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Preparation and characterization of surface active *N*-butyrated low molecular weight chitosan

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ABSTRACT

New classes of natural surfactants have been prepared through the reaction of low molecular weight chitosans (1.3, 6, 10, 18 and 30 kDa) with butyric anhydride in aqueous medium. The new compounds were characterized by ^{1}H NMR and FT-IR spectroscopy. Their surface tension and particle size were also determined. The degree of substitutions was in the range of 44-57% as confirmed by ^{1}H NMR spectra. These compounds possess low aggregate size 12-18 nm with higher surface activity on comparison with native chitosan. The prepared compounds are found to be soluble in all pH media in contrast to native chitosan. This may be due to the breaking up of chitosans inter-strand hydrogen bonding upon substitutes.

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1. Introduction

Chitin (β(1→4)-linked-2-acetamido-2-deoxy-β-D-glucose (*N*-acetylglucosamine)) and chitosan (α-(1→4)-linked-2-amino-2-deoxy-β-D-glucose) (Figure 1) are natural polymers extracted from various plants and animals [1,2]. Synthesis of chitosan derivatives has recently become of great interest, with tailoring of the polymer to specific applications such as wastewater treatment [3], the food industry [4] and in medicine as wound dressings [5], gel implants [6], scaffolding materials [7] and drug delivery systems [8,9].

Chitosan exhibits a large number of amine groups which are believed to be involved in the interaction between chitosan and many other substances [10-12]. Due to the easy availability of free amino groups in chitosan, it carries a positive charge and thus in turn reacts with many negatively charged surfaces/polymers [13]. It was reported that chitosan, because of its high amino content, has been found to possess good sorptive abilities for many heavy metal ions, through complexation with the amino groups [14]. Chitosan is a weak base and insoluble in water and organic solvents, although, it is soluble in dilute aqueous acidic solution (pH < 6.5), which convert the glucosamine units into a soluble form R-NH_3^+ [15], rendering the polysaccharide a polyelectrolyte in acidic media [16]. The main parameters influencing the characteristics of chitosan are its molecular weight (MW) and degree of deacetylation (DD), representing the proportion of deacetylated units [17].

There is a remarkable interest in food science and cosmetics concerning the application of chitosan due to its property to act as natural preservative. Chitosan showed antimicrobial activity on the hypothesis that protonated amine groups of chitosan at C-2 interact with anionic constituents on the surface of microorganisms causing cell damage [18].

Chitosan by itself have weak surface activity since it has no hydrophobic segments. Chemical modifications of chitosan could improve such surface activity. This is achieved by introducing hydrophobic substituents in its glucosidic group. Several examples of chitosan derivatives with surfactant activity have been surveyed. The surface active polymers form micelles and aggregates which have enormous importance in the entrapment of water-insoluble drugs and consequently applications in the controlled drug delivery and many biomedical fields. Chitosan also interacts with several substrates by electrostatic and hydrophobic interactions with considerable biomedical applications [18].

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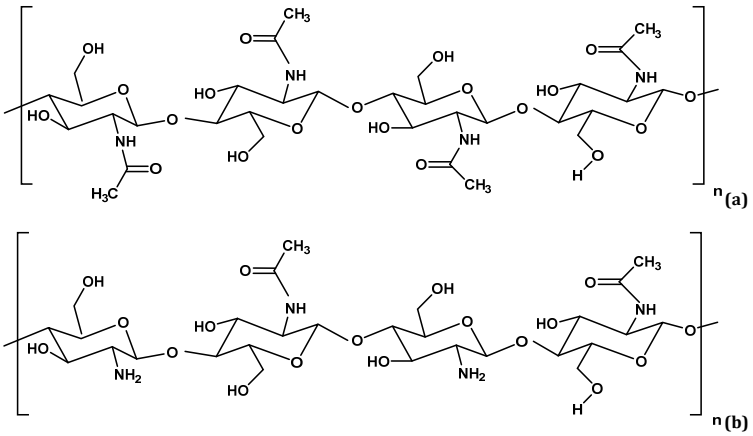

Figure 1. The structure of chitin (a) and the structure of partially deacetylated chitosan (b).

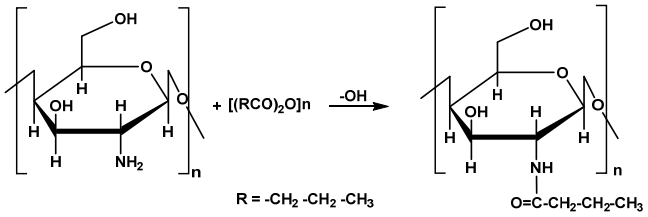

Figure 2. Synthesis of *N*-butyrated chitosan.

In this work, *N*-butyrated chitosan (NBCh) from different low molecular weights chitosan (LMWC) were prepared by using butyric anhydride, and their structures were clarified by means of ^{1}H NMR and FT-IR techniques. Their self-aggregation behaviors were examined by dynamic light scattering (DLS) and surface tension.

2. Experimental

2.1. Materials

High molecular weight chitosan (HMWC) of a viscosity average molecular weight of 250 kDa was obtained from Hongo Chemical Company Ltd., China. The degree of deacetylation (DA) is 93%. Butyric anhydride ($C_8H_{14}O_3$) obtained from Merck Schuchardt OHG Hohenbrunn, Germany. Water used to prepare the aqueous solutions was double distilled water. All reagents were of analytical grade and used without further purifications.

2.2. Methods

2.2.1. Depolymerization procedure of chitosan

The high molecular weight chitosan (10 g, 250 kDa) were dissolved in 830 mL (0.1 M) HCl, and then 170 mL of concentrated HCl (37%) were added to dissolve chitosan. The dissolved chitosan was vigorously stirred (1000 rpm) with heating under reflux for the different depolymerization times. At the end of every reaction, each mixture was allowed to cool and around two liters of 96% ethanol were added to the depolymerized chitosan to enhance the precipitation of hydrochloric salt of chitosan oligomers. The hydrolysis reaction time for 1, 2, 3.5, 6 and 24 hours gives different molecular weights (30, 18, 10, 6 and 1.3 kDa) respectively. Finally the precipitated low molecular weight chitosan was filtered and centrifuged, the solid residue was washed several times using ethanol, and dried using the freeze dryer. Low molecular weight chitosans were stored as powder in glass vials at room temperature [19].

The viscosity average molecular weight of chitosan oligomers was calculated according to Mark-Houwink equation (1) [12].

$$[\eta] = K \times M^a \quad (1)$$

where [η] is the intrinsic viscosity, M is the average molecular weight. K and a are constants which had been determined by many authors. K = 1.38×10^{-4} and a = 0.85 [20] and K = 2.14×10^{-3} and a = 0.657 [21] another values were K = 1.4×10^{-4} and a = 0.83 [22]. Another formula for liquid chitosan has been adapted [23] with K = 8.93×10^{-4} and a = 0.71.

2.2.2. Synthesis of N-butyrated chitosan

The depolymerized chitosan (0.5 g) of 1.3, 6, 10, 18 and 30 kDa were dissolved in 50 mL of distilled water. The reaction mixture was adjusted to at pH (6.5-7.0) with 0.2 M NaOH solution. Equivalent molar ratio (1:1) of butyric anhydride was added with mechanical stirrer to the chitosan solution, then, the solution was kept at room temperature overnight (25 °C). The resulted solution was dried in oven at temperature 40 °C for two days. The resulted films were crusted and kept in tightly closed vials. The synthetic *N*-butyrated chitosan was prepared as shown in Figure 2.

2.3. Characterization

LMWC and *N*-butyrated LMWC were characterized by using different techniques, such as ^{1}H NMR and FT-IR spectroscopy, particle size, surface tension, and comparison between the native chitosan and *N*-butyrated chitosan (NBCh).

Table 1. Assignments of chemical shift (δ) for chitosan and *N*-butyrated chitosan (NBCh).

Sample	δ of H-1 peak	δ of ($H_{3,4,5,6}$) peaks	δ of H-2 peak	δ of CH_2 (c) peak	δ of CH_2(b) peak	δ of CH_3 (a) peak	DS %
NBCh	5.294	3.61-4.32	3.113	-	-	-	0.000
1.3 kDa	5.150	4.16-4.63	3.432	2.510	1.948	1.242	55.05
6 kDa	5.259	4.12-4.69	3.550	2.703	2.040	1.351	44.55
10 kDa	4.908	3.99-4.68	3.132	2.214	1.896	1.185	57.69
18 kDa	5.263	4.15-4.67	3.539	2.721	2.073	1.394	50.33
30 kDa	5.238	4.09-4.21	3.525	2.679	2.011	1.326	49.83

2.3.1. Viscosity measurement

Viscosity measurements were performed using a Sine-wave Vibro SV-10/SV-100 viscometer (KSV Instruments, Helsinki, Finland).

2.3.2. ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded on a Bruker AV 300 spectrometer. About 5 g/L of chitosan/*N*-butyrated chitosan was dissolved in 5 mm diameter tubes with deutrated water (D_2O) at temperature 70 °C. The peaks for the completely deacetylated chitosan and *N*-acylated chitosan and the degree of substitution (DS) was calculated from the area peaks.

2.3.3. FT-IR spectroscopy

Infrared spectroscopy can be used to investigate the composition of chitosan sample due to its simplicity, relative instrument availability and independence of sample solubility. IR spectroscopy is the one of the most studied methods for characterization of chitosan and *N*-butyrated chitosan. Infrared spectroscopy offers the possibility to measure different types of interatomic bond vibrations at different frequencies. The FT-IR spectra were measured in KBr pellets in the transmission mode in the range 4000-400 cm^{-1}.

2.3.4. Surface tension measurements

Surface tension measurements were carried out using a Fisher Surface Tensiomat, which employs the De Nouy method. Before each measurement, the platinum ring was thoroughly cleaned and rinsed three times with double distilled water, then with absolute ethanol and burned on benzene flame for five minutes. The measurements were carried out at 25±3 °C and the accuracy of the measurements is also controlled by the surface tension measurements of water before each measurement. After equilibrium, the surface tension of *N*-butyrated solutions of different concentrations (1.3 and 10 kDa) was determined.

2.3.5. Particle size measurements

The particle size of *N*-butyrated chitosan of different concentrations was measured using the Dynamic Light Scattering (DLS) method using a Malvern Zetasizer (Malvern, UK). Samples of different molecular weight chitosan (native and butyrated) (1.3, 6, 10 and 15 kDa) at different concentrations were prepared. Each sample was filtered through 0.45 μm and 0.2 μm syringe filters and each measurement was repeated eight times.

3. Results and discussion

The poor solubility of chitosan in water is mainly due to its high crystallinity and strong inter- or intra-molecular hydrogen bonding. Therefore, introduction of appropriate substituents into chitosan backbone is likely to disrupt the inter- or intra-molecular hydrogen bonding and weaken its crystallinity, this favoring solvating of chitosan in water. However, an excessive hydrophobic substitution would generate water-insoluble derivatives due to strong hydrophobic interaction following a "hydrophobic self-assembling" model [16].

3.1. Viscosity-average molecular weight determination

The average molecular weight of prepared chitosan was determined using intrinsic viscosity measurement, and then applying equation (1) as mentioned in experimental part. We applied our published procedure [24]. Five types of low molecular weight chitosan were obtained, namely 1.34, 5.92, 9.85, 17.6, 29.4 kDa.

3.2. ^{1}H NMR spectra of chitosan and N-butyrated chitosan

In this work, *N*-butyrated chitosan (NBCh) is prepared from different molecular weight chitosans (1.3, 6, 10, 18 and 30 kDa). We used a prefixes 1.3, 6, 10, 18, and 30 in NBCh to refer molecular weight of *N*-butyrated chitosan. By using butyric anhydride, the monomolar ratio 1:1 (butyric anhydride/NH_2 on repeating unit of chitosan). The ^{1}H NMR spectra (Figures 3-5) of low molecular weight chitosan (LMWC) polymers (1.3, 6, 10, 18 and 30 kDa) were dissolved in D_2O at 70 °C with peak assignments are shown in Table 1. The degree of substitution can be estimated according to the Equation (1) [25].

$$\%DS = \left[1 - \frac{I_{H\text{-}1}}{I_{H\text{-}1} + \frac{I_{CH_3}}{3}}\right] \times 100\% \quad (1)$$

where $I_{H\text{-}1}$ and I_{CH3} are integrals of the signals corresponding to H-1 of chitosan and CH_3 in butyl group, respectively.

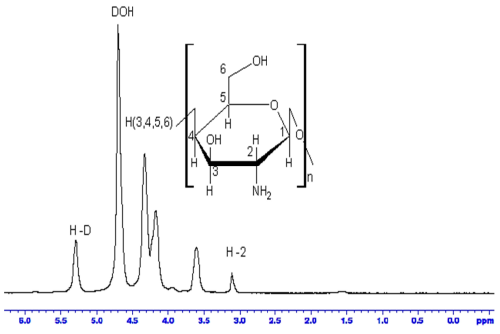

Figure 3. ^{1}H NMR spectra of chitosan (NBCh) structure in D_2O at 70 °C.

3.3. FT-IR spectra of chitosan and N-butyrated chitosan

Analysis of FT-IR spectra allows insight into what type of functional group are present in the sample. The specificity of chitosan based polymeric surfactants (CBPSs) consists of possessing both hydrophilic (-NH_2 and -OH) and hydrophobic groups (substituted butyl chains) on the chitosan macro-molecule, which allows efficient substitution of the product from reactions under homogenous conditions leading to random substitution along the main chain of chitosan.

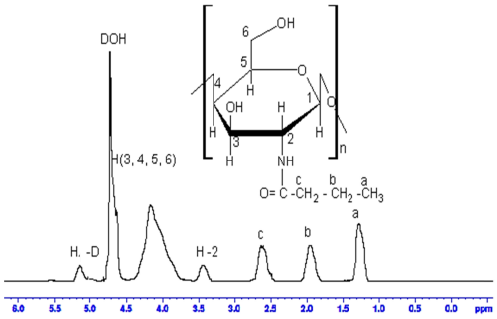

Figure 4. ^{1}H NMR spectra of 1.3 kDa NBCh in D_2O at 70 °C.

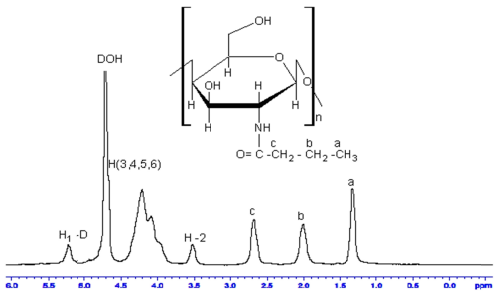

Figure 5. ^{1}H NMR spectra of 30 kDa NBCh, in D_2O at 70 °C.

Chitosan exhibits main characteristic peaks of amine group (-NH_2) at 1622 and 1514 cm^{-1}; these bands originate from asymmetrical and symmetrical NH_3^+ bending vibrations. The broad band observed at 3400-3500 cm^{-1} might be attributed to stretching vibration of the H-bonded N-H and O-H groups. The peaks at 1000-1200 cm^{-1} are attributed to C-O stretching of the saccharide structure of chitosan.

Comparing the spectra of chitosan and 1.3 kDa NBCh, it can be seen a significant shift of amine peak from 1622 cm^{-1} of IR spectrum of chitosan to a higher value, 1649 cm^{-1}, due to amide carbonyl group stretching frequency. The presence of *N*-butyl ester groups is confirmed by the absence of any peaks in the range 1710-1760 cm^{-1}. This supports the fact that the reaction occurs at -NH_2 groups rather than the -OH groups, which leading to the *N*-butyrated chitosan only.

3.4. The surface tension measurements

When comparing the surface tension of 1.3 and 10 kDa samples, it was found that the lowest molecular weight chitosan (1.3 kDa) had a lower surface tension, which might be due to higher solubility in aqueous medium. When the measured surface tension is plotted against the concentration, the critical micelle concentration (CMC) is determined, as shown in Figures 6 and 7 for chitosan 1.3 and 10 kDa, respectively. The results showed that the critical micelle concentration of the lower molecular weight chitosan (1.3 kDa = 0.67 g/10 mL) was greater than that of the higher molecular weight chitosan (10 kDa = 0.32 g/100 mL).

3.5. The particle size measurements

The particle size of chitosan and *N*-butyrated chitosan at different concentrations were used to determine the critical micelle concentration (CMC). The CMC was estimated from different concentrations of *N*-butyrated chitosans (1.3 kDa NBCh and 10 kDa NBCh). Their critical micelle concentrations were estimated to be 0.67 g/100 mL and 0.32 g/100 mL, respectively, which is similar to their critical micelle concentration which calculated by surface tension versus concentrations. These results can be clearly seen in the following Figures 8-10.

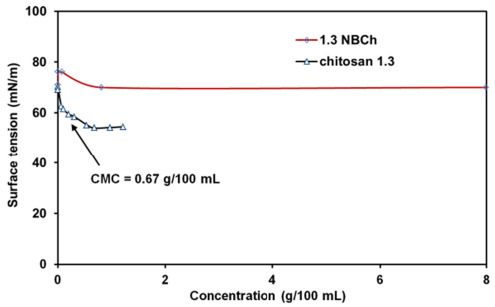

Figure 6. Surface tension *vs* concentration for chitosan and 1.3 kDa NBCh at 25 °C.

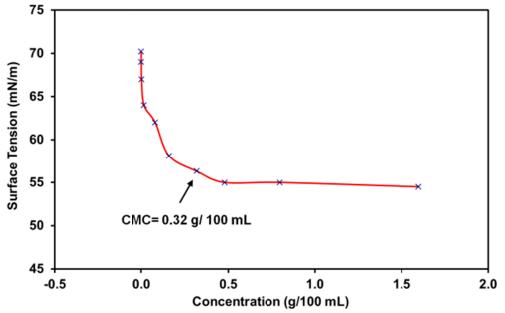

Figure 7. Surface tension *vs* concentration for 10 kDa NBCh at 25 °C.

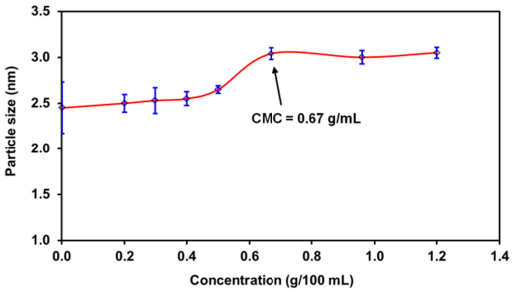

Figure 8. Particle size *vs* concentration for *N*-butyrated chitosan 1.3 kDa NBCh.

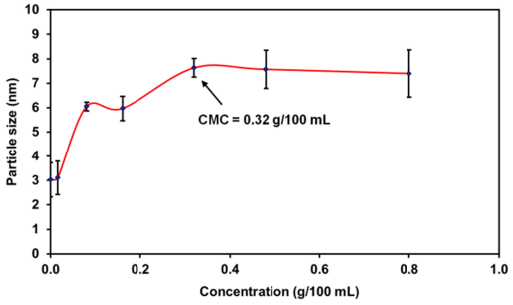

Figure 9. Particle size *vs* concentration for *N*-butyrated chitosan 10 kDa NBCh.

The particle size of different molecular weight chitosan (1.3, 6, 10, 18, 30 kDa), when compared with their *N*-butyrated chitosan (1.3/B, 6/B, 10/B, 18/B, 30 kDA NBCh), it was observed that the particle size of *N*-butyrated is lower than the native (Tables 2-6).

Table 2. Particle size of 1.3 kDa chitosan and 1.3 kDa NBCh at different concentrations.

Chitosan concentration (g/100 mL)	Average particle size Dp (n) [nm]	Relative std. dev.
1.3 (0.5)	54.00	9.20
1.3 (0.5)/B	2.65	0.43
1.3 (0.4)	33.70	10.20
1.3 (0.4)/B	2.72	0.35
1.3 (0.3)	62.80	5.40
1.3 (0.3)/B	2.43	0.31
1.3 (0.2)	67.00	5.43
1.3 (0.2)/B	2.59	0.10

Table 3. Particle size of 6 kDa chitosan and 6 kDa NBCh at different concentrations.

Chitosan concentration (g/100 mL)	Average particle size Dp (n) [nm]	Std. dev.
6 (0.5)	59.40	7.27
6 (0.5)/B	4.63	0.46
6 (0.4)	20.20	3.30
6 (0.4)/B	3.60	0.23
6 (0.3)	76.60	6.40
6 (0.3)/B	2.98	0.35
6 (0.2)	19.70	2.05
6 (0.2)/B	2.77	0.40

Table 4. Particle size of 10 kDa and 10 kDa NBCh at different concentrations.

Chitosan concentration (g/100 mL)	Average particle size Dp (n) [nm]	Relative std. dev.
10 (0.5)	73.50	11.00
10 (0.5)/B	7.44	0.53
10 (0.4)	70.20	2.77
10 (0.4)/B	4.10	0.60
10 (0.3)	109.00	3.09
10 (0.3)/B	4.44	0.27
10 (0.2)	119.00	7.12
10 (0.2)/B	4.06	0.33

Table 5. Particle size of 18 kDa and 18 KDa NBCh at different concentrations.

Chitosan concentration (g/100 mL)	Average particle size Dp (n) [nm]	Relative std. dev.
18 (0.5)	80.90	5.87
18 (0.5)/B	7.90	0.70
18 (0.4)	52.40	5.20
18 (0.4)/B	4.82	0.52
18 (0.3)	113.00	9.50
18 (0.3)/B	4.45	0.44
18 (0.2)	129.00	11.20
18 (0.2)/B	4.66	0.67

Table 6. Particle size of 30 kDa and 30 kDa NBCh at different concentrations.

Chitosan concentration (g/100 mL)	Average particle size Dp (n) [nm]	Relative std. dev.
30 (0.5)	73.10	12.70
30 (0.5)/B	8.37	0.78
30 (0.4)	52.40	5.20
30 (0.4)/B	5.25	0.64
30 (0.3)	121.00	8.84
30 (0.3)/B	4.85	0.53
30 (0.2)	128.00	12.90
30 (0.2)/B	6.15	0.56

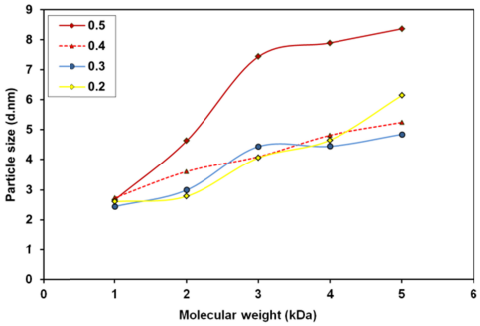

Figure 10. Particle size of different molecular weight of *N*-butyrated chitosan at different concentrations (0.2-0.5 g/100 mL).

This due to the chitosan in solutions exists in the form of quasi-globular conformation stabilized by extensive intra- and inter-molecular hydrogen bonding. The hydrogen bonding caused chitosan to aggregate into large supramolecular systems. Therefore, the introduction of *N*-butyl in chitosan backbone is likely to disrupt the inter- or intra-molecular hydrogen bonding of chitosan and weaken its crystallinity as well, and thus the *N*-butyrated chitosan tends to aggregate in small molecules systems. The values of particle size are listed in Tables 2-6.

4. Conclusion

New natural surfactants have been prepared through the reaction of different molecular weight chitosans (1.3, 6, 10, 18 and 30 kDa) with butyric anhydride in aqueous medium. The new compounds were characterized by ^{1}H NMR and FT-IR spectroscopy. Their surface tension and particle size were also determined. The degree of substitutions (DS) were in the range 44-57% as confirmed by ^{1}H NMR spectra. These compounds are highly water soluble in contrast to native

chitosan, this is may be due to breaking up of chitosans inter-strand hydrogen bonding. The new surfactants have a reduced particle size.

Acknowledgements

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