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Preparation of *N*-alkyl-*O*-sulfate chitosan derivatives and micellar solubilization of taxol

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Abstract

A series of novel chitosan derivative carrying long chain alkyl groups ($n = 8, 10, 12$) as hydrophobic moieties and sulfated groups as hydrophilic moieties were synthesized. The chemical structure of chitosan derivatives were characterized by elemental analysis, FTIR, ^{1}H NMR and ^{13}C NMR. Some physical properties of chitosan derivative were measured by TG and WAXD. Polymeric micelles of size around 100–400 nm were formed in water from the derivative. Taxol, a water insoluble anticancer drug, was solubilized into the polymeric micelle by physical entrapment. The result shows that the taxol concentration in the *N*-octyl-*O*-sulfate chitosan (OCS1) micellar solution was 2.01 mg/ml, which was much higher than that in water (<0.001 mg/ml). So *N*-octyl-*O*-sulfate chitosan (OCS1) micelle may be useful as a carrier for taxol.

Keywords: Chitosan derivatives; Polymeric micelle; Characterization; Solubilization; Taxol

1. Introduction

In recent years, polymeric micelles have been utilized as a potential drug carrier for poorly water soluble drugs because the micelles primarily solubilize these drugs in their inner core and generally offer some attractive characteristics, such as a small size (<100 nm) (Bader, Ringsdorf, & Schmidt, 1984; Kataoka, Kwon, Yokoyama, Okano, & Sakurai, 1993; Yokoyama, 1998). They were also considerably more stable than surfactant micelles (Kwon & Kataoka, 1995). In addition, they were reported to prolong circulation times in vivo and to accumulate in tumor tissues due to both their small size and the properties of hydrophilic shell (Firestone, 1994).

Polymeric micelles have a hydrophilic shell and a hydrophobic core. Pharmaceutical research on polymeric micelles has been mainly focused on copolymers having an A–B diblock structure. Numerous studies reported that the core which generally consists of a biodegradable polymer such as poly(β-benzyl-l-aspartate) (PBLA) (La, Okano, & Kataoka, 1996), poly(dl-lactic acid) (PDLLA) (Connor, Norley, & Huang, 1986; Emoto, Nagasaki, & Kataoka, 1999) or poly(ε-caprolactone) (PCL) (Shin, Kim, Lee, Cho, & Sung, 1998), serves as a reservoir for an insoluble drug, and thereby protects the drug from the aqueous environment. In contrast, the shell normally consists of water-soluble biocompatible polymer, such as PEG (Yokoyama et al., 1990; Zhu, Lin, & Yang, 1990) and PEO (Batrakova, Han, Alakhov, Miller, & Kabanov, 1998). It has been reported that polymeric micelles could also be formed by modification of natural polymer (Yoshioka, Nonaka, Fukuda, & Kazama, 1995).

Chitosan is a non-toxic, biocompatible and biodegradable polymer that has been prepared from *N*-deacetylation of chitin. Chitosan consists of 2-amino-2-deoxy-(1-4β)-D-glucopyranose residues (D-glucosamine units) and little or no *N*-acetyl-D-glucosamine units. Chitosan is soluble in acqueous solutions of various acids, but chitosan molecules have no amphiphilicity and cannot form micelles in water.

In the report a series of chitosan derivatives were prepared. The aim was primarily to modify chitosan molecules by attaching long chain alkyl groups ($n = 8$, 10, 12) to amino groups providing the hydrophobic moieties as the core and sulfate groups to hydroxyl groups providing

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the hydrophilic moieties as the shell. The chemical structures and physical properties of the modified chitosan were characterized by elemental analysis, FTIR, ^{1}H NMR, ^{13}C NMR, TG and X-ray diffraction. The possibility of increasing the solubility of sparingly soluble drugs in the polymeric micelles of the modified chitosan was examined using taxol as a model.

2. Experiments

2.1. Materials

Two chitosan samples were provided by the Nantong Suanglin Biochemical Co. Ltd (China) with deacetylation degrees of 97 % and viscosity average molecular weight of 25000 and 65000 D. Taxol was supplied by Taihua Natural Plant Pharmaceutical CO. Ltd (China). All commercially available solvents and reagents were used without further purification.

2.2. Synthesis of N-alkyl-O-sulfate chitosan derivatives

Chitosan (65000 D) (1.0 g) was suspended in 50 ml methanol with stirring at room temperature, then octaldehyde (1.02 g) was added. After 24 h, KBH_4 (0.5 g) dissolved in 5 ml water was slowly added to the solution. After a further 24 h continuous stirring, the reaction solution was neutralized with 2N hydrochloric acid and the product was precipitated with methanol. The precipitate was filtered and repeatedly washed with methanol and water. The product, *N*-octyl-chitosan (OC1) was dried under vacuum at 60 °C overnight.

OC2 was prepared with chitosan (25000 D) as the starting material using the same method as above.

Different *N*-alkyl substituents DC1 (chitosan 65000 D), DC2 (chitosan 25000 D), LC1 (chitosan 65000 D) and LC2 (chitosan 25000 D) were synthesized using decanal and lauryl aldehyde, respectively.

OC1 (1.05 g) was suspended in DMF (40 ml), and magnetically stirred overnight. Chlorsul hanic acid (20 ml) was added dropwise into DMF (40 ml) with stirring at 0 °C under N_2 atmosphere. After completely dripped, the solution was kept in agitation for 1 h, and then the suspension of OC1 and DMF was added to the above solution. The mixture was reacted at 10 °C under N_2 atmosphere for 24 h. The reaction solution was neutralized with 20% NaOH to pH 7, and the filtered solution was dialyzed (MWCO 10000) against distilled water, then lyophilized and the OCS1 powder was obtained.

Different chitosan derivatives, OCS2, DCS1, DCS2; LCS1, LCS2 were obtained using the same method.

2.3. Characterization of chitosan derivatives

^{1}H NMR and ^{13}C NMR spectra were performed on a Bruker (AVACE) AV-500 spectrometer, chitosan was dissolved in the mixed solvent D_2O and F_3CCOOD. The chitosan derivative was dissolved in D_2O.

Elemental analysis was determined using an Element Vario EL III analyzer.

IR spectra were recorded on Fourier-transform infrared spectrometer (Nicolet 2000) in KBr discs.

X-ray diffraction spectrometry was obtained using an XD-3A powder diffraction meter with Cu Kα radiation in the range 5–40°(2θ) at 40 kV and 30 mA.

Thermogrammetry (TG) analysis was obtained with NETZSCH TG 209 equipment. The temperature range was 30–550 °C and the heating rate is 20 °C/min.

2.4. Preparation of taxol-loaded chitosan derivative micelles

Taxol loaded chitosan derivative micelles were prepared by dialysis (Kwon, Naito, Yokoyama, Okano, Sakurai, & Kataoka, 1995). Chitosan derivative 40 mg was dissolved in 7 ml water with stirring for 30 min at 50 °C. 15.6 mg Taxol was dissolved in 1 ml ethanol, then the taxol solution and the polymer micellar solution were mixed and sonicated for 30 min at room temperature (JY 92-II ultrasonic processor, China), followed by dialysis against distilled water overnight at room temperature using a dialysis membrane with 100,000 cut-off was used. The micellar solution was centrifuged at 3000 rpm for 5 min, and filtered with a 0.22 μm pore-sized microfiltration membrane.

Diameter and polydispersity (V) of chitosan derivatives micelle were measured by a Zetasizer 3000 HS instrument (Malvern Instruments, Malvern, UK) with 633 nm He–Ne lasers at 25 °C.

2.5. Measurement of taxol concentration in chitosan derivative micellar solution

The concentration of taxol in the micellar solution was determined by high-performance liquid chromatography (LC-6A, Shimadzu, Japan). The mobile phase was a mixture of methanol, acetonitrile and water (30:40:32 v/v).The column was a Diamohsil™ C18 (250 × 4.6 mm) with 5 μm particles. The flow rate was 1.0 ml/min, the detection wavelength was 227 nm (SPD-10A, UV detector, Shimadzu, Japan), and the column temperature was 30°C. Injected volume of the sample was 20 μl.

3. Results and discussion

3.1. Synthesis and characterization of chitosan derivatives

The substitution degree of the chitosan derivative was calculated from the elemental analysis data (Senso, Franco, Oliveros, & Minguillon, 2000): Anal. Calcd for $[C_6H_8NO_4(SO_3H)_{1.6}(H)_{0.815}(C_8H_{17})_{0.5}(C_2H_3O)_{0.085}\cdot(H_2O)_{1.7}]_n$: C, 31.88; N, 3.54; H, 5.77. Found: C, 32.18; N, 3.69; H, 5.99.

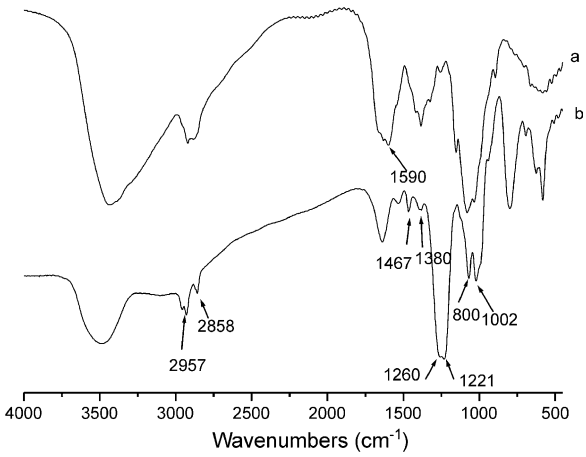

Fig. 1. IR spectra of (a) chitosan and (b) OCS1.

Structure changes of chitosan and its derivative were confirmed by FTIR spectra (Fig. 1). The IR spectra of chitosan derivative shows new peaks at 2957, 2858, 1467, 1380, 800 cm^{-1} attributed to long alkyl chain and 1260, 1221 and 1002 cm^{-1} assigned to the O=S=O group. The signal from the $-NH_2$ group at 1590 cm^{-1} disappeared. These results suggest that sulfate group and long alkyl group were part of the OCS1 structure. The amino group of chitosan was substituted.

Compared with chitosan, the ^1H -NMR spectra of the OCS1 (Fig. 2) was shown that the signals at 3.0–3.2 (m, 2H) were assigned to the methene hydrogen ($-NH-CH_2-(CH_2)_6-CH_3$) of the *N*-alkyl group. The signals of another six methene hydrogen ($-NH-CH_2-(CH_2)_6-CH_3$) belong to 1.0 ~ 2.2 (m, 6H). The triple peaks at 0.81 ppm were attributed to the methyl hydrogen ($-NH-CH_2-(CH_2)_6-CH_3$). The results evidenced that the modified chitosan contained *N*-octyl groups.

Fig. 3 shows the ^{13}C NMR spectra of the OCS1 in D_2O as compared with the starting material chitosan in F_3CCOOD/D_2O. The signals of the latter at 55.6(C_2), 60(C_6), 70(C_3), 75(C_5), 76.5(C_4) and 97.5(C_1) (ppm) were detected (Dung, Milas, Rinaudo, & Desbriers, 1994; Hiral, Odani, & Nakajima, 1991). To OCS1, the peaks at 13.5, 13.7 were assigned to the methyl carbon ($-NH-CH_2-(CH_2)_6-CH_3$) of the *N*-alkyl group. The signals of the six methene carbon ($-NH-CH_2-(CH_2)_6-CH_3$) and another methene carbon ($-NH-CH_2-(CH_2)_6-CH_3$) of the *N*-alkyl group were shown at 21.9–31.4 and 48.3 (ppm), respectively. The signals shown at 55 (C_2), 61.4 (C_6), 66.8 (C_3), 75.4 (C_5), 76.4 (C_4) and 97 (C_1) (ppm) were attributed to the polysaccharide structures. The results indicated that OCS1 contained *N*-octyl group again.

These evidence of elemental analysis, FTIR, ^1H-NMR and ^{13}C-NMR obviously support the amino group of chitosan was converted to *N*-octyl and the hydroxyl group was sulfated.

3.2. Physical properties of chitosan derivatives

X-ray diffraction diagrams of chitosan and its derivative OCS1 are show in Fig. 4. Chitosan has three reflection fall at $2\theta = 11°$, $2\theta = 20°$, $2\theta = 22°$. Samuels reported that the reflection fall at $2\theta = 11°$ was assigned to crystal forms I. The strongest reflection appears at $2\theta = 20°$ which correspond to crystal forms II (Samuels, 1981). OCS1 shows only one broad peak at around $2\theta = 20°$. It suggested that the ability of forming hydrogen bond was decreased after chemical modification. The crystalline structure of modified chitosan appears amorphous.

Thermographs of chitosan and OCS1 are shown in Fig. 5. Chitosan shows slow weight loss starting from

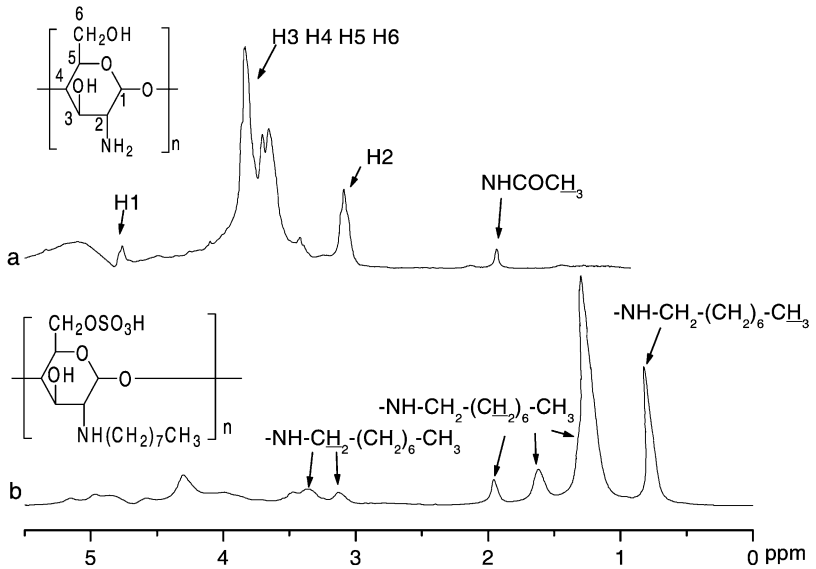

Fig. 2. ^1H NMR spectra of (a) chitosan and (b) OCS1.

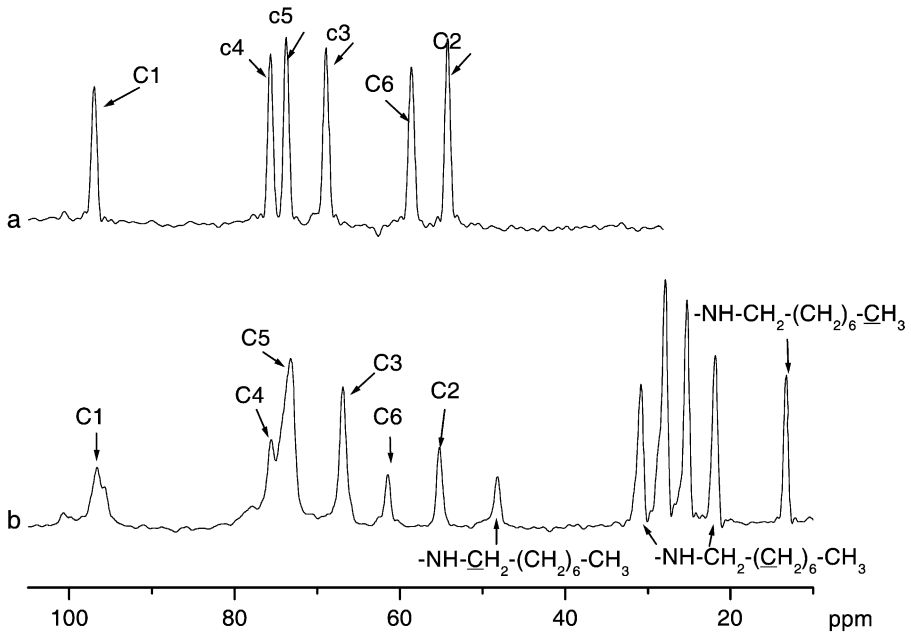

Fig. 3. ^{13}C NMR spectra of (a) chitosan and (b) OCS1.

140 to 200 °C due to the decomposition of polymer with low molecular weight, followed by more obvious loss of weight, attributed to the decomposition of the polymer with high molecular weight starting from 200 to 310 °C. A fast process of weight loss appears in the TG response for the chitosan derivative (OSC1) decomposing from 170 to 230 °C, due to removing a part of the polymer with low molecular weight by dialysis. All these results show some decrease of the thermal stability for OSC1 relative to the original chitosan. Introduction of substituents into polysaccharide structures should disrupt the crystalline structure of chitosan, especially by the loss of the hydrogen bonding.

3.3. Measurement of diameter and polydispersity of chitosan derivative micelles

Micelle size was determined by dynamic light scattering (Table 1). Based on the experiment observations, it is obvious that the modified chitosan when dissolved in water could form the micelles with sizes in the range of 100–400 nm depending on the type of derivatives.

3.4. The solubility of taxol in chitosan derivative micelles

The solubility of taxol in chitosan derivative micellar solution is recorded in Table 1. It is seen that the taxol

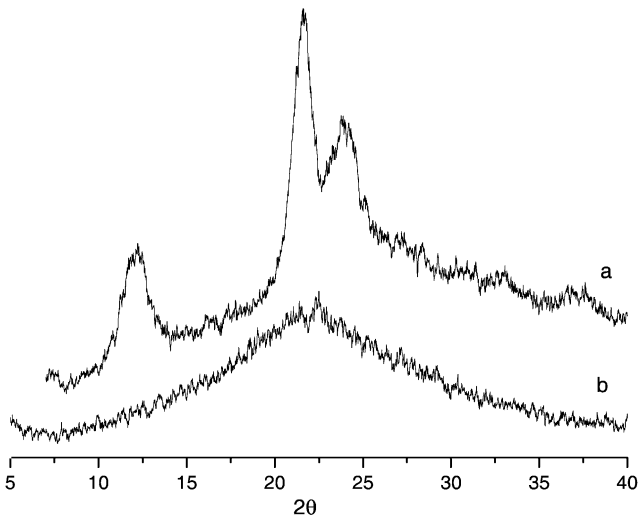

Fig. 4. WAXD patterns of (a) chitosan and (b) OCS1.

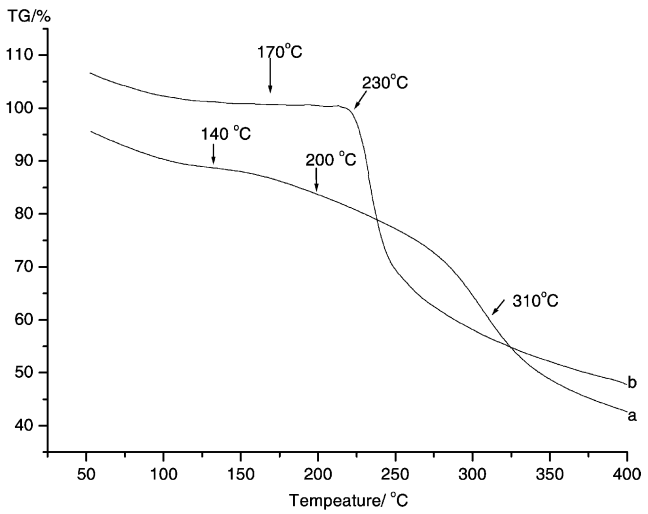

Fig. 5. TG thermograms of (a) chitosan and (b) OCS1.

Table 1
The micelle size and taxol concentration

Sample	Polydispersity	Average size (nm)	Taxol concentration (in micelle), μg/ml
OCS1	1	318	2010
OCS2	1	100	119
DCS1	1	344	11
DCS2	1	140	8
LCS1	1	376	138
LCS2	1	160	38

Derivative concentration in water was 0.568 g/100 ml.

Table 2
Taxol concentration in the OCS1 micellar solution

Derivative concentration (in water) g/100 ml	Taxol concentration (in micelle) μg/ml
0.568	2010
0.554	1950
0.252	1540
0.063	120

concentrations in the micelles is related to the types of the derivatives prepared and to the molecular weight of chitosan. The higher the molecular weight of chitosan, the greater the formed micelle size and thereby the higher the taxol concentrations in the micelles.

Table 2 shows that the solubility of taxol in OCS1 micellar solution was greatly enhanced. When the derivative was 0.568 g /100 ml, the concentration of taxol is high up to 2.01 mg/ml, and even for the derivative concentration of 0.063 g/100 ml, the concentration of taxol is still up to 120 times that of free taxol concentration in water (<0.001 mg/ml).

4. Conclusions

A series of novel *N*-alkyl-*O*-sulfate chitosan derivatives which can form the micelles were prepared, and the micelle size were around 100 ~ 400 nm. The chemical structure of chitosan derivatives and some of physical properties were characterized by elemental analysis, FTIR, ^{1}H NMR, ^{13}C NMR, WAXD and TG. The solubility of taxol in *N*-octyl-*O*-sulfate chitosan (OCS1) micellar solution was increased largely. So *N*-octyl-*O*-sulfate chitosan may be used as a potential drug carrier.

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