

Unprecedented ureidyl-chitosan derivatives: An interesting tool for anchoring aromatic moieties

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Abstract

The synthesis in water of new ureidyl chitosan derivatives and their characterization by NMR techniques both in solution and solid state is described. Aliphatic and aromatic diisocyanates have been used as crosslinking agents to obtain a chitosan network with potential physicochemical properties in gel formation as well as biomaterial for chiral sensing.

Keywords

Chitosan; Isocyanates; urea containing biopolymers.

Introduction

Chitosan is a polysaccharide of $\beta(1-4)$ linked D-glucosamine with a number of advantages such as non-toxicity, biocompatibility, and biodegradability.¹ The control of their chemical and physical properties allows Chitosan derivatives to be used for different biomedical and industrial applications. Degree of deacetylation (DD) and polymer chain length (molecular weight, M_w) can be considered the most important features to determine the physico-chemical properties of these systems and consequently decisive factors to give desired results in formulations and applications.

Chromatographic enantioseparation by high-performance liquid chromatography (HPLC) has become the most powerful method not only for analyzing enantiomers, but also for obtaining them in a pure form. Advances in this method depend on the development of chiral stationary phases (CSPs) capable of effective chiral recognition. These CSPs can be classified into two types: one consists of small chiral molecules which are usually attached to a support and the other is derived from optically active polymers which can be used as a porous gel or with silica gel.^{2,3}

We have previously reported the synthesis of a series of *N*-substituted Chitosan derivatives from functionalized aromatic aldehydes, some of them showing fluorescence, others being hydrophobic molecules and others antimicrobial.⁴ The syntheses were carried out by reductive amination of chitosan with substituted aromatic aldehydes and sodium cyanoborohydride as reducing agent.

In this communication, we have focused on the reaction of a variety of aromatic isocyanates, including diisocyanates, with the Chitosan amino groups in water to obtain new ureidyl Chitosan derivatives. Some of the Chitosan-urea derivatives prepared here are not soluble in chloroform or other organic solvents, and therefore, have been characterized by solid state ¹³C CPMAS RMN.

Experimental Methods

All chemicals were purchased and used without further purification. Evaporations were conducted under reduced pressure. TLC was performed on silica gel plates (DC-Alufolien F254, E. Merck) by using MeOH as eluent. Detection of compounds was accomplished with UV light (254 nm) and by charring with H₂SO₄ and characterization with FTIR, UV-visible and NMR spectroscopy both in solution and cross-polarized solid state under magic angle spinning.

For the synthesis of ureidyl-Chitosan derivatives were carried out from commercially available isocyanates both aromatic and aliphatic.

General procedure to obtain Ureidyl-Chitosan derivatives

To a solution of chitosan (120 mg) in aq. AcOH/MeOH 1:1 (40 mL) at final pH value of 6.2 was added the commercial isocyanate (1 equiv.). The mixture was stirred at room temperature until the reaction was completed monitoring by TLC. Then, the solution was adjusted at pH 12.0 by addition of 4 M NaOH. The precipitate obtained was filtered off and washed successively with H₂O, MeOH, CH₂Cl₂ and acetone to give the corresponding ureidyl-Chitosan derivative as a white solid in good to excellent yields.

E.g. Spectral data for compound **2**:

IR: ν_{\max} 3301, 2866, 1662, 1637, 1610, 1561, 1432, 1303, 1231, 1151, 1061, 1031, 883, 840 and 684 cm⁻¹. UV-vis (AcOH): 243 nm. ¹H NMR (700 MHz,

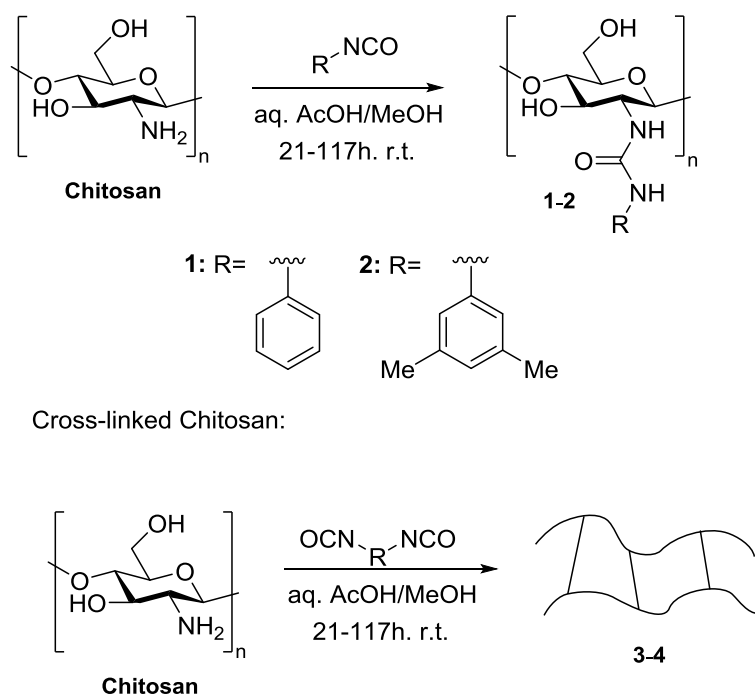
CD₃COOD/D₂O): δ = 6.97 (br, 2H), 6.79 (br, 1H), 4.94 (s, 1H), 4.67 (br, 2H), 3.95-3.40 (br., m) and 3.25 (br, 1H) ppm. ¹³C CPMAS NMR (150.9 MHz): δ = 174.4, 164.9, 158.2, 139.5, 126.4, 116.8, 104.5, 75.3 and 21.6 ppm.

Results and Discussion

Ureidyl-Chitosan (**1-4**) with donor-acceptor-donor properties were synthesized in a heterogeneous mixture from corresponding isocyanate and Chitosan (Scheme 1). Starting polymeric material has been characterized in order to obtain the degree of deacetylation (DD, 84 %) and molecular weight (M_w , 87875 gmol⁻¹). Both parameters are the essential fingerprint for polymers characterization. For DD calculation relative integrals of protons in ¹H NMR spectra were used. Moreover, M_w was determined by using GPC-SEC technique.

In all cases, acetic acid was employed to solubilize the polymer due to protonation of amino groups (pK_a ~ 6.5). The pH value used in the syntheses (pH 6.2) was chosen to ensure that amino groups preserve their nucleophilic properties without the precipitation of polymer may occur. In these conditions, the degrees of *N*-substitution (DS) were the maximum as expected by pH value.

Characterization of ureides was realized by using FTIR, ¹H NMR and ¹³C CPMAS NMR techniques. FTIR spectra for synthesized compounds show a band at 1638 cm⁻¹ (overlapped with C=O of amide moiety) for C=O stretching vibration. Bands in the range 1558-1537 cm⁻¹ are assigned to C=C of aromatic moieties, as well as, bands at 838-744 cm⁻¹ corresponding to out-of-plane C-H bends of aromatic rings introduced.



Scheme 1. Synthesis of ureidyl-Chitosan (**1-4**)

In ^1H NMR spectra for all obtained compounds the typical signals corresponding to Chitosan backbone were observed. At 4.92 ppm peak assigned to anomeric proton (H-1) is observed. Furthermore, another interesting proton (H-2) used to DS calculation (Table 1) shows a singlet at 3.23 ppm. When reaction between Chitosan and isocyanates take place new peaks for aromatic protons were observed. For instance, compound **1** shows in ^1H NMR spectrum two broad singlet at 7.35 and 7.14 ppm, one of them for *meta* and *ortho* protons and the another one for proton in *para* position, respectively. On the other hand, compound **2** presents a singlet for methyl groups at 2.23 ppm..

Table 1. Degrees of N-substitution calculated both in solution and solid state NMR

Compound	Recovery (%)	DS (^1H NMR)	DS (^{13}C CPMAS NMR)
1	94	24.1	- ^a
2	87	21.3	19.4
3	85	5.6	2.9
4	97	NS ^b	29.2

^aNon determined. ^bNon soluble

For compound **3**, DS calculated (5.6 %) was unexpected the lowest. Results of DS values in both techniques (^1H NMR and ^{13}C CPMAS NMR) can be correlated to

equation type $y = mx + b$ enabling a plausible predictions for this parameter in those derivatives where is not possible to be analysed due to solubility properties, among others.

Finally, in compounds **3** and **4** the use of diisocyanate involves an effective cross-linked process. Aromatic and aliphatic diisocyanate show the same chain length varying the constraint of system (bismethylenediisocyanate and hexamethylene diisocyanate, respectively).

Conclusions

In summary, we have successfully synthesized a variety of *N*-substituted derivatives of Chitosan, containing an urea functionality, in an easy and cost-efficient way that provide a broad range of possible applications, in particular those related with chiral recognition. A detailed study on the degree of *N*-substitution (DS) evaluation and characterization of by using solid state ^{13}C CPMAS NMR for the new poor soluble Chitosan derivatives is included.

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