Design of Aromatic Aldehyde Chitosan Derivatives for biological and Industrial Applications

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ABSTRACT

Our research focuses on the modification of chitosan, a natural-based polymer, nontoxic, biocompatible, and biodegradable. We have synthesized *N*-substituted chitosan derivatives **2-8 (a, b)** from diversely functionalized aromatic aldehydes, some of them showing fluorescence, others being hydrophobic molecules and others antimicrobial. The synthesis has been carried out by reductive amination of chitosan with substituted aromatic aldehydes and sodium cyanoborohydride as reducing agent. We have also modified the primary hydroxyl group of chitosan derivatives moiety into a carboxymethyl group for some biological applications in which water solubility is required. We have also studied the non-covalent and covalent interactions between the derivatives and chitosan through the IR, NMR, and HPLC/SEC techniques. Substitution degree of each derivative was quantified by liquid 1H NMR and/or solid ^{13}C CP-MAS NMR.

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Introduction

The chemical modification of polymers confers them new interesting properties.[1] Attaching fluorescent probes to a biopolymer for instance allows the use of the polymeric material as a biosensor or for half-life determination purposes of the material in which the fluorescent probe is incorporated. Furthermore, conferring hydrophobic properties to a dilute acid soluble polymer renders it insoluble in water and might give better structural qualities for industrial purposes.[2]

Our research focuses on the modification of chitosan, a natural-based polymer obtained by alkaline deacetylation of chitin, which is nontoxic, biocompatible, and biodegradable. Numerous papers have been published on chitosan revealing multiple applications for this biopolymer.[3] We believe that we can expand or improve the properties of chitosan by chemical modification. For instance, we can improve or modulate better the release of a drug by binding either hydrophylic or hydrophobic molecules to chitosan[1]. We can improve resistance of chitosan to acid hydrolysis, making it a good excipient. The half life of a material can be studied by binding fluorescent molecules covalently. However, before producing a chitosan derivative it is important to study the conditions in which chitosan reacts best so that we can able to know which molecule and functionality should be attached to chitosan and control the incorporation degree.

Many of the published articles on chitosan derivatives do not give precise detail about synthesis or characterization [2-3], and because of the nature of chitosan reactions and solvents used it is easy to obtain misleading results or interpretations related to actual chemical reactions. In our experience, working with other functional groups besides aldehydes, we can establish that some insoluble byproducts might be detected together with chitosan that might be taken erroneously as incorporation into chitosan molecules. We have

therefore set the goal of reaching to an unequivocal synthesis and characterization of chitosan derivatives, and control of the degree of incorporation of the derivative in the chitosan molecule.

Methods - Procedure

Six different commercial (Sigma Aldrich) or previously prepared aldehydes were employed (4,*N*-diphenylaminobenzaldehyde, 4-*N*-dimethylaminobenzaldehyde, biphenylcarboxaldehyde, *p*-nitrobenzaldehyde , p-hydroxybenzaldehyde, and pyrenecarboxaldehyde in the reaction of chitosan and aldehydes. Low molecular weight chitosan (MW 97 kDa) with degree of acetylation of 85% was purchased from Sigma-Aldrich.

To determine the exact procedure of reductive amination of the chitosan and aldehyde reaction, reduction of each aldehyde in the presence of sodium cyanoborohydride in MeoH was checked firstly. Secondly, a preliminary reaction of chitosan and 4-*N*-diphenylaminobenzaldehyde was carried out at different pH and solvents to establish the optimal reaction conditions.

From these results, our final procedure of reductive amination was to split the formation of chitosan – aldehyde derivatives into imine and amine chitosan derivative (Figure 1). The imine formation was set to 24 hours in MeOH at 0.12 M acetic acid and the reduction step was set to 24 hours and/or 96 hours in MeOH at 0.12 M acetic acid. Elaboration of the amine chitosan derivative was carried out so as to eliminate traces of $NaBH_3CN$. The degree of incorporation of the amine derivatives after 24 hours of reduction was quantified with solid ^{13}C NMR, and the reactions with 96 hours of reduction were quantified by liquid ^{13}C NMR. Liquid and solid NMR acquisition parameters were carried out as suggested by Lavertu [4] and Guinesi, [5] respectively.

Results and discussion

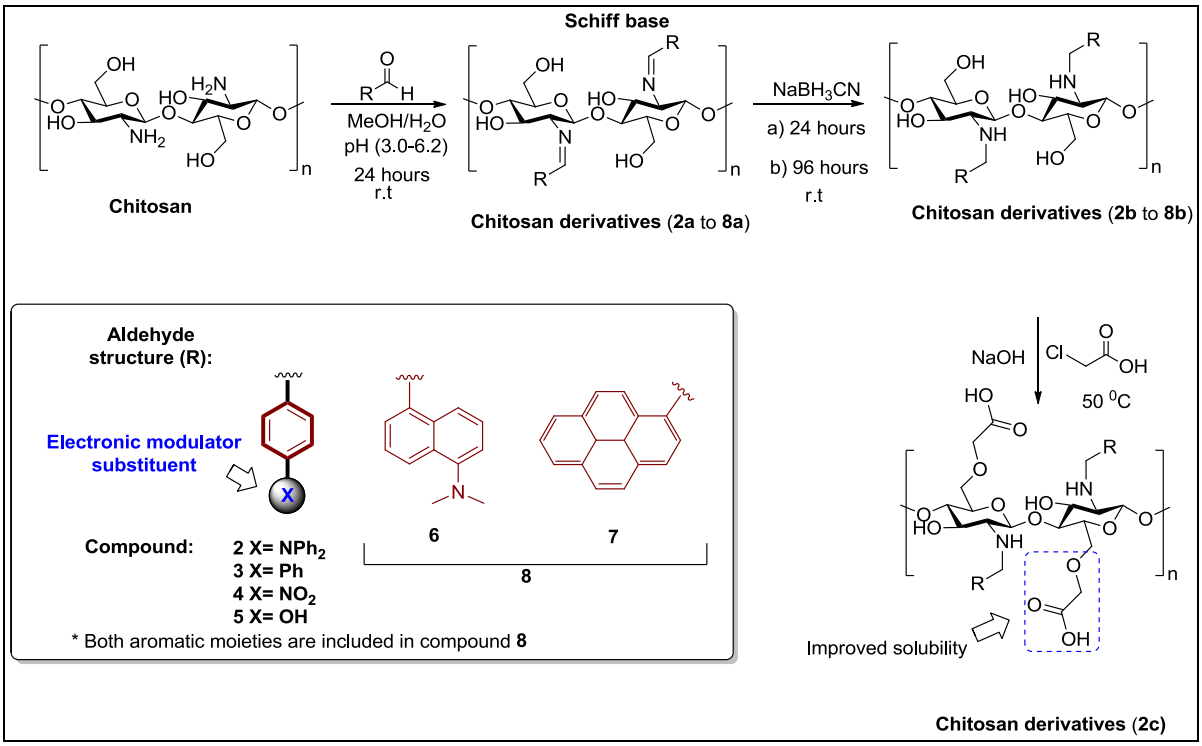

Figure 1. Synthesis of imino and amino chitosan derivatives **2-8**, including water soluble products.

To study the formation of the imino and the amino chitosan derivatives, we used the following criteria:

a) Presence of the C=N band at approximately 1643 cm^{-1} and disappearance of the aldehyde band.
b) Observation of the C-H stretch at around 1062 cm^{-1} for the amine compared to the imine derivative.
c) Presence of the imine signal at 163-168 ppm in solid ^{13}C NMR.

Table 1. Characteristic IR bands of the starting aldehyde, imine and amine chitosan derivatives.

Starting Aldehyde	ν C=O Aldehyde	ν C=N (Imine)	ν C-H (Amine)
4-Nitrobenzaldehyde	1702.84	1643.05	1062.59
4-Hydroxybenzaldehyde	1633.3	1637.27	1060.66
Biphenilcarboxaldehyde	1693.19	1638.23	1061.62
4-N,N-Diphenylaminobenzaldehyde	1685.48	1637.2	1064.51
4-N,N-Dimethylaminonaphtaldehyde	1672.95	1635.34	1063.02
Pyrenecarboxaldehyde	1677.77	1623.02	1057.76

The imines **2a-8a** were not possible to be studied in liquid ^{1}H NMR RMN since it is hydrolyzed in CD_3COOD. However, incorporation degrees for amine chitosan derivatives **2b-8b** were determined by ^{1}H NMR in some cases.

Due to the inconvenience of poor solubility of some amine derivatives in CD_3COOD and hydrolysis of the imine derivative we resorted to solid state ^{13}C CP-MAS NMR. This technique offered the advantage of avoiding the drawback of gel formation and the possibility of comparing both imine and amine chitosan derivatives. In Figure 2, it is seen the comparison of imino and amino chitosan products derived from the reaction with *p*-nitrobenzaldehyde. The curve in purple corresponds to the amine chitosan derivative and the blue is the imine chitosan derivative.

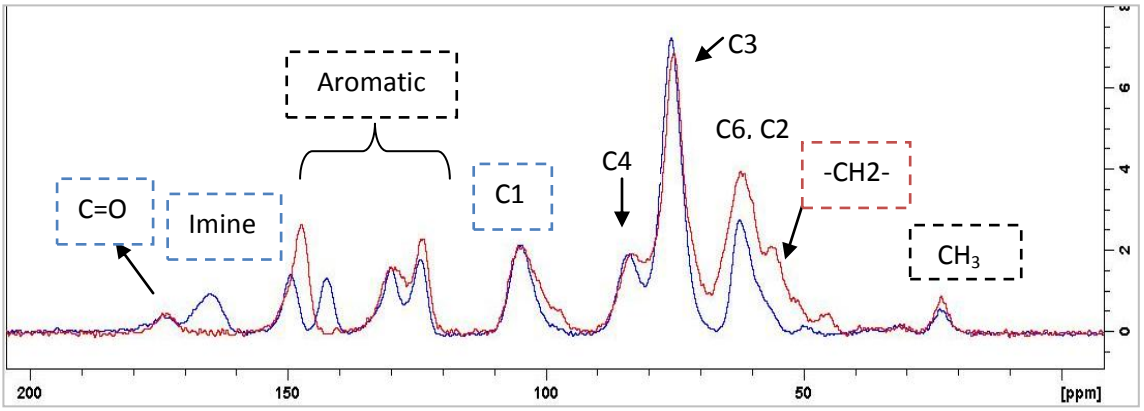

Figure 2. Comparison of imine **4a** and the corresponding amine **4b** chitosan derivatives in solid state ^{13}C CP-MAS NMR.

Average incorporation values of chitosan derivative are stated in Table 2. Deacetylation degree was calculated integrating the acetamido CH_3 signal (23 ppm) and/or C=O region (173 ppm) compared to the integral of chitosan C-1.

Table 2. Average incorporation and deacethylation degree (DD) for imine and amine chitosan derivatives[4] determined by liquid ^{1}H NMR solid state ^{13}C CP-MAS NMR.

Chitosan Derivative	*C*=N (ppm)	DD (%)	Average incorporation (%)	
			By liquid ^{1}H NMR	By solid state ^{13}C CP-MAS NMR
2a	162.81	84		11.9
2b[1]			50	
2b[2]			40	
2b[3]			35	
2b		84		14
3a	168.44	84		25.8
3b		85		34.4
4a	164.14	85		30
4b		85		33.1
5a	167.21	88		22.7
5b		85		26.6
6a	*	*		*
6b			34	
6b		85		26.6
7a	165.56	86		14
7b		86		17.7

[1] Obtained in Methanol at pH 3.0; [2] Obtained in Methanol at pH 6.0, 96 h; [3] Obtained in Ethanol at pH 4.0; [4] Obtained in Methanol at pH 6.0, 24 h; *Spectrum to be acquired.

Loooking at Table 2, we realize that in both diphenylaminobenzaldehyde and pyrenecarboxaldehyde, the steric effect could explain the lower degree of incorporation compared to the rest of aldehydes. However, when diphenylaminobenzaldehyde is reduced for 96 hours we reached much higher degree of incorporation, since in this case the reducing agent does not act on the aldehyde. Biphenyl carboxaldehyde has the highest difference in incorporation comparing the imine to the respective amine.

Finally, to determine the possibility of having a chitosan-aldehyde derivative with two or more aldehydes in the same chitosan molecule, a reaction of chitosan with three aldehydes was carried out and the incorporation degree of the derivative quantified with solid NMR (Figure 3).

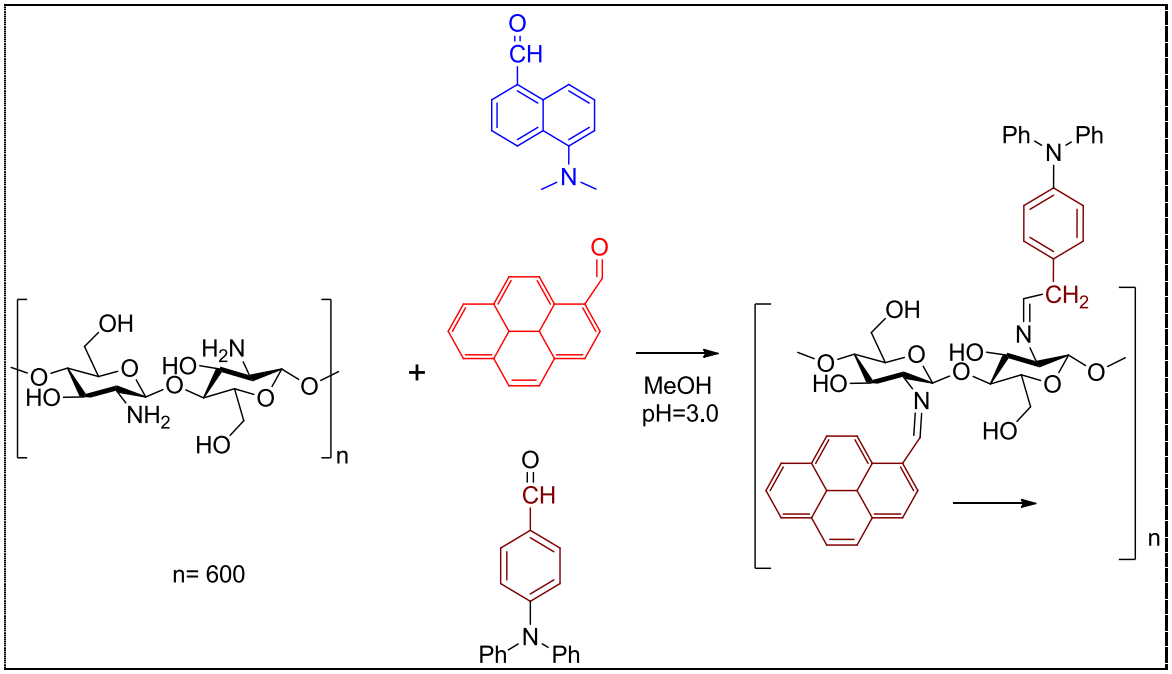

Figure 4. Synthesis of a chitosan derivative incorporating two different aldehydes.

The result of this reaction was surprising since equimolar amount of these aldehydes were added to chitosan and neither solid state NMR nor FT-IR spectrum reveal the presence of 4-*N*-dimethylaminobenzaldehyde. It can only be speculated at the moment that these aldehydes might interact with each other in a previous step before reaction with chitosan or since the formation of the imine is reversible, it could be possible that 4-*N*-dymethilaminobenzaldehyde reacts first but it is replaced by either pyrenecarboxaldehyde or 4-*N*-diphenylaminobenzaldehyde. More experiments will be carried out with different combinations of aldehydes in order to obtain the answer of this question.

Conclusions

In the reactions of chitosan and aldehydes studied, characterization of imino and amino chitosan derivatives has been analyzed by comparison of different sample preparations and NMR techniques. Incorporation degrees of these imino and amino chitosan derivatives ranged from 13 to 60 % depending on starting aldehyde and reaction time. We are presently characterizing water soluble amine chitosan derivatives.[6] The ability of incorporating more than one aldehyde in the same chitosan molecule opens the possibility of modulating more precisely chitosan applications.

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