the first time the capability of ILs to dissolve cellulose, overcoming the main hurdle to its modification. From that moment on, several different biopolymers, including chitin, were solubilized and modified in ILs. Nowadays, this is representative of biorefinery, namely their transformation into value-added sustainable materials.<sup>15-17</sup> Among the biopolymers available in large amounts, chitosan, which is an aminopolysaccharide consisting of β-(1→4)-linked D-glucosamine units (Figure 1), is directly obtained by de-O-acetylation of chitin, the second most abundant biopolymer on earth after cellulose.

Figure 1. Structure of chitosan



Aside from being biodegradable and non-toxic, chitosan displays remarkable intrinsic properties such as antifungal, mucoadhesive and haemostatic properties, and antibacterial activity, all making chitosan and chitosan-based materials of interest for developing future biomedical applications.<sup>18,19</sup>

While ILs have been under investigation for quite some time as CO<sub>2</sub> capture systems,<sup>20</sup> only a few recent reports studied CO<sub>2</sub>, ILs, and chitosan, at the same time. In particular, in one case CO<sub>2</sub> was used as antisolvent to regenerate chitosan from ILs-chitosan solutions,<sup>21</sup> while in other works, ILs-chitosan systems were studied either as reversible CO<sub>2</sub> sorbents, in solutions<sup>22,23</sup> or after immobilization on silica gel,<sup>24</sup> or as a catalytic system for the conversion of CO<sub>2</sub> and MeOH in dimethylcarbonates.<sup>25</sup> A slightly different approach instead employed a hybrid chitosan functionalized IL as a supported catalyst for cycloaddition of CO<sub>2</sub>.<sup>26</sup>

Based on the aforementioned findings, herein the attempt to prepare an ureido cross-linked chitosan in IL using CO<sub>2</sub> as the cross-linking agent is reported as a way to use the greenhouse gas and modify the biopolymer in order to prepare a new material to be studied in the biomedical field. (Scheme 1)

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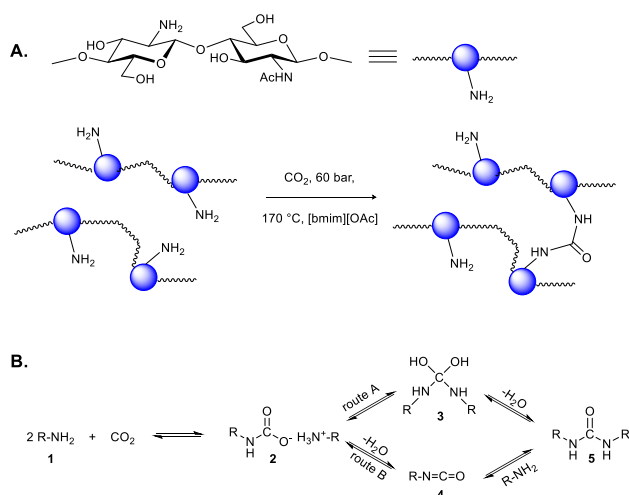
† Footnotes relating to the title and/or authors should appear here. Electronic Supplementary Information (ESI) available: Experimental procedures, FT-IR spectra, and TGA. See DOI: 10.1039/x0xx00000x

Two different chitosans, 85% deacetylated and medium molecular weight, were purchased from Aldrich and used as model systems without further purifications. 1-Butyl-3-methyl imidazolium acetate [bmim][OAc] was purchased from IoLiTech.

The reaction conditions were extrapolated from the literature<sup>4-9</sup> which suggested high temperatures (150–180 °C), together with high pressures of CO<sub>2</sub> (40–100 bar), were necessary in order to form the ureido linkage.

Regarding the chitosan concentration, it should be noted that not only CO<sub>2</sub> has an antisolvent effect on ILs-chitosan solutions at high pressure as stated above,<sup>21</sup> but also that a simple CO<sub>2</sub> atmosphere negatively affects the solubility of the polysaccharide into IL, with this effect decreasing with the temperature.<sup>21</sup> This is why a 6% w/w chitosan-[bmim][OAc] ratio, which should definitely fall into the solubility range at the working temperatures, was chosen.

**Scheme 1.** A. Synthesis of ureido cross-linked chitosan. B. Proposed mechanism.



A possible mechanism for the ureido linkage formation has been already postulated<sup>4</sup> and is based on a two-step sequence. (Scheme 1, B.) First a primary amine reacts with CO<sub>2</sub> giving a carbamic acid that is neutralized by a second amino group forming thus the ammonium alkyl carbamate **2** (Scheme 1, B.). The subsequent dehydration reaction can then occur in two possible ways, either through the tetrahedral intermediate **3** or the isocyanate **4**, affording the final urea derivative **5**.

Formation of **2** is fast and takes place at relatively low temperatures, while the second part of the sequence is the rate-determining step which requires high temperatures. For this reason, the reactor containing chitosan and [bmim][OAc] was charged with CO<sub>2</sub> (35 bar) at 25 °C and the temperature was slowly increased to 170 °C [at this temperature the system reached the desired pressure (60 bar) which was maintained during the course of the reaction]. The idea was to fix CO<sub>2</sub> onto chitosan while this was gradually dissolving as soon as the intramolecular hydrogen bonding were disrupted by the effect of bmimOAc and heating, and the primary amino groups were exposed. (Table 1, entries 1 and 2)

**Table 1.** Different reaction conditions used for the chitosan cross-linking reaction

<sup>a</sup>The polymer was regenerated from a solution of chitosan MW in [bmim]Cl (6% w/w, 100 °C, 4h)

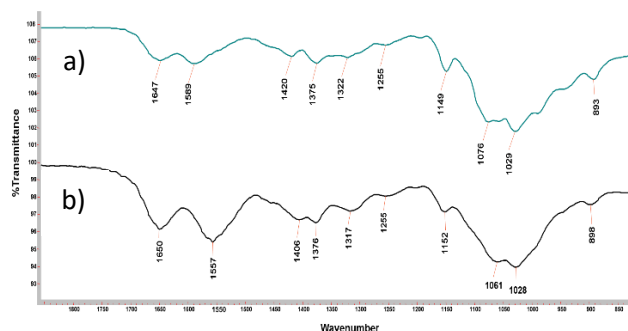
<sup>b</sup>Chitosan-[bmim][OAc] ionogel.

Entry	Chitosan	Time (h)	CO <sub>2</sub> (bar)	Temperature	Solvent
1	Chitosan 85%	4	60	25 °C → 170 °C	[bmim][OAc]
2	Chitosan MW	4	60	25 °C → 170 °C	[bmim][OAc]
3	Chitosan 85%	4	60	25 °C → 170 °C	NMP
4	Chitosan MW <sup>a</sup>	4	60	25 °C → 170 °C	[bmim][OAc]
5	Chitosan <sup>b</sup>	4	60	25 °C → 170 °C	[bmim][OAc]
6	Chitosan	4	--	25 °C → 170 °C	[bmim][OAc]

After 4h at 170 °C, the vessels were cooled to 25 °C and carefully depressurized. As expected, the brown viscous solution continued to bubble for 10 minutes, releasing the CO<sub>2</sub> either absorbed on the chitosan or physically absorbed by [bmim][OAc]. It has been reported before that imidazole based ILs (for instance [emim][OAc], [bmim]Cl, [bmim][OAc])<sup>4,22,23</sup> are able to adsorb substantial amounts of CO<sub>2</sub>. At the bottom of the two reactors, some solids were present, which were filtered off, washed the first three times with hot water, then three time with hot ethanol, and finally dried in the oven (60 °C for 12h).

The dry solids obtained accounted for around 85% of the amount of the starting polysaccharide. From the two different chitosans, similar types of solid were obtained, both not soluble in acid water, DMSO or DMF. This precluded the possibility to analyze them by liquid NMR as usually done for chitosan and chitosan derivatives. The two modified chitosans were then characterized by FTIR spectroscopic analysis which supported the successful modification on the main chain. (Figure 2 and Figure S4).

**Figure 2.** FTIR spectra of pristine chitosan MW (a), and ureido cross-linked chitosan (b)

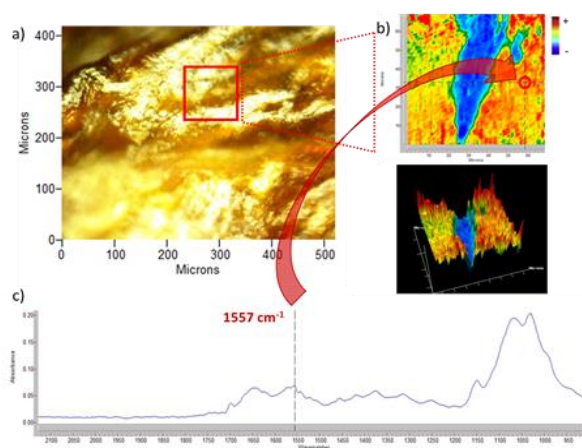


From the chitosan spectrum (curve a), distinctive absorption bands appeared at 1647 cm<sup>-1</sup> (amide I), 1589 cm<sup>-1</sup> (-NH<sub>2</sub> bending), and 1375 cm<sup>-1</sup> (amide III). The absorption bands at 1149, 1076, and 1029 cm<sup>-1</sup> are peculiar for the saccharidic portion of the polymer. The spectrum of cross-linked chitosan (curve b) showed new signals at 1650 cm<sup>-1</sup> (partially overlapping with the amide I of chitosan) and at 1557 cm<sup>-1</sup>, which were assigned to the ureido group (amide I, and amide II respectively), and the concomitant disappearance of the -NH<sub>2</sub> bending band due to the formation of the ureido bond.

The ureido group signals are fully consistent with what was obtained for an ureido-linked disaccharide prepared by reacting 1,3,4,6-tetra-*O*-acetyl-β-D-glucosamine with 1,3,4,6-tetra-*O*-acetyl-

2-isocyanyl- $\beta$ -D-glucopyranose<sup>27</sup> and with the signals of chitosan alkyl urea derivatives recently reported,<sup>28</sup> in turn synthesized as intermediates for chitosan-based enantioseparation materials.<sup>29</sup> Also, the absence of the signal at 1666  $\text{cm}^{-1}$ , and of the broad band centered around 3623  $\text{cm}^{-1}$ , consistent with a carbamate C=O stretch and an ammonium N-H stretch respectively,<sup>30,31</sup> confirmed that no ammonium carbamate type-2 was still present on the cross-linked chitosan. This means that all the ammonium carbamates were either converted further into the ureido linkage during the reaction or switched back to  $\text{CO}_2$  and primary amine at the end of the reaction. The distinctive signals of [bmim][OAc], namely the bands at 1574, 1378, and 1174  $\text{cm}^{-1}$  (Figure S5) which have been assigned to the antisymmetric elongation of the acetate anion and the twisting and rocking modes of the imidazolium ring respectively,<sup>32</sup> were also not detectable. The surface of the obtained solids was further analyzed by FTIR imaging combined with a FPA detector. The acquisition was centered on the distinctive band of the cross-linked chitosan at 1557  $\text{cm}^{-1}$ , and clearly shows the presence of the ureido linkage all over the surface (Figure 3).

**Figure 3.** a) Microscope image, b) FTIR imaging (2D and 3D) with a FPA detector at 1557  $\text{cm}^{-1}$ , and c) FTIR spectrum of ureido cross-linked chitosan



MW.

The degree of cross-linking of the obtained material (around 13%) was determined by ninhydrin (NHN) assay.<sup>32</sup>

To the best of our knowledge, no ureido cross-linked chitosan of the type here reported has been prepared thus far, while there is only one other example of a chitosan cross-linking reaction in ILs<sup>33</sup> using glutaraldehyde as cross-linking agent, a reaction that has been thoroughly studied in aqueous media.

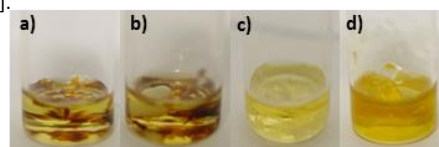
To ascertain the role of the IL, the reaction was also run in *N*-methyl-2-pyrrolidone (NMP), which is the solvent of choice for transforming primary amines into alkyl ureas by reaction with  $\text{CO}_2$  (Table 1, entry 3). After 4h, only unreacted chitosan was recovered which proves that the IL plays an essential part. In the following attempt, the regenerated version of the chitosan MW was used. (Table 1, entry 4). Its preparation involves first the dissolution of the chitosan into an IL ([bmim]Cl was used as IL) at high temperature (100 °C for 4h) followed by the regeneration step by means of an antisolvent (water or ethanol) until no IL adsorbed on the

polysaccharide is detectable. The reaction performed on the regenerated chitosan afforded the same cross-linked solid as above (Figure S8).

To analyze to what extent the ability of [bmim][OAc] to disrupt the chitosan network affects the process, the reaction has been performed on a previously solubilized chitosan: *i.e.* the polysaccharide was first dissolved in [bmim][OAc] at 100 °C in the autoclave vessel, then cooled to room temperature affording an ionogel which was finally submitted to the standard reaction conditions (Table 1, entry 5). After 4 h, the pressure was released and only a dark-brown solution was obtained with no traces of the cross-linked solid. Attempts to regenerate the chitosan with water and ethanol failed, which suggests that the degradation of the polymer occurred in the condition used. The same result was obtained when chitosan was heated at 170 °C in [bmim][OAc] without  $\text{CO}_2$ . (Table 1, entry 6)

The characteristics of the cross-linked chitosans were further investigated, and dissolution attempts of the isolated solids in [bmim][OAc] (Figure 4) were carried out (6% w/w). While the pristine chitosans, dissolved in IL by heating at 100 °C, afforded an ionogel after subsequent cooling to room temperature [Figure 4, d)], the modified chitosans remained unchanged even after longer periods and higher temperatures [Figure 4, a) and b)].

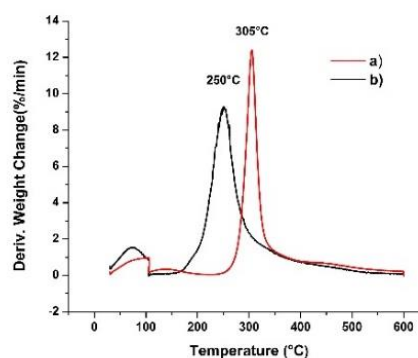
**Figure 4.** Solubility tests for the ureido cross-linked chitosan and the pristine chitosan 85%: starting solutions in [bmim][OAc] [ a) and c), respectively], and after 3h at 100 °C and subsequent cooling to 25 °C [ b) and d), respectively].



From the results obtained, it seems that [bmim][OAc] acts more as a catalyst of the reaction than a solvent for the polysaccharide. It is possible to speculate that the superficial chitosan primary amino groups react with  $\text{CO}_2$  thanks to the presence of [bmim][OAc], and that the formed superficial ureido bonds shield the solid from dissolution and ultimately degradation at high temperature.

The thermal behavior of the cross-linked chitosans were studied by thermogravimetric analysis (TGA) and compared to pristine chitosans. (Figure 5, Figure S9 and Figure S11)

**Figure 5.** Derivatives of thermal gravimetric analysis (TGA) of pristine chitosan 85% (a) and its ureido cross-linked chitosan (b)



In both cases there are two major mass loss events, the first one corresponds to the water release (up to 100 °C), while the second and main one represents the polysaccharide backbone depolymerization and pyrolytic decomposition. The two pristine chitosans display practically the same decomposition temperatures (~305 °C), while the ureido cross-linked chitosans are characterized by lower decomposition temperatures (278 °C for medium molecular weight chitosan-derived solid, and 250 °C for 85% deacetylated chitosan-derived solid)(Figure S11 and S12). This effect is well known for cross-linked chitosans<sup>34-36</sup> and derives from the partial disruption of the hydrogen bonding networks originally present in the chitosans. A similar, albeit less pronounced effect, has been observed on the chitosan sample regenerated from [bmim]Cl where the decomposition temperature decreases from 305 °C to 299 °C. (Figure S13) Overall, the ureido cross-linking reaction has a dual effect on chitosan: on the one hand, it decreases the thermal stability of the polymer, on the other hand, it increases the resistance toward its dissolution in acid water, polar organic solvents, and [bmim][OAc].

## Conclusions

In summary, the overall process utilizes a natural renewable polysaccharide available in large quantities (chitosan), a solvent with unique physicochemical properties and greener characteristics than the conventional organic solvents ([bmim][OAc]), and a chemically safe cross linking reagent (CO<sub>2</sub>), to prepare a new material based on a bio-feedstock with potential application in the biomedical field. In particular, a promising feature appears to be its stability in different conditions which is ideal for certain drug delivery systems (i.e. nasal, vaginal drug delivery).<sup>37</sup> Reaction parameter variations that could affect cross-linking, for instance the use of different, less basic ILs (based on NMR analysis, 2% of [bmim][OAc] decomposed at the end of the reaction), either as solvents or additives, and of different concentrations of chitosan, together with the study of ILs recycling and of the potential applications of the modified chitosans as drug delivery systems are in progress.

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