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Full Length Research Paper

Relationships between the molecular structure and moisture-absorption and moisture-retention abilities of succinyl chitosan

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***N*-Succinyl chitosan (NSC) with various degrees of deacetylation (DD 6 – 94%) and substitution (DS 0.14 – 0.79) were successfully prepared from *N*-acetylchitosans in varying reaction time. Infrared spectroscopy (IR), ^{1}H NMR and gel-permeation chromatographic (GPC) techniques were used to characterize their molecular structures. The moisture-absorption (R_a) and -retention (R_h) abilities of NSC are closely related to the DD and DS values. Under conditions of high relative humidity, the maximum R_a and R_h were obtained at DD values of about 50%, and when the DD value deviated from 50%, R_a and R_h decreased. Under dry conditions, when the DD value was 50%, the R_h was the lowest. The NSC with DS 0.65 to 0.79 exhibited better R_h than that of HA, and has the potential to prepare HA-like substances in pharmaceutical and cosmetics industries.**

Key words: Chitosan, moisture-absortion, moisture-retention and succinic anhydride.

INTRODUCTION

Chitosan (CS) is the fully or partially deacetylated product of chitin, the second most abundant natural resource next to cellulose. It is non-toxic, biocompatible and biodegradeable (Borzacchiello et al., 2001). Recently, much attention has been paid to utilize chitosan in biomedical applications, such as a wound dressing, skin grafting template, hemodialysis membrane and drug delivery vehicle, etc (Niekraszewicz et al., 1997; Kiyozumi et al., 2006; Lin et al., 2004; Verestiuc et al., 2006). However, chitosan is a pH-sensitive polycation and it is insoluble at neutral and basic pH.

A special emphasis has been placed on the *N*-succinyl-chitosan (NSC). NSC is synthesized via introduction of succinyl groups at the N-position of the glucosamine units. Succinylation degree of NSC could be easily modified by changing reaction conditions using succinic anhydride (Yamaguchi et al., 1981). Furthermore, the molecular weight of NSC was inexpensively reduced using hydrochloric acid (Kato et al., 2002). NSC has unique characteristics, such as water soluble, low toxic (Felt et al., 1998; Illum, 1998) and fewer biodegradables in the body (Yamaguchi et al., 1981). Therefore, it has applied as colonic delivery, bioadhesive polymer, wound dressing materials (Kuroyanagi et al., 1994), cosmetic materials (Maeda and Goto, 2006), drug carrier (Kamiyama et al., 1999), artificial blood vessels and hemostatics (Kuroyanagi and Horiuchi, 1991). Currently, new wound dress-ings composed of NSC and gelatin were also developed (Tajima et al., 2000). In addition, NSC was applied for a patent as a treatment of arthritis in Japan (Nakagawa et al., 1994).

Hyaluronic acid (HA), an important functional biomaterial in cosmetics and clinical medicine, is unique for its excellent moisture-retention ability and large viscosity. However, its total amount currently available is very limited and its price is too high (Bakos et al., 1999). As the fundamental chemical structure of NSC is close to that of HA and CS is an inexpensive biopolymer, NSC appears to be more suitable than others for preparing biomaterials to substitute for HA. However, published studies on the relationship between the moisture-retention ability of NSC and its molecular structural parameters (degree of deacetylatuin and succinylation)

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Table 1. *N*-Acetylchitosan of different degrees of deacetylation (DD).

Sample	MR	DD
CS1	3.0	0.06
CS2	2.0	0.15
CS3	1.25	0.30
CS4	0.9	0.43
CS5	0.6	0.52
CS6	0.2	0.73
CS7	0.1	0.82
CS8	0	0.94

MR: mol ratio of Ac_2O anhydride to chitosan.

are scarce. In this paper, NSC of various degrees of deacetylation and substitution were prepared. The relationship between degree of deacetylation and succinylation of NSC and its moisture-absorption and retention ability were quantitatively investigated.

MATERIALS AND METHODS

Materials

Chitosan (M_W = 1,200,000, DD = 94%) was supplied by Yuhuan Ocean Biochemical Co., China. HA was purchased from Sigma Chem. Co. (M_W = 800,000) Dextran standard for GPC were purchased from Pharmacia Biochemical Co. All other chemicals were analytical grade. N-Acetylation of chitosan (Vachoud et al., 2000).

A solution of chitosan (20 g) in aqueous 10% (w/w) HOAc (400 mL) was diluted with MeOH (1600 mL), and Ac_2O was added with stirring at room temperature. The reaction mixture was stored overnight at room temperature to give a rigid gel or clear solution. The gel was stirred with 0.5 M KOH in EtOH at room temperature overnight. The product was precipitated by addition of concentrated NH_4OH solution and filtered off. Each product was then washed neutral with 75% EtOH and Et_2O, and vacuum dried to give *N*-acetyl chitosan. Table 1 lists the N-acetylation conditions along with the degree of deacetylation (DD) for the chitosan samples obtained. The degree of deacetylation was shown to vary with the Ac_2O volume.

Preparation of N-succinyl chitosan (Wang et al., 2004)

A solution of *N*-Acetyl chitosan (2 g) in aqueous 4.8% (w/w) lactic acid (40 mL) was diluted with MeOH (160 mL), and then succinic anhydride (6g) was added. After stirring for several hours at room temperature, 1 M NaOH was added to the mixture to adjust the pH to 5. The NSC salt was collected after filtration, and was dissolved in the deionized water (50 mL) quickly. The solution was neutralized (to pH = 10) by addition 1 M NaOH solution. After dialyzed against deionized water for 2 days, the product was freeze-dried finally.

Characterizations

FTIR spectra were recorded as KBr pellets on a Nicolet Thermo avatar 370 FTIR spectrometer (U.S.A). CS samples were prepared as KBr pellets (40 mg in 120 mg of KBr). The DD value was measured with the integrated intensity of the absorption at wave number of 1655 cm^{-1} (amide I band) (Brugnerotto et al., 2001). ^1H NMR spectra were recorded at 400 MHZ, with a Varian Unity NMR instrument (U.S.A), using D_2O as the solvent. DS of each sample was estimated from ^1H NMR (Hitoshi and Yoshihiro, 1999).

Gel permeation chromatography (GPC) was performed at room temperature using a Shimadzu LC-10AT instrument(Shimadzu .Co.), equipped with a Shim-pack Diol-150 column (7.9 × 250 mm), LC-10AS pump and an Alltech ELSD 2000 evaporative light scattering detector. Each sample was dissolved in 0.1 M phosphate buffer, which was the eluent. The flow rate was 1.0 mL/min. Dextran (M_W 2×10^6, 4.82×10^5, 1.1×10^5, 7×10^4, 4×10^4, 1×10^4) were used as M_W markers. The weight-average molecular weight (*Mw*) was calculated by the following equation:

$$\mathrm{Lg}\ (M_W) = -0.7840Ve + 10.0903 \qquad (1)$$

Moisture absorption and retention test

Prior to the moisture-absorption testing, the samples were dried over P_2O_5 in vacuo oven. for 24 h. The water-absorption ability was evaluated by the percentage of weight increase of dry sample (R_a):

$$R_a\ (\%) = 100 \times (W_n - W_0)/W_0 \qquad (2)$$

Where W_0 and W_n are the weights of sample before and after putting it into a saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) and in a saturated K_2CO_3 desiccator (43% relative humidity) at 20℃ for 24 h.

In the moisture-retention test, wet samples were prepared by adding 10% water to each sample. The moisture-retention ability was evaluated by the percentage of residual water of wet sample (R_h):

$$R_h\ (\%) = 100 \times (H_n/H_0) \qquad (3)$$

Where H_0 and H_n are the weights of water in sample before and after putting in the silica gel and in a saturated K_2CO_3 desiccator (RH 43%) at 20℃ for 24 h.

RESULTS AND DISCUSSION

Succiylation of chitosan

A series of succinylation reactions were conducted in this paper to determine the effect of the DD value of chitosan and reaction time on the succinylation yield. As shown in Table 2, the degree of substitution of succinyl groups had no relation with the DD value of chitosan. While the reaction time was an important factor to regulate succinylation yield of chitosan (Table 3). When reaction time increased from 8 to 24 h, the DS value increased from 0.10 to 0.73 at a DD value of 43%, and from 0.14 to 0.78 at a DD value of 82%. Whereas, further increase of reaction time slightly promote further succinylation reaction, and the DS stayed at 0.74 at a DD value of 43%, and at 0.79 at a DD value of 82%. As a result, 24 h seems to be provided the optimum reaction time to prepare high DS of product in the succinylation process. Moreover, it was practically impossible to prepare NSC with a DS value higher than 0.79 by changing the reaction time.

When the reaction time was 8 or 12 h, a small part of the resulting products were not soluble, but they did swell

Table 2. NSC of different degrees of deacetylation (DD).

Sample	DD	DS	M_w ($\times 10^5$)
NSC1	0.06	0.62	52.6
NSC2	0.15	0.66	60.8
NSC3	0.30	0.67	73.3
NSC4	0.43	0.65	88.1
NSC5	0.52	0.64	105.3
NSC6	0.73	0.67	138.5
NSC7	0.82	0.68	147.7
NSC8	0.94	0.68	190.5

DS: the area ratio of succinyl group protons and methyl proton of N-acetyl group.

Table 3. NSC of different degrees of substitution (DS).

Sample	DD	Reaction time (h)	DS	M_w ($\times 10^6$)
NSC4-1	0.43	8	0.16	-
NSC4-2	0.43	12	0.36	-
NSC4-3	0.43	16	0.47	83.4
NSC4-4	0.43	20	0.65	88.1
NSC4-5	0.43	24	0.73	91.7
NSC4-6	0.43	28	0.74	91.8
NSC7-1	0.82	8	0.14	-
NSC7-2	0.82	12	0.34	-
NSC7-3	0.82	16	0.43	143.1
NSC7-4	0.82	20	0.68	147.7
NSC7-5	0.82	24	0.78	148.8
NSC7-6	0.82	28	0.79	147.6

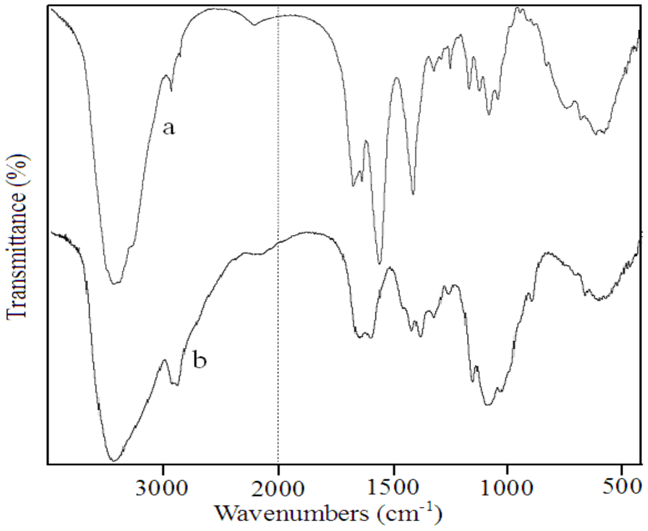

Figure 1. FTIR of NSC (a) and chitosan (b).

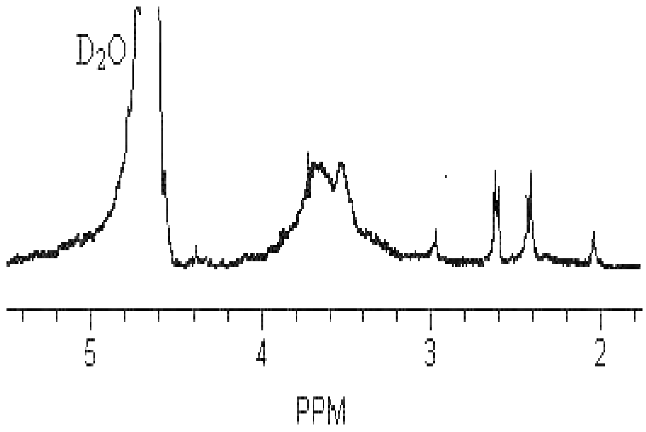

Figure 2. ^{1}H NMR of NSC.

in water. Due to succinyl group introduced into the amino group of chitosan, the water solubility has been greatly enhanced. As shown in Table 3, NSC was water-soluble at DS values above 0.47 when the DD was either 43 or 82%. Therefore, the critical DS value at which NSC becomes soluble in water may exist in the range of 0.36 to 0.43

Structural analysis of chitosan derivatives

The infrared spectra of both biopolymers, including chitosan for comparison, are shown in Figure 1. By comparing the infrared spectra of chitosan (Figure 1b), it is clear that the chitosan modified with succinic anhydride (Figure 1a) presented additional medium intensity bands, one at 1628 and the other one at 1551 cm^{-1}, which are the characteristic bands derived from carbonyl groups, that were formed during the opening of the anhydride, as it reacted with the amino group of chitosan (Lima and Airoldi, 2003).

Further evidence supporting a successful succinylation of chitosan at $-NH_2$ position is provided by the ^{1}H-NMR spectrum of NSC. The ^{1}H NMR spectrum of N-MACH are shown in Figure 2: $-NH(CO)CH_3$ 2.02 ppm, CH_2CH_2 of the succinyl group 2.41 and 2.62 ppm, H-2 of glucosamine unit 2.98 ppm, chitosan backbone hydrogen 3.4 and 4.0 ppm, H-1 of glucosamine unit 4.4 ppm. The results indicated that $-NH_2$ is the only site for formation of NSC in this reaction. Both the IR and NMR spectra of NSC prepared herein were in agreement with that of authoritative sample by conventional methods (Hitoshi and Yoshihiro, 1999; Hirano and Moriyasu, 2004; Yoshinori et al., 2000).

Moisture-absorption and retention properties

The moisture-absorption and retention ability of NSC obviously depends on the DD and DS value. As shown in

Table 4. Moisture-absorption and -retention abilities of NSC with different DD values.

		R_a/%		**R_h/%**	
Sample	**DD**	**RH81**	**RH43**	**RH43**	**silica gel**
NSC1	0.06	30.6	9.1	223.7	32.3
NSC2	0.15	32.8	9.7	230.4	32.0
NSC3	0.30	35.9	10.2	234.3	31.5
NSC4	0.43	38.3	11.4	249.8	30.8.
NSC5	0.52	45.5	13.7	279.1	30.1
NSC6	0.73	40.3	12.9	251.3	31.5
NSC7	0.82	35.0	11.3	246.8	32.1.
NSC8	0.94	31.0	9.5	231.4	32.7
HA	-	34.8	8.9	201.7	32.5

DD: Different degrees of deacetylation.

Table 5. Moisture-absorption and -retention ability of NSCwith different DS values.

			R_a/%		**R_h/%**	
Sample	**DD**	**DS**	**RH81**	**RH43**	**RH43**	**silica gel**
NSC4-1	0.43	0.10	15.9	5.8	57.4	7.9
NSC4-2	0.43	0.23	19.8	7.1	97.1	18.7
NSC4-3	0.43	0.47	27.1	8.6	156.7	24.6
NSC4-4	0.43	0.65	38.3	11.4	249.8	32.8.
NSC4-5	0.43	0.73	45.2	12.6	261.6	33.9
NSC4-6	0.43	0.74	45.3	12.8	262.4	34.1
NSC7-1	0.82	0.12	13.0	5.3	54.9	8.3
NSC7-2	0.82	0.28	15.2	6.1	90.3	19.8
NSC7-3	0.82	0.51	23.7	7.6	150.5	26.5
NSC7-4	0.82	0.68	35.0	11.3	246.8	32.1.
NSC7-5	0.82	0.78	36.4	12.0	257.8	35.7
NSC7-6	0.82	0.79	36.5	12.2	260.3	35.9
CH4	0.43	-	11.0	3.0	15.6	2.3
CH7	0.94	-	8.7	2.3	11.5	3.2

DS: Different degrees of substitution.
DD: Different degrees of deacetylation.

Table 4, when RH was 81%, the moisture-absorption ability increased until the DD value of 52%, reached the maximum at 45.5% and then decreased rapidly to 31.0% at the DD value of 94%. When RH was 43%, the maximum R_a (13.7%) and R_h (279.1%) was also obtained at a DD value of 52%, when the DD value deviated from 52%, the R_a and R_h value decreased. However, the order of R_h of NSC in silica gel was the opposite: the lowest R_h was obtained at a DD value of 52%, and when the DD deviated from 52%, R_h increased. This suggests that the intermolecular hydrogen bonds of molecular chains, which affects the interaction between water and polymer chains directly, may be a very important factor to regulate moisture-absorption and retention ability of NSC under high relative humidity conditions (RH 81 and 43%), water molecules are accessible to the surface of the sugar residues and readily develop hydrogen-bond interactions with the NSC molecular chains. As a result, they intervene between chains or chain sheets in the network [24]. When the DD value was about 50%, most molecular chains are bonded together by intermolecular hydrogen bonds to form a spacious network structure, thus, water molecules are kept inside more easily. Whereas in the case of dry conditions (in silica gel), moisture in the polymer network will diffuse to the environment. When the DD value was 30 - 82%, most of the -COOH and –

NH_2 groups were occupied by inter chain interactions between NSC molecular chains, which decreased the hydrogen bonds between such hydrophilic groups and water. Thus moisture is released more quickly. When the RH was 43%, all samples showed better R_a and R_h than HA. When RH was 81%, the R_a of NSC with DD value of 30 – 82% was better than that of HA. While in silica gel, NSC with a DD value of 0.94 demonstrated better moisture-retention ability.

As shown in Table 5, all NSC samples showed better R_a and R_h than chitosan, indicating introduction of the succinyl group is a convenient and effective method to give chitosan its moisture-retention ability. With an increase in DS, in moisture-absorption and retention ability of NSC increased accordingly. Especially with the DS value changing from 0.47 to 0.65, R_a and R_h values increased rapidly. However, further increasing of the DS value from 0.65 to 0.74, the increasing rate of the R_a and R_h value slowed down. This can be explained that with increasing carboxyl groups, on one hand, hydrogen bonds between water and COOH groups increased, while on the other hand, the intermolecular hydrogen bonds between NSC molecular chains increased at the same time, which helps to bond large amounts of water and retain them in a spacious network (Jiang, 1995). While, when the DS value was higher than 0.67, the hydrogen bonds between the molecular chains of NSC increased sharply. Thus the hydrogen bonds between –COOH and water were weak and resulted in a slow down of the increasing rate of R_a and $R_{h.}$ Attracting, NSC with the DS value from 0.65 to 0.79 showed better R_a and R_h than that of HA.

Conclusion

By the moisture-absorption and –retention test, it is considered that introduction of the succinyl group is a convenient and effective method to give chitosan its moisture-retention ability. Under conditions of high relative humidity, the maximum R_a and R_h were obtained at DD values of about 50%. Under dry conditions, when the DD value was 50%, the R_h was the lowest. The NSC with DS 0.65 to 0.79 exhibited better R_h than that of HA, and has the potential to prepare HA-like substances in pharmaceutical and cosmetics industries.

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