Carbohydrate Polymers 75 (2009) 390–394

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesize and characterization of organic-soluble acylated chitosan

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ARTICLE INFO

Article history:
Received 20 March 2008
Received in revised form 28 July 2008
Accepted 31 July 2008
Available online 9 August 2008

Keywords:
Acylated chitosan
Organic-soluble chitosan
Thermogravimetric

ABSTRACT

Acylated chitosan was synthesized by reaction of chitosan and stearoyl chloride. The chemical structures and physical properties of the prepared compounds were confirmed by Fourier transform infrared (FT-IR), ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy, X-ray diffraction (XRD) and Thermogravimetric (TG) techniques. The degree of substitution (DS) was calculated by ^{1}H NMR and ranged from 1.8 to 3.8. The synthesized compounds exhibited an excellent solubility in organic solvents. XRD analysis showed that they had high crystalline structure. TG results demonstrated that thermal stability of the prepared compounds was lower than that of chitosan, the weight loss decreased with increase of DS. This procedure could be a facile method to prepare organic-soluble chitosan derivatives.

1. Introduction

Chitosan is a linear polysaccharide of β-(1,4)-2-amino-2-deoxy-D-glucopyranose (GlcN) and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) residues. Chitosan is one of the most important partially deacetylated derivatives obtained from chitin which is the second most abundant polysaccharide only next to cellulose in nature. Based on its biological properties, such as antibacterial (Ding, Eweis, Elkholyb, & Elsabeeb, 2006; Xie, Xu, Wang, & Liu, 2002), non-toxicity, biodegradability (Park, Cho, Chung, Kwon, & Jeong, 2003), haemocompatible (Zhu, Shan, Yuan, & Shen, 2003) and biocompatibility (Renbutsu et al., 2005), it is an attractive biomaterial and utilized in a number of biomedical applications, including gene delivery (Chan, Kurisawa, Chung, & Yang, 2007), tissue engineering (Kim et al., 2008), drug delivery systems (Ding, Huang, Li, & Liu, 2007; Jiang, Quan, Liao, & Wang, 2006) and wound dressings (Chen, Wang, Chen, Ho, & Sheu, 2006). Although chitosan is an attractive biomacromolecule, it is normally insoluble in aqueous solutions above pH 7 because of its rigid crystalline structure which limited its application. However, it is especially interesting in the presence of amino group which may be modified by controlled chemical reactions. In an attempt to improve the water solubility of chitosan, numerous works have been published on the chemical modification of chitosan, leading to various derivatives with improved functional properties. Recently, various kinds of water-soluble chitosan derivatives were synthesized by using heterocyclic aldehydes reaction (Fahd & Tirkistani, 1998a, 1998b), alkylation reaction (Lim & Hudson, 2004; Sashiwa, Yamamor, Ichinose, Sunamoto, & Aiba, 2003), quaternary reaction (Ignatova, Manolova, & Rashkov, 2007; Murata, Ohya, & Ouchi, 1996), carboxymethyl reaction (Sreedhar, Aparna, Sairam, & Hebalkar, 2007). There are also other chemistry modifications about chitosan (Badawy, 2008). But there is little research about the organic-soluble chitosan derivatives. Zong, etc. reported a method to prepare the acylated chitosan (Zong, Kimura, Takahashi, & Yamane, 2000), Artphop Neamnark prepared and electrospun the hexanoyl chitosan (Neamnark, Rujiravanita, & Supaphol, 2006), Keisuke Kurita synthesized organosoluble derivatives of chitin and studied its bioactivity (Kurita, 1998). But the procedure of the methods was complex, the cost was high and toxic segment was introduced during the process.

In this paper, the successful preparation of organic-soluble chitosan by simple *N*,*O*-acylation in the mixed solvent of triethylamine and acetone was reported, and its structure and properties were characterized in detail. The XRD of acylated chitosan has been carried out for characterization of crystalline structure. The solubility of acylated chitosan was tested in the series organic solvent at 25 °C. The thermal stability of the acylated chitosan was studied by TG analysis.

2. Experimental

2.1. Materials

Chitosan from crab shell was purchased from Yuhuan Ocean Biochemical Co. Ltd. (Zhejiang, China) and used as received. According to the company analysis, its molecular weight was 200 kDa and its degree of deacetylation was about 88.0%. Stearoyl

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0144-8617/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2008.07.035

chloride was bought from Geel (New Jersey, USA) and used without further purification. All other reagents were purchased from Beijing Chemical Reagent Company.

2.2. Synthesis of acylated chitosan derivates

Chitosan (4.0 g) was soaked in a mixed solvent of triethylamine (100 mL) and acetone (60 mL) for 48 h at 50 °C, then cooled and stirred for 2 h at 0 °C. Stearoyl chloride (25.0 g) which could be calculated by the mol ratio of stearoyl chloride to the reactive group (–NH_2, –OH) of chitosan dissolved in acetone (40 mL) was added dropwise into the mixed solution in 3 h, and then the temperature of the mixed solution was increased to 80 °C in 4 h and refluxed at this temperature for 3 h. The reacted solution was filtrated and poured into 400 mL of methanol. The precipitate was dissolved in acetone and precipitated by methanol for three times, then dried under vacuum at 30 °C overnight to obtain the acylated chitosan (Scheme 1). The different substitute degrees (DS) of acylated chitosan could be got by adjusting mol ratio of stearoyl chloride to the reactive group (–NH_2, –OH) of chitosan.

2.3. FT-IR spectroscopy

Fourier transform infrared (FT-IR) spectrum was recorded on Nicolet 5700 instrument (Nicolet Instrument, Thermo Company, USA). Samples were prepared as KBr pellet and were scanned against a blank KBr pellet background at wavenumber range 4000–600 cm^{-1} with resolution of 4.0 cm^{-1}.

2.4. ^{1}H NMR spectroscopy

^{1}H NMR spectra were carried out on a Bruker AV600MHz (Bruker, Germany). Chitosan was dissolved in a mixed solvent of CD_3COOD and D_2O, and acylated chitosan derivates were dissolved in CD_3Cl. Degrees of substitution (DS) was calculated from the peak area at about 0.9 ppm of –CH_3 proton against 2.03 ppm of NHAc proton.

2.5. Solubility test

The solubility of acylated chitosan derivates was evaluated in organic solvent such as acetone, pyridine, benzene and dichloromethane at the concentration of 5 mg/mL at 25 °C.

2.6. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of the sheet samples was recorded on a X-ray diffractometer (D/Max2500VB2+/Pc, Rigaku, Japan) with area detector operating at a voltage of 40 kV and a current of 50 mA using Cu Kα radiation (λ = 0.154 nm). The scanning rate was 5°/min and the scanning scope of 2θ was from 3° to 50° at room temperature.

2.7. Thermogravimetric analysis (TG, DTG)

Thermogravimetric (TG, DTG) analysis was carried out with a Netzsch TG 209 C Iris system. All analysis was performed with a 5-mg sample in an open aluminum pans under argon atmosphere, and the gas flow rate was 25 mL/min. Then the sample was heated at constant rates of 10 °C/min during the analysis and the final temperature was raised to 600 °C.

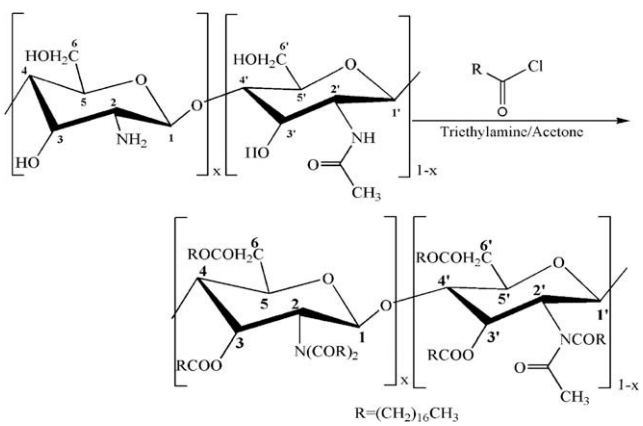
3. Results and discussion

3.1. FT-IR analysis

The FT-IR spectra of chitosan and acylated chitosan was shown in Fig. 1. The broad band at around 3443 cm^{-1} attributed to the inter- and intra-molecular hydrogen bonding of –NH_2 and –OH stretching vibration of chitosan molecules, and was absent or decreased in the acylated chitosan derivatives. New peaks at 1720 and 1747 cm^{-1} were assigned to the C═O of –NCOR group and C═O of –OCOR group which were brought by the acylated reaction between stearoyl chloride and the groups (–NH_2, –OH) of chitosan, respectively. Other prominent peaks at 2920 and 2850 cm^{-1} were assigned to the asymmetrical and symmetrical bending vibrations of methylene groups which were the introduction of long chains after acylated reaction. With the increase of stearoyl chloride content, the –NH_2 group was firstly transform into –NCOR and then the –OH group converted to –OCOR. The relative intensities of the absorbance of COO– group (1747 cm^{-1}) depended upon the degree of substitution (DS) values. The activeness of amino group was stronger than that of hydroxy group according to Fig. 1, the amino group was priority to convert to –NCOR group, compared with the hydroxy group was to convert to –OCOR group, when the DS of acylated chitosan was improved to 3.8, it suggested that all the hydroxy and amino groups on the monosaccharide structure of the chitosan could be fully acylated.

3.2. ^{1}H NMR analysis

The typical ^{1}H NMR spectrum of the chitosan in CD_3COOD/D_2O and acylated chitosan in $CDCl_3$ were shown in Figs. 2 and 3. In



Scheme 1. Synthesis of acylated chitosan.

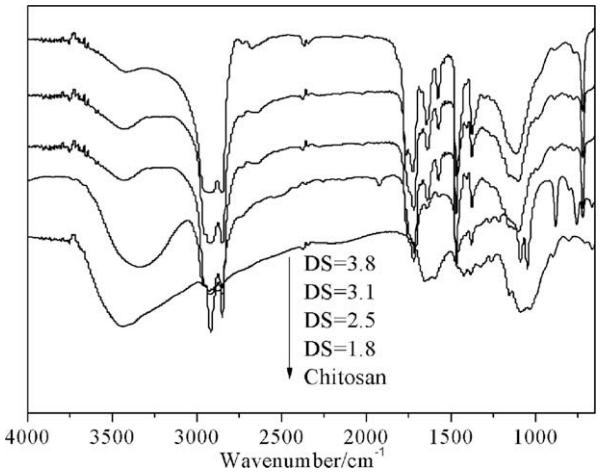


Fig. 1. FT-IR spectra of chitosan and acylated chitosan.

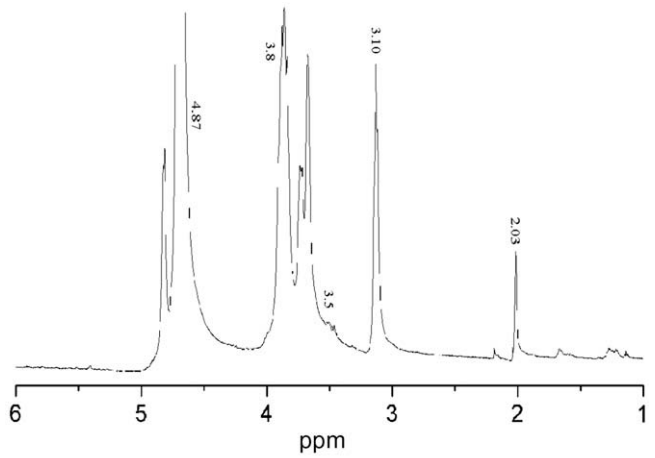


Fig. 2. Typical ^{1}H NMR spectra of chitosan.

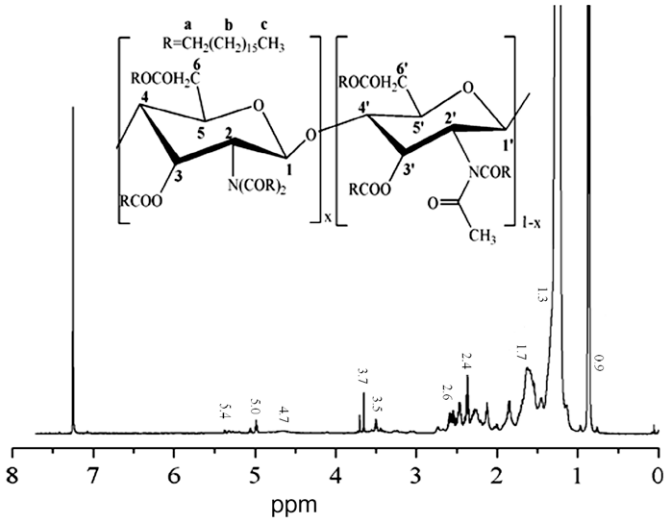


Fig. 3. Typical ^{1}H NMR spectrum of acylated chitosan.

Fig. 2, a small peaks at 2.03 ppm existed because of the presence of $-CH_3$ of *N*-alkylated GlcN residue. A singlet at 3.10 ppm assigned to H_2 of GlcN and *N*-alkylated GlcN, multiplets from 3.5 to 3.8 ppm corresponded to H_3, H_4, H_5 and H_6 of the methine protons of GlcN and *N*-alkylated GlcN. A small peak at 4.87 ppm attributed to H_1 of GlcN and *N*-alkylated GlcN. Fig. 3 showed that signals of newly formed at 5.4, 5.0, 4.7 ppm attributed to H_1, H_3 and H_4 protons of the polysaccharide, respectively. Other new signals at 3.5 and 3.7 ppm assigned to H_6 and H_5 due to the protons of ring. Compared to signals of chitosan, these shifts of acylated chitosan were due to the substitution of the N,O-acylated group to the amino group of the GlcN and *N*-alkylated GlcN residue. Furthermore, the typical multiplet peak about at 2.6 ppm attributed to the H_2 and signal at 2.40 ppm was due to Ha. The broad multiplet peaks from 1.3 to 1.7 ppm and a typical peak at 0.9 ppm attributed to the $-CH_2-$ groups and $-CH_3$, respectively, which were assigned to stearoyl chloride chains. The DS values calculated from ^{1}H NMR spectra equaled to the mol ratio of stearoyl chloride to the group ($-NH_2$, $-OH$) of chitosan.

3.3. Solubility analysis

Table 1 listed the solubility of chitosan and acylated chitosan (DS = 2.5) in organic solvents. Chitosan could not dissolve in water or any selected organic solvent, and the acylated chitosan was insoluble in aqueous acid solutions. As Table 1 showed, when the substitute degrees of acylated chitosan was 2.5, it could be soluble in halogenated hydrocarbons, ether and aromatic solvents such as tetrahydrofuran, pyridine, petroleum ether, trichloromethane, dichloromethane and cyclohexane, but poorly soluble in polar solvents like dimethylsulfoxide and *N*,*N*-dimethylformamide, could not dissolve in ethanol and *N*-methyl-2-pyrrolidone. This could be due to the introduction of long chain which is hydrophobic group.

3.4. X-ray diffraction (XRD)

Fig. 4 illustrated the XRD patterns of chitosan and acylated chitosan with different degrees of substitution. The chitosan showed three characteristic peaks around $2\theta = 10.3°$, 15.9° and 20.1°, indicating the high degree of crystallinity of chitosan as the previous reports (Samuels, 1981; Zhang & Neau, 2001). The reflection fall at $2\theta = 10.3°$ was assigned to crystal forms I and strongest reflection appeared at $2\theta = 20.1°$ corresponding to crystal forms II. The XRD patterns of acylated chitosan were significantly different from the chitosan, the acylated chitosan showed a strong reflection at about $2\theta = 21°$ and a weak reflection at around $2\theta = 6°$. With the increase of the DS, the reflection at around $2\theta = 21°$ became acute and the reflection at around $2\theta = 23°$ and $2\theta = 9°$ was disappeared. Even acylated chitosan have hindered the formation of inter- and extra-molecular hydrogen bonds, they could form the new kind of crystallinity because of the perfect arrangement after chemical modification. The crystallinity of acylated chitosan might ascribe to the presence of long chain residue. The increase number of long chains and the improving of arrangement of the long chains led to the increase of crystallinity. When the $-NH_2$ and –OH groups of chitosan were substituted completely, the acylated chitosan has only one strong reflection at $2\theta = 21°$ indicated its high crystalline structure.

3.5. Thermogravimetric (TG, DTG)

TG and DTG curves of chitosan and acylated chitosan were shown in Figs. 5 and 6. It showed that thermogram of chitosan

Table 1
Solubility of chitosan and acylated chitosan in organic solvent

Solubility					
	N,*N*-Dimethylformamide	Dichloromethane	Tetrahydrofuran	Cyclohexane	Ethanol
Chitosan	–	–	–	–	–
Acylated CS	±	+	+	+	–
	Carbon tetrachloride	Trichloromethane	Petroleum ether	*n*-Hexane	Pyridine
Chitosan	–	–	–	–	–
Acylated CS	+	+	+	+	+
	N-Methyl-2-pyrrolidone	Dimethylsulfoxide	Methylbenzene	Benzene	Acetone
Chitosan	–	–	–	–	–
Acylated CS	–	±	+	+	+

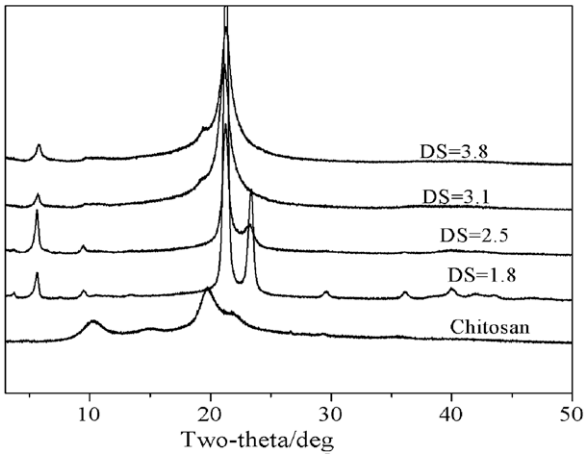


Fig. 4. XRD pattern of chitosan and acylated chitosan.

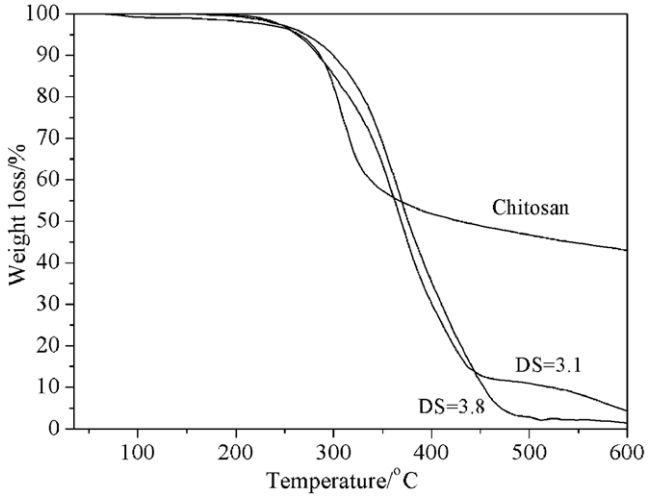


Fig. 5. TG thermograms of chitosan and acylated chitosan.

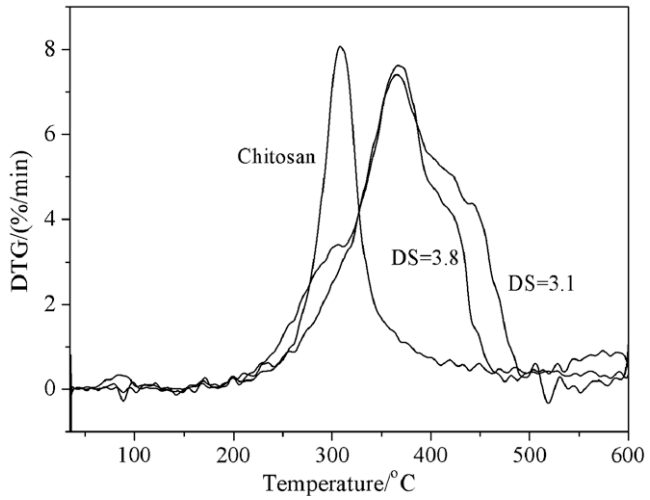


Fig. 6. DTG curves for chitosan and acylated chitosan.

had two stage of weight loss in which, first stage at about 92 °C with weight loss about 6.1% corresponded to water in materials, however, the second stage started at about 260 °C and reached a maximum with weight loss of 57% was due to the decomposition of chitosan. The chitosan had a high speed of decomposition between the temperature of 250 and 350 °C and it had the decomposition peak at 308 °C. The acylated chitosan (DS = 3.8) also showed two degradation stages. The first stage started at about 103 °C with a weight loss of 8.2% ascribed to the volatile low molecular products and water. The second degradation stage of acylated chitosans at about 247 °C and reached a maximum at 483 °C with weight loss of 97.3%. The acylated chitosan had a mild speed of decomposition during the temperature of 275 and 460 °C, and it had the decomposition peak at 365 °C. Thermal stability of acylated chitosan was lower than that of the pure chitosan, but the scope of acylated chitosan decomposition temperature was wider than that of chitosan, and the weight loss of acylated chitosan was shifted to lower temperatures than that of chitosan. Such a shift to lower temperature was attributed to a decrease in thermal stability as a consequence of increase in acylated chain of acylated chitosan. The result was similar to that of Tang, Wang, and Chen (2005). From the thermograms it was clear that the thermal stability of acylated chitosan increased with increase DS of the acylated chitosan. As the DS of acylated chitosan increased from 3.1 to 3.8, the onset of degradation of acylated chitosan showed a shift from 253 to 246 °C. This indicated that all the acylated chitosan was less stable than the chitosan due to the weakening of inter- and extra-molecular hydrogen bonding.

4. Conclusions

The acylated chitosan with different degree of substitution from 1.8 to 3.8 were prepared by reaction of chitosan and stearoyl chloride. The structure was defined by FT-IR and ^{1}H NMR. The acylated chitosan exhibited an excellent solubility in organic solvents at 25 °C such as acetone, pyridine, benzene and dichloromethane. The thermal stability of acylated chitosan was lower than that of chitosan and the thermal stability of acylated chitosan decreased with increase of DS. The acylated chitosan had different crystallinity compared to that of chitosan. This procedure could be a facile chemical modification method to prepare organic-soluble, and it could extent the application scope of chitosan.

Acknowledgments

This study was supported by Key Laboratory of Beijing City on Preparation and Processing of Novel Polymer Materials and The Program for Changjiang Scholars and Innovative Research Team in University.

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