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A series of novel chitosan derivatives: Synthesis, characterization and micellar solubilization of paclitaxel

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Abstract

A series of novel chitosan derivatives with octyl, sulfate and polyethylene glycol monomethyl ether (mPEG) groups as hydrophobic and hydrophilic moieties, respectively, were synthesized. These PEGylated amphiphilic chitosan derivatives were characterized with ^{1}H NMR, ^{13}C NMR, FTIR and elemental analysis. And their physical properties were measured by wide angle X-ray diffraction (WAXD) and thermogravimetric analysis (TG). The critical micelle concentrations (CMCs) of the modified chitosans determined by using pyrene as a hydrophobic probe in fluorescence spectroscopy were found to be 0.011–0.079 mg/ml, and the log CMC was linearly relative to four structure parameters, that is the degree of substitution (DS) of chitosan unit, sulfate group, PEG unit and octyl group by mole per kilogram. Paclitaxel, a water-insoluble anticancer drug, was solubilized into the polymeric micelles formed by these derivatives utilizing physical entrapment method, with micellar particle size around 100–130 nm, and the highest paclitaxel concentration of 3.94 mg/ml was found in *N*-mPEG-*N*-octyl-*O*-sulfate chitosan (mPEGOSC) micellar solution, which was much higher than that in water (less than 0.001 mg/ml). Therefore, *N*-mPEG-*N*-octyl-*O*-sulfate chitosan micelles may be useful as a prospective carrier for paclitaxel.

Keywords: Chitosan; Polymeric micelles; Paclitaxel; Solubilization

1. Introduction

The blood–brain barrier (BBB) is formed at the level of the endothelial cells of the cerebral capillaries and essentially comprises the major interface between the blood and the brain. The brain blood vessel endothelial cells are characterized by having tight continuous circumferential junctions between them. It is a vital element in the regulation of the constancy of the internal environment of the brain. However, it is also an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents, and a variety of central nervous system (CNS)-active drugs (Keruter, 2001). Many attempts have been made to overcome the above barrier including osmotic opening of the tight junctions (Gummerloch & Neuwalt, 1992), application of prodrugs or carrier systems such as antibodies (Pardridge, Buciak, & Friden, 1991), liposomes (Chen & Lee, 1993; Huwyler, Wu, & Pardridge, 1996; Zhou & Huang, 1992), and nanoparticles, in which, the utilization of nanoparticles was one of most promising methods. Drugs that have successfully been transported into the brain by nanoparticles include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the NMDA receptor antagonist MRZ 2/576, and doxorubicin. Two representative brain-targeting nanoparticle examples are polysorbate 80-coated polybutylcyanoacrylate (PBCA) nanoparticles (Alyautdin, Gothier, Petrov, Kharkevich, & Kreuter, 1995; Alyautdin et al., 1997, 1998; Friese, Seiller, Quack, Lorenz, & Kreuter, 2000; Gulyaev et al., 1999; Keruter, 2001; Lockman, Mumper, Khan, & Allen, 2002; Schroeder, Sommerfeld, Ulrich, & Sabel, 1998) and PEGylated (mPEG2000) polycyanoacrylate nanoparticles (Calvo et al., 2001). In fact, a polysorbate 80 molecule includes

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three PEG groups, and the molecular weight of each PEG group is about 880 Da. It suggests that the PEG group is the critical functional group for their brain delivery and the mechanism lies on two points: the PEG group can add long-circulating properties to the carrier and it provides suitable appropriate surface characteristics allowing the carrier to interact with BBB endothelial cells leading to its uptake by these cells (Keruter, 2001).

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-(1-4*b*)-D-glucopyranose residues (D-glucosamine units) and little or no *N*-acetyl-D-glucosamine units. In recent years, several researches on modified chitosan micelles have been carried out. Most of these micelles can solubilize water-insoluble drugs to a high concentration in water by self-assembly (Jiang, Quan, Liao, & Wang, 2006; Miwa et al., 1998; Sui, Yin, Chen, Zhang, & Kong, 2006; Uchegbu et al., 2001; Yoshioka, Nonaka, Fukuda, & Kazama, 1995; Zhang, Ping, & Zhang, 2004; Zhang, Ping, Zhang, & Shen, 2003). However, the chitosan derivatives with both amphiphilic group as well as brain-targeting group have not been reported.

In this paper, a series of novel chitosan derivatives were designed and prepared, which were designated to have the solubilization and self-assembly characteristics of polymeric micelles, and were expected at same time to have brain-targeting characteristics as polysorbate 80-coated PBCA nanoparticles and PEGylated polycyanoacrylate nanoparticles. Such a new series of chitosan derivatives with multi-functions was obtained primarily by attaching octyl to amino groups, sulfate to hydroxyl groups, and mPEG (mPEG1100, mPEG2000 and mPEG5000) to amino groups of chitosan molecules, providing hydrophobic, hydrophilic, and both brain-targeting and hydrophilic moieties, respectively. The chemical structures and physical properties of the modified chitosan were characterized with ^{1}H NMR, ^{13}C NMR, FTIR, elemental analysis, WAXD and TG. The critical micelle concentrations (CMCs) of the modified chitosans were evaluated for their micelle-forming properties and some morphological characteristics of modified chitosan micelles were observed with fluorescence microscope and atomic force microscope (AFM). In addition, the potential of PEGylated amphiphilic chitosan derivatives in entrapping paclitaxel, a lipophilic chemotherapy drug, in the polymeric micelles and enhancing its water solubility was investigated. This study may lead to a kind of novel carriers for improving the efficacy of water-insoluble anticancer drugs.

2. Experimental

2.1. Materials

Chitosan was provided by Nantong Shuanglin Biochemical Co. Ltd (China), with a degree of deacetylation of 90% and viscosity average molecular weight of 65,000 Da. Polyethylene glycol monomethyl ether was obtained from Sigma Co., with number average molecular weight of 1100, 2000 and 5000 Da. Paclitaxel was supplied by Taihua Natural Plant Pharmaceutical Co. Ltd (China). All commercially available solvents and reagents were used without further purification.

2.2. Methods

2.2.1. Synthesis of N-mPEG-N-octyl-O-sulfate chitosan derivatives

2.2.1.1. Preparation of mPEG aldehyde (mPEGCHO). mPEGCHO was prepared by the oxidation of mPEG with DMSO/acetic anhydride (Harris et al., 1984). Acetic anhydride (14.5 ml, 150 mmol) was added to the solution of mPEG2000 (25 g, 12.5 mmol) in 100 ml anhydrous dimethyl sulfoxide containing 10% chloroform. The mixture was stirred for 9 h at room temperature (25 °C). The reaction mixture was then poured into 1000 ml diethylether. The precipitate was filtered and reprecipitated twice from chloroform solution with diethylether. After drying, 18.8 g of white powder (mPEGCHO2000) was obtained.

mPEGCHO1100 and mPEGCHO5000 were synthesized following the same procedure by using mPEG1100 and mPEG5000 as the starting material, respectively.

2.2.1.2. Preparation of N-mPEG chitosan (mPEGC). The preparation of mPEGC was improved from the method of Harris et al. (1984). Chitosan (1.0 g, 6 mmol as monosaccharide residue containing 5.4 mmol amino groups) was dissolved in a mixed solvent of aqueous 2% acetic acid solution (40 ml) and methanol (20 ml). A 14 ml of aqueous solution of mPEGCHO2000 [5.54 g, 2.79 mmol, –CHO: 1.5 mmol (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998)] was added to above chitosan solution and stirred for 30 min at room temperature. Then the pH value of the chitosan/mPEGCHO2000 solution was adjusted to 6.5 with 1 M aqueous NaOH solution and stirred for 60 min at room temperature. $NaBH_3CN$ (0.952 g, 15 mmol) in 14 ml water was added to the reaction mixture dropwise for 20 min and the solution was stirred for 12 h at room temperature. The reaction solution was dialyzed with dialysis membrane (molecular weight cut-off, MWCO 10,000) against aqueous 0.05 M NaOH solution and distilled water alternately for 2 days then against distilled water four times in 2 days. The inner solution was concentrated under reduced pressure, and then the obtained concentrated solution was lyophilized and washed twice with 200 ml of acetone. After drying in vacuum at 40 °C, 1.26 g of light yellow powder (mPEGC2000M) was obtained.

mPEGC2000L and mPEGC2000H were prepared using the same method with 0.5 and 1.5 times weight of mPEGCHO2000, respectively. L, M, and H represented low, middle, and high degree of mPEG group's substitution, respectively.

mPEGC1100s (including mPEGC1100L, mPEGC1100M, and mPEGC1100H) and mPEGC5000s (including

mPEGC5000L, mPEGC5000M, and mPEGC5000H) were prepared using the same procedure of mPEGC2000s, respectively.

2.2.1.3. Preparation of N-mPEG-N-octyl chitosan (mPEGOC). mPEGC2000M (0.5 g) was dissolved in 50 ml of aqueous 1% HCl solution with stirring at room temperature. After the pH value of the solution was adjusted to 7 with aqueous 1 M NaOH solution, 50 ml of methanol was added and stirred for 30 min. Octanal (1.6 ml, 10.3 mmol) was added to the reaction mixture and the solution was stirred for 48 h at room temperature. Then KBH_4 (1.67 g, 30.9 mmol) was added in batch. After a further 24 h of continuous stirring, the reaction solution was neutralized with aqueous 1 M HCl solution. Then the mixture was concentrated under reduced pressure and ethanol 100 ml 3× was added to ensure complete removal of water from the mixture in the concentration course. Subsequently, diethyl ether 100 ml was added to the obtained residue. After stirring vigorously for 2 h, the gained suspension was filtered and the filter cake was washed with diethyl ether three times. 4.40 g of white powder (mPEGOC2000M and inorganic salts) was obtained after drying in vacuum at 50 °C overnight.

mPEGOC1100L, mPEGOC1100M, mPEGOC1100H, mPEGOC2000L, mPEGOC2000H, mPEGOC5000L, mPEGOC5000M, and mPEGOC5000H were prepared following the same procedure using the corresponding mPEGC as starting material, respectively.

2.2.1.4. Preparation of N-mPEG-N-octyl-O-sulfate chitosan (mPEGOSC). Chlorosulfonic acid (4 ml, 60 mmol) was added dropwise into DMF (25 ml) with stirring at 0 °C. After completely dripped, the temperature of the mixture was heightened to 40 °C. Then mPEGOC2000M (4.40 g, including inorganic salts) was added and the reaction mixture was stirred vigorously for 10 h. The reaction solution was neutralized with aqueous 20% NaOH solution to pH 7 at 0 °C, and the filtered solution was dialyzed (MWCO 10,000) against distilled water (6× 20 L) for 3 days, then lyophilized and 0.487 g of white powder (mPEGOSC2000M) was obtained.

Different chitosan derivatives, mPEGOSC1100L, mPEGOSC1100M, mPEGOSC1100H, mPEGOSC2000L, mPEGOSC2000H, mPEGOSC5000L, mPEGOSC5000M, and mPEGOSC5000H, were obtained with the corresponding mPEGOC as the starting material, respectively.

2.2.2. Characterization of chitosan derivatives

^1H NMR and ^{13}C NMR spectra were performed on a Bruker (AVACE) AV-500 spectrometer. Chitosan and mPEGC were dissolved in the mixed solvent D_2O and CF_3COOD. mPEGOSC was dissolved in D_2O.

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

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

WAXD spectra were obtained using an XD-3A powder diffraction meter with Cu Kα radiation in the range of 5–40° (2θ) at 40 kV and 30 mA.

TG spectra were obtained with NETZSCH TG 209 equipment. The temperature range was 30–350 °C and the heating rate is 10 °C/min.

2.2.3. Measurement of CMC

The CMC of the mPEGOSC was determined by using pyrene (Fluka, >99%) as a hydrophobic probe in fluorescence spectroscopy (Shimadzu RF-5301 PC, Japan) (Astafieva, Zhong, & Eisenberg, 1993; Chae, Son, Lee, Jang, & Nah, 2005; Wilhelm, Zhao, Wang, Xu, & Winnik, 1991). Briefly, a known amount of pyrene in acetone was added to each of a series of 10 ml vials and the acetone was evaporated, 5 ml of various concentrations of mPEGOSC solutions (1×10^{-4}–1 mg/ml) were added to the vials (the final concentration of pyrene was controlled to 6×10^{-7} M), then sonicated for 30 min at room temperature. The sample solutions were heated at 65 °C for 3 h to equilibrate pyrene and the micelles, and then left to cool overnight at room temperature. Fluorescence excitation spectra were measured at the emission wavelength of $\lambda_{em} = 390$ nm, excitation wavelength was 300–360 nm for excitation spectra. Both excitation and emission bandwidths were set at 3 nm. Based on the pyrene excitation spectra and red shift of the spectra with increasing mPEGOSC concentrations, the CMC of the mPEGOSC was measured.

2.2.4. Preparation of paclitaxel-loaded chitosan derivative micellar solutions

Paclitaxel-loaded chitosan derivative micellar solutions were prepared by dialysis (Kwon et al., 1995; Miwa et al., 1998; Zhang et al., 2003, 2004). Briefly, mPEGOSC 12 mg was dissolved in 2 ml water. Ten milligrams of paclitaxel was dissolved in 0.2 ml ethanol, and then the paclitaxel solution was injected into mPEGOSC solution with stirring at room temperature, followed by dialysis against distilled water overnight at room temperature using a dialysis membrane (MWCO 10,000). The micellar solution was filtered with a 0.22 μm pore-sized microfiltration membrane.

Particle size and polydispersity index of mPEGOSC micelles were measured with a Zetasizer 3000 HS instrument (Malvern Instruments, Malvern, UK) with 633 nm He–Ne lasers at 25 °C.

2.2.5. Measurement of paclitaxel concentration in mPEGOSC micellar solution

The concentration of paclitaxel in the micellar solution was determined by HPLC (HP1100, Agilent, USA). The mobile phase was a mixture of methanol and water (75:25 v/v). The column was a Diamohsile C18 (4.6 mm × 250 mm, 5 μm, Dikma, Beijing, China). The flow rate was 1.0 ml/min, the detection wavelength was 227 nm (UV detector, HP1100, Agilent, USA), the column temperature was 40 °C, and the injected volume of the sample was 20 μl.

2.2.6. Morphological observation

The mixed solution of pyrene and mPEGOSC was observed with inverted fluorescence microscope (IX51 Olympus, Japan).

The AFM morphological observation was performed with SPA-300 HV (Seiko, Japan) and the paclitaxel-loading micellar solution was dropped on a silicon slice and dried under the pressure.

3. Results and discussions

3.1. Synthesis and characterization of chitosan derivatives

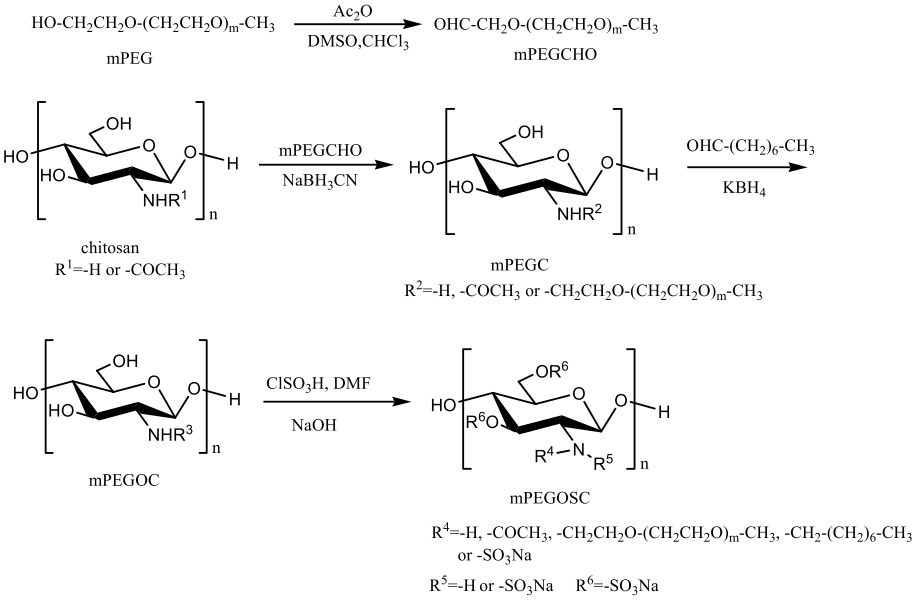
The synthetic route of mPEGOSC was briefly summarized in Scheme 1.

mPEGOC was not dissolved in aqueous 80% methanol solution. In general, in the after-treatment of the preparation of mPEGOC, the reaction mixture could be poured into sufficient amount of methanol then filtered and washed with 80% methanol to remove inorganic salts and excess octanal. But mPEGOC was largely swollen against the water in the mixed solvent of water and methanol, so that the filtration could not be carried out. Therefore, we removed all the water in the reaction mixture when the reaction was over and then washed out excess octanal with diethylether. In the end the mixture of mPEGOC and inorganic salts was obtained, which was used directly in the following reaction without further treatment because inorganic salts in the mixture would not influence the following reaction.

Structure changes of chitosan derivatives were characterized with ^{1}H NMR spectra (Fig. 1), ^{13}C NMR spectra (Fig. 2) and FTIR spectra (Fig. 3).

Compared with chitosan, the ^{1}H NMR spectrum of the mPEGC2000M (Fig. 1) showed the signals at δ (ppm) 3.6–3.9 (m, mH) and the signals at δ (ppm) 3.4 (s, 3 H), which were assigned to the methene hydrogen (–CH_2CH_2O–) of the *N*-mPEG groups and the methyl hydrogen (–OCH_3) of the *N*-mPEG groups, respectively. And it was evidenced that the mPEGC2000M contained *N*-mPEG groups. Compared with mPEGC2000M, the ^{1}H NMR spectrum of the mPEGOSC2000M showed the signals at δ (ppm) 3.1–3.3 (m, 2 H), the signals at δ (ppm) 1.3, 1.7 and 2.2 (m, 12H) and the signals at δ (ppm) 0.89 (t, 3H), which were attributed to the methene hydrogens (–NH–CH_2– $(CH_2)_6$–CH_3) of the *N*-octyl groups, another twelve methene hydrogens (–NH–CH_2– $(CH_2)_6$-CH_3) of the *N*-octyl groups and the methyl hydrogen (–NH–CH_2–$(CH_2)_6$– CH_3) of the *N*-octyl groups, respectively. The results certified that the chitosan derivative carried *N*-octyl groups.

Fig. 2 showed the ^{13}C NMR spectra of chitosan, mPEGC2000M and mPEGOSC2000M. The signals of chitosan at δ (ppm) 55.6 (C2), 60 (C6), 70 (C3), 75 (C5), 76.5 (C4) and 97.5 (C1) were detected (Dung, Milas, Rinaudo, & Desbriers, 1994). To mPEGC2000M, the peak at δ (ppm) 72.2 was assigned to methene carbon (–CH_2CH_2O–) of the *N*-mPEG groups. The peaks of polysaccharide structures were not obvious because the signals were inhibited by the signals of *N*-mPEG groups. Compared with mPEGC2000M, the ^{13}C NMR spectrum of the mPEGOSC2000M showed the signal at δ (ppm) 16.3 assigned to the methyl carbon (–NH–CH_2–$(CH_2)_6$–CH_3) of the *N*-octyl groups, the signal at δ (ppm) 48.2 and the signals at δ (ppm) 28.5, 29, 30.7, 33.8 attributed to the methene carbon (–NH–CH_2–$(CH_2)_6$–CH_3) and another six methene carbons (–NH–CH_2–(CH_2)$_6$–CH_3) of the *N*-octyl groups, respectively (Zhang et al., 2003). The results indicated that



Scheme 1. Synthesis of *N*-mPEG-*N*-octyl-*O*-sulfate chitosan (mPEGOSC).

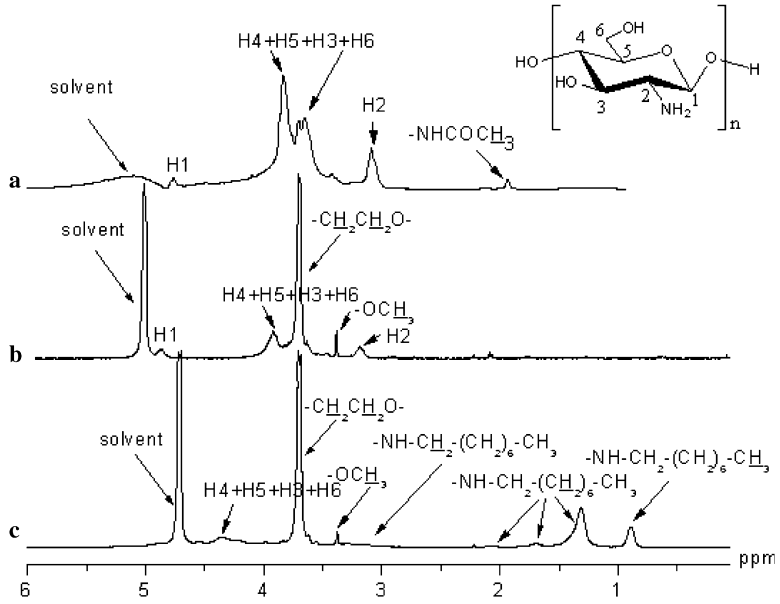


Fig. 1. ^{1}H NMR spectra of (a) chitosan, (b) mPEGC2000M and (c) mPEGOSC2000M (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively).

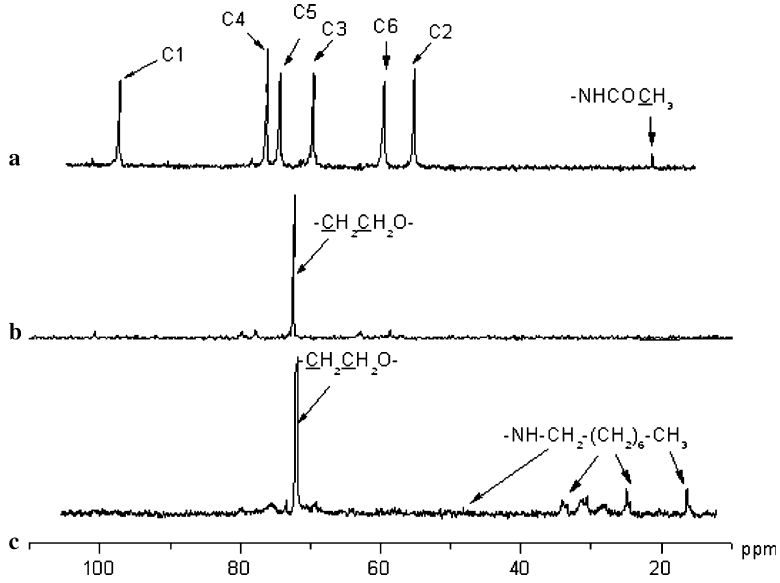


Fig. 2. ^{13}C NMR spectra of (a) chitosan, (b) mPEGC2000M and (c) mPEGOSC2000M (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively).

mPEGOSC2000M contained *N*-mPEG groups and *N*-octyl groups.

Compared with chitosan, the FTIR spectrum of mPEGOC2000M showed new peaks at 2926, 2862, 1462 and 1379 cm^{-1} attributed to the *N*-octyl groups and 1078 cm^{-1}assigned to C–O–C bonds of *N*-mPEG groups. In addition the signal from the amino groups at 1597 cm^{-1} disappeared (Fig. 3). These results suggested that octyl groups and mPEG groups were introduced into 2-NH_2 groups of chitosan. In the FTIR spectrum of mPEGOSC2000M, the peaks at 1034 and 1097 cm^{-1} from 3-OH and 6-OH of mPEGOC2000M disappeared, and the new peaks at 1258, 1228, 1007 and 806 cm^{-1} were assigned to the O=S=O bonds of *N*-sulfate groups. These results indicated that sulfate groups were mainly introduced into 3-OH and 6-OH groups of mPEGOC2000M.

The DS of different group was calculated by comparing the C, N molar ratio (C/N (mol)) and S, N molar ratio (S/N (mol)) of each derivative from elemental analysis using the following equations (Miwa et al., 1998):

$$\text{DS of mPEG group} = \text{C/N(mol)}_{\text{mPEGC}} - \text{C/N(mol)}_{\text{chitosan}}, \quad (1)$$

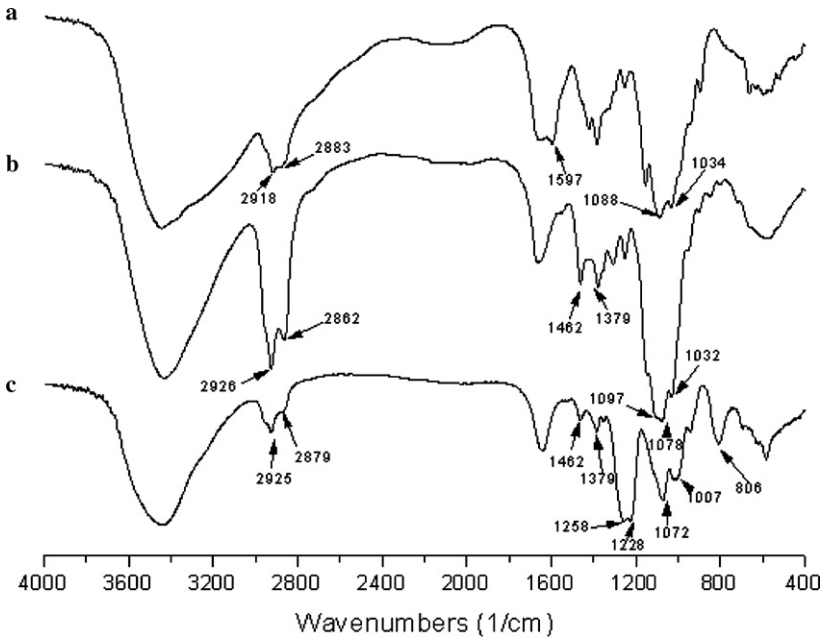


Fig. 3. FTIR spectra of (a) chitosan, (b) mPEGOC2000M and (c) mPEGOSC2000M (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively).

$$\text{DS of octyl group} = \text{C/N(mol)}_{\text{mPEGOSC}} - \text{C/N(mol)}_{\text{mPEGC}}, \quad (2)$$

$$\text{DS of sulfate group} = \text{S/N(mol)}_{\text{mPEGOSC}}. \quad (3)$$

The DS of mPEG, octyl and sulfate group was estimated to be 0.05–0.42, 0.41–0.83 and 2.19–2.61, respectively, as shown in Table 1.

3.2. Physical properties of chitosan derivatives

The WAXD spectra of chitosan, mPEGOSC1100H, mPEGOSC2000H and mPEGOSC5000H were shown in Fig. 4. Chitosan had three reflections falling at $2\theta = 11°$, $2\theta = 20°$ and $2\theta = 22°$. Samuels reported that the reflection falling at $2\theta = 11°$ was assigned to crystal form I. The strongest reflection falling at $2\theta = 20°$ corresponding to crystal form II (Samuels, 1981). mPEGOSC1100H showed only one broad peak at $2\theta = 20°$ and the peak at $2\theta = 20°$ of mPEGOSC2000H was hardly appreciable. It suggested that the ability of forming hydrogen bond of them was decreased after chemical modification. And their crystalline structures appeared amorphous. But mPEGOSC5000H showed new diffraction peaks at $2\theta = 18°$ and $2\theta = 27°$. It corresponded to the crystallization of mPEG block when the molecular weight of mPEG increased.

The TG spectra of chitosan, mPEGOSC1100H, mPEGOSC2000H and mPEGOSC5000H were shown in Fig. 5. Chitosan showed slow weight loss starting from 140 to 275 °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 275 °C. Because most of the polymer with low molecular weight was removed by dialysis, chitosan derivatives did not have the slow weight loss process. However, a fast process of weight loss appeared in the TG spectra of chitosan derivatives starting from 225 to 275 °C, which was attributed to the abolition of octyl and sulfate groups. The following weight loss after 275 °C was assigned to the decomposing of chitosan backbone, and mPEG groups was scarcely depolymerized.

3.3. Measurement of CMC

At low concentration (C) of mPEGOSC ($C < CMC$), there were small or negligible changes in total fluorescence intensity and the location of the band at 333 nm. As the concentration increased, a remarkable increase in the total fluorescence intensity and a red shift of the band from 333 to 336 nm were observed (Fig. 6). Fig. 7 showed the intensity ratio (I336/I333) of the pyrene excitation spectra versus the logarithm of the mPEGOSC2000M concentrations. Based on the intensity ratio data, the CMC value of mPEGOSC2000M was calculated by the crossover point and the CMC values of other mPEGOSCs were obtained by the same method. All the CMC values were listed in Table 2.

To reveal the relationship of CMC and the structure of mPEGOSC, four structure parameters, DS of octyl group, PEG unit, sulfate group and chitosan unit by mole per kilogram (mol/kg), were introduced. The different degree of substitution (mol/kg) could be calculated by the following equations. The obtained values of four structure parameters were listed in Table 2.

$$\text{DS of octyl group (mol/kg)} = 1000 * (\text{DS of octyl group})/\text{MW}_{(\text{mPEGOSC unit})}, \quad (4)$$

Table 1
Elemental analyses and the DS of each group of chitosan derivatives

Compound	N (%, W)	C (%, W)	S (%, W)	C/N (mol)	C/N (mol) mean	S/N (mol)	S/N (mol) mean	DS of mPEG group	DS of octyl group	DS of sulfate group
Chitosan	7.73	41.10		6.20	6.20					
	7.73	41.12		6.21						
mPEGC1100L	3.57	45.10		14.74	14.81					
	3.53	45.05		14.89						
mPEGC1100M	2.58	46.23		20.91	20.98					
	2.55	46.01		21.05						
mPEGC1100H	2.02	47.82		27.62	26.87					
	2.13	47.70		26.13						
mPEGC2000L	4.56	43.18		11.05	11.02					
	4.59	43.28		11.00						
mPEGC2000M	3.36	44.01		15.28	15.27					
	3.37	44.08		15.26						
mPEGC2000H	2.73	47.17		20.16	20.23					
	2.69	46.82		20.31						
mPEGC5000L	2.05	49.92		28.41	28.53					
	2.03	49.86		28.66						
mPEGC5000M	1.05	52.31		58.12	57.80					
	1.06	52.22		57.47						
mPEGC5000H	0.79	54.24		80.10	80.12					
	0.79	54.26		80.13						
mPEGOSC1100L	1.84	32.07	9.32	20.33	20.17	2.22	2.21	0.17	0.67	2.21
	1.87	32.07	9.40	20.01		2.20				
mPEGOSC1100M	1.65	37.05	9.02	26.20	25.86	2.39	2.40	0.30	0.61	2.40
	1.69	36.97	9.33	25.52		2.42				
mPEGOSC1100H	1.49	39.14	8.88	30.65	30.62	2.61	2.61	0.42	0.47	2.61
	1.49	39.07	8.90	30.59		2.61				
mPEGOSC2000L	2.00	27.65	10.34	16.13	15.95	2.26	2.27	0.05	0.62	2.27
	2.05	27.71	10.71	15.77		2.29				
mPEGOSC2000M	1.79	33.15	9.67	21.61	21.94	2.36	2.36	0.10	0.83	2.36
	1.73	33.03	9.35	22.27		2.36				
mPEGOSC2000H	1.55	34.40	8.69	25.89	25.30	2.45	2.44	0.16	0.63	2.44
	1.61	34.09	8.93	24.70		2.43				
mPEGOSC5000L	1.28	37.29	6.54	33.99	33.18	2.24	2.19	0.10	0.58	2.19
	1.33	36.90	6.51	32.37		2.14				
mPEGOSC5000M	0.73	41.12	4.34	65.72	63.35	2.60	2.55	0.23	0.69	2.55
	0.79	41.30	4.50	60.99		2.49				
mPEGOSC5000H	0.61	43.50	3.44	83.20	83.41	2.47	2.46	0.33	0.41	2.46
	0.61	43.72	3.42	83.62		2.45				

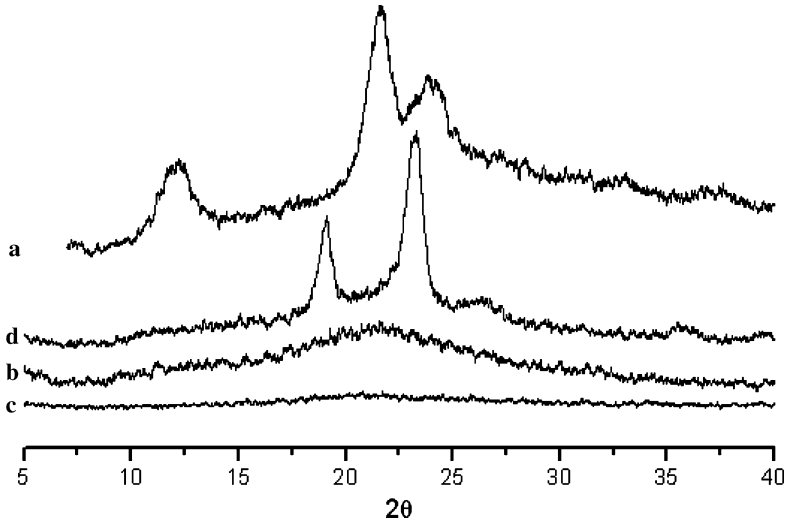


Fig. 4. WAXD spectrta of (a) chitosan, (b) mPEGOSC1100H (DS of mPEG group, octyl group and sulfate group is 0.42, 0.47 and 2.61, respectively), (c) mPEGOSC2000H (DS of mPEG group, octyl group and sulfate group is 0.16, 0.63, and 2.44, respectively), and (d) mPEGOSC5000H(DS of mPEG group, octyl group and sulfate group is 0.33, 0.41 and 2.46, respectively).

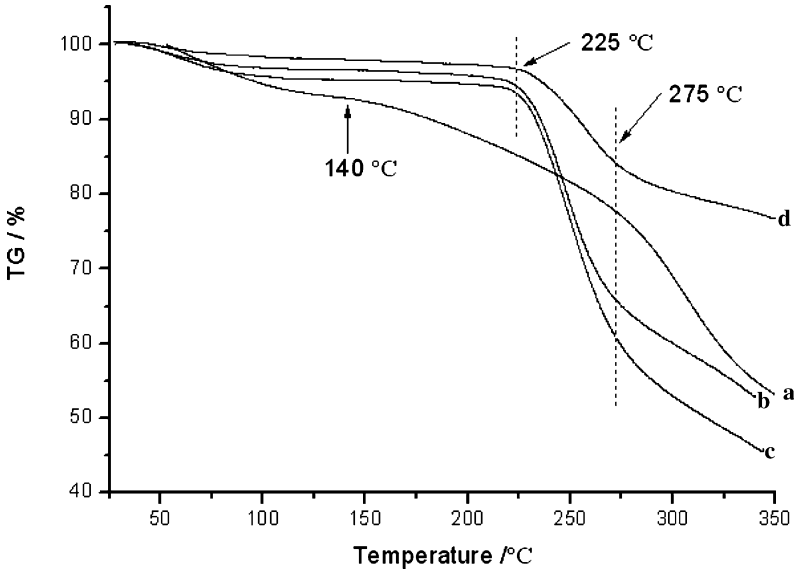


Fig. 5. TG spectra of (a) chitosan, (b) mPEGOSC1100H (DS of mPEG group, octyl group and sulfate group is 0.42, 0.47 and 2.61, respectively), (c) mPEGOSC2000H (DS of mPEG group, octyl group and sulfate group is 0.16, 0.63, and 2.44, respectively), and (d) mPEGOSC5000H (DS of mPEG group, octyl group and sulfate group is 0.33, 0.41 and 2.46, respectively).

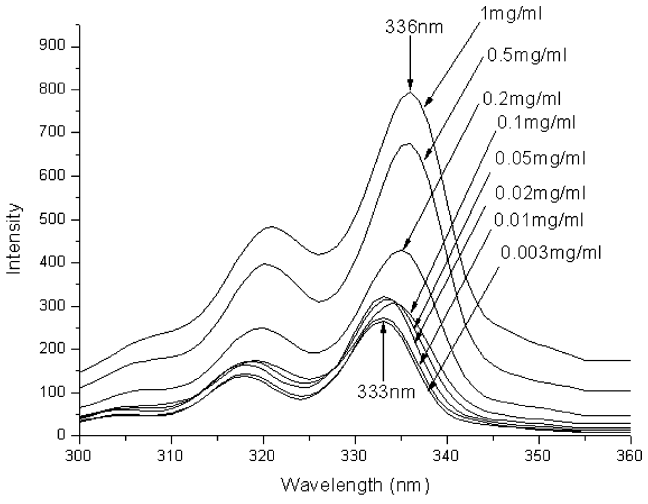


Fig. 6. Pyrene excitation spectra ([pyrene] = 6×10^{-7} M) of mPEGOSC2000M (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively) aqueous solutions (emission wavelength of 390 nm).

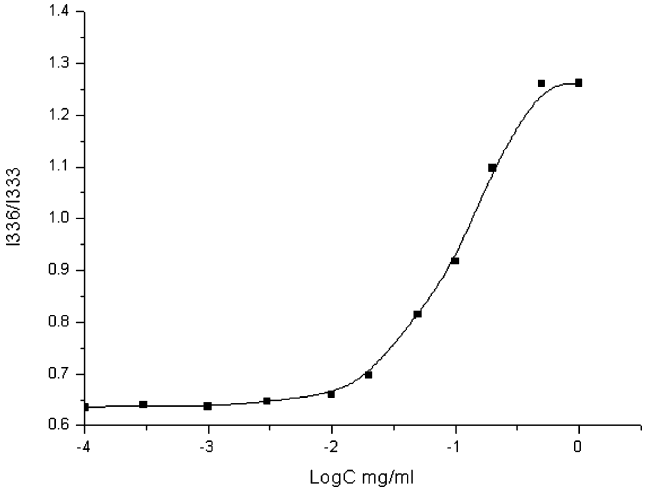


Fig. 7. Intensity ratio plots of I336/I333 versus logC for mPEGOSC2000M in water (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively).

$$\text{DS of PEG unit (mol/kg)} = 1000 * (\text{DS of mPEG group}) * N_{(\text{PEG unit})}/\text{MW}_{(\text{mPEGOSC unit})}, \quad (5)$$

$$\text{DS of sulfate group (mol/kg)} = 1000 * (\text{DS of sulfate group})/\text{MW}_{(\text{mPEGOSC unit})}, \quad (6)$$

$$\text{DS of chitosan unit (mol/kg)} = 1000/\text{MW}_{(\text{mPEGOSC unit})}, \quad (7)$$

where $N_{(\text{PEG unit})}$ and $\text{MW}_{(\text{mPEGOSC unit})}$ represented the number of CH_2CH_2O in mPEG and the molar weight of mPEGOSC unit, respectively.

Table 3 listed the results of linear regression with every two ones of logCMC, DS of chitosan unit, sulfate group, PEG unit and octyl group by mole per kilogram, and all the absolute values of correlation coefficients (r) were larger than 0.9, which suggested that they were all linear relative. The linear relationship of the four structure parameters of mPEGOSCs resulted from the substitution restriction of every group by each other in our reaction conditions. The linear regression curves of logCMC versus DS of octyl group, PEG unit, sulfate group and chitosan

Table 2
The DS of each group, CMC and logCMC of chitosan derivatives

Compound	DS of octyl group (mol/kg)	DS of PEG unit (mol/kg)	DS of sulfate group (mol/kg)	DS of chitosan unit (mol/kg)	logCMC	CMC (mg/ml)
mPEGOSC1100L	1.02	6.46	3.38	1.53	−1.83	0.015
mPEGOSC1100M	0.76	9.06	2.99	1.25	−1.82	0.015
mPEGOSC1100H	0.50	10.84	2.79	1.07	−1.65	0.022
mPEGOSC2000L	1.08	4.15	3.98	1.75	−1.82	0.015
mPEGOSC2000M	1.19	6.40	3.38	1.43	−1.95	0.011
mPEGOSC2000H	0.80	8.74	3.08	1.26	−1.71	0.019
mPEGOSC5000L	0.62	11.75	2.33	1.06	−1.52	0.030
mPEGOSC5000M	0.42	15.68	1.56	0.61	−1.30	0.050
mPEGOSC5000H	0.20	17.64	1.18	0.48	−1.10	0.079

Table 3
The results of linear regression with every two ones in logCMC, DS of chitosan unit, sulfate group, PEG unit and octyl group by mole per kilogram

r[a], slope	DS of octyl group (mol/kg)	DS of PEG unit (mol/kg)	DS of sulfate group (mol/kg)	DS of chitosan unit (mol/kg)	logCMC
DS of octyl (mol/kg)	–	–	–	–	–
DS of PEG unit (mol/kg)	−0.9470, −12.672	–	–	–	–
DS of sulfate (mol/kg)	0.9118, 2.4928	−0.9941, −0.2031	–	–	–
DS of chitosan unit (mol/kg)	0.9303, 1.1612	−0.9952, −0.0928	0.9879, 0.4509	–	–
logCMC	−0.9166, −0.7742	0.9469, 0.0598	−0.9449, −0.2919	−0.9217, −0.6237	–

[a] r, correlation coefficient.

unit by mole per kilogram were shown in Fig. 8. The CMC values of mPEGOSCs could be estimated by every equation listed in Fig. 8, which could provide quantitative directions for further structural optimization of mPEGOSC, at the same time, supplied a successful example of QSCMCR (quantitative structure CMC relationship) research on graft copolymeric surfactants.

3.4. Characterization of paclitaxel-loading chitosan derivative micelles

Table 4 listed paclitaxel concentration, paclitaxel-loading rate, entrapment efficiency and particle size of paclitaxel-loading micelles, and paclitaxel-loading rate and entrapment efficiency could be calculated by Eqs. (8)

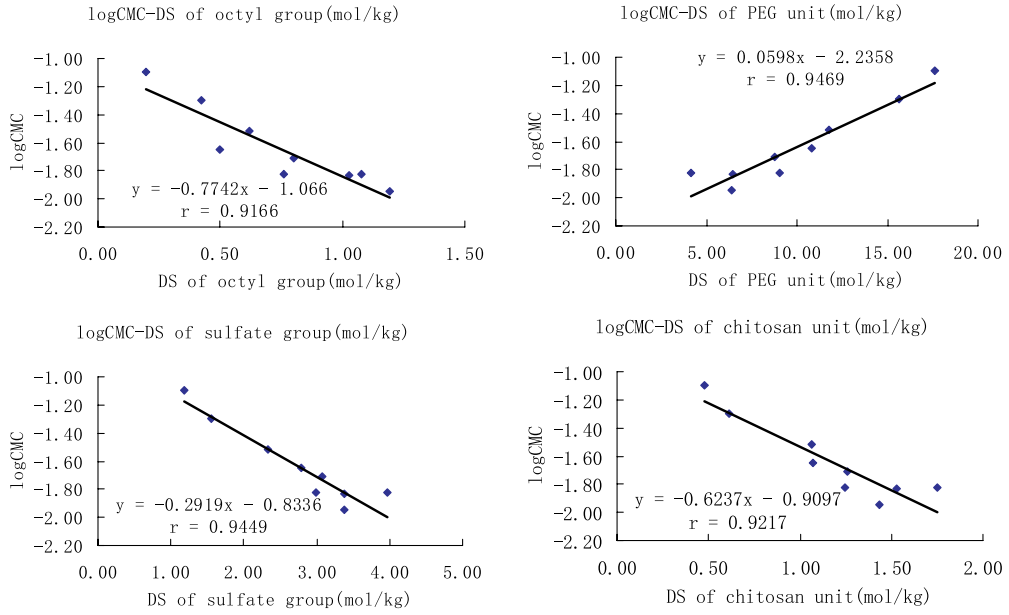


Fig. 8. The linear regression curves of logCMC versus DS of octyl group, PEG unit, sulfate group and chitosan unit by mole per kilogram, respectively.

Table 4
Paclitaxel concentration, paclitaxel-loading rate, entrapment efficiency and particle size of paclitaxel-loading micelles ($n = 3$)

Compound	Paclitaxel concentration (mg/ml)	SD	Paclitaxel-loading rate (% (w/w))	SD	Entrapment efficiency (%)	SD	Particle size (nm)	SD	Polydispersity index	SD
mPEGOSC1100L	3.27	0.15	37.6	1.0	72.3	2.5	119.0	1.9	0.28	0.02
mPEGOSC1100M	3.49	0.11	38.9	0.8	77.2	2.4	117.7	1.1	0.30	0.01
mPEGOSC1100H	3.34	0.21	37.9	1.5	74.8	4.4	119.6	2.2	0.28	0.05
mPEGOSC2000L	3.88	0.03	41.8	0.2	84.4	2.9	108.5	0.6	0.34	0.04
mPEGOSC2000M	3.77	0.30	41.1	2.0	82.3	4.9	104.3	5.8	0.39	0.12
mPEGOSC2000H	3.94	0.14	42.6	0.9	85.6	3.0	110.6	1.2	0.34	0.05
mPEGOSC5000L	3.37	0.12	38.5	0.5	76.4	1.8	133.4	0.6	0.34	0.03

and (9). In addition, mPEGOSC5000M and mPEGOSC5000H could not solubilize the paclitaxel. It was seen that these data were related to the molecular weight of mPEG group introduced to chitosan. mPEGOSC2000s had the highest paclitaxel concentrations (3.77–3.94 mg/ml), paclitaxel-loading rates (41.1–42.6%), entrapment efficiencies (82.3–85.6%) and the smallest particle sizes (104.3–110.6 nm). Moreover the concentration of paclitaxel in mPEGOSC2000H micellar solution was 3.94 mg/ml, which was up to about 4000 times higher than that of free paclitaxel in water (less than 0.001 mg/ml). It suggested that the solubilization performances of chitosan derivatives were influenced by the crystallinities of them. The lower the degrees of crystallinity of the compounds (Fig. 4), the better the solubilization performances of them were. The crystallinity of polymer was owing to orderly arrangement of its stable conformations. The higher the degrees of crystallinity of polymer, the more its stable conformations were, and the harder the rotation of the polymer chain was. Chitosan was a D-glucosamine polysaccharide polymerized with β-1,4 linkages, and the reactive amino and hydroxyl groups were arranged in the two sides of chitosan backbone alternately, so hydrophobic and hydrophilic moieties of chitosan derivatives were located in both sides too. When modified chitosan surfactants solubilized drugs, the backbone of them must rotated to ensure the formation of drug-loading micelles, then the polymer, which had lower degree of crystallinity and could rotate easier, would have higher entrapment efficiency and solubilize more drugs.

$$\text{paclitaxel-loading rate} = C * V/(C * V + W_{\text{mPEGOSC}}) * 100\%, \quad (8)$$

$$\text{entrapment efficiency} = C * V/(W_{\text{paclitaxel}}) * 100\%, \quad (9)$$

where C, V, W_{mPEGOSC} and $W_{\text{paclitaxel}}$ represented the paclitaxel concentration of micellar solution, the volume of micellar solution, the weight of mPEGOSC added and the weight of paclitaxel added, respectively.

3.5. Morphological observation

The inverted fluorescence microscope was used to observe the mixed solution of pyrene and mPEGOSC

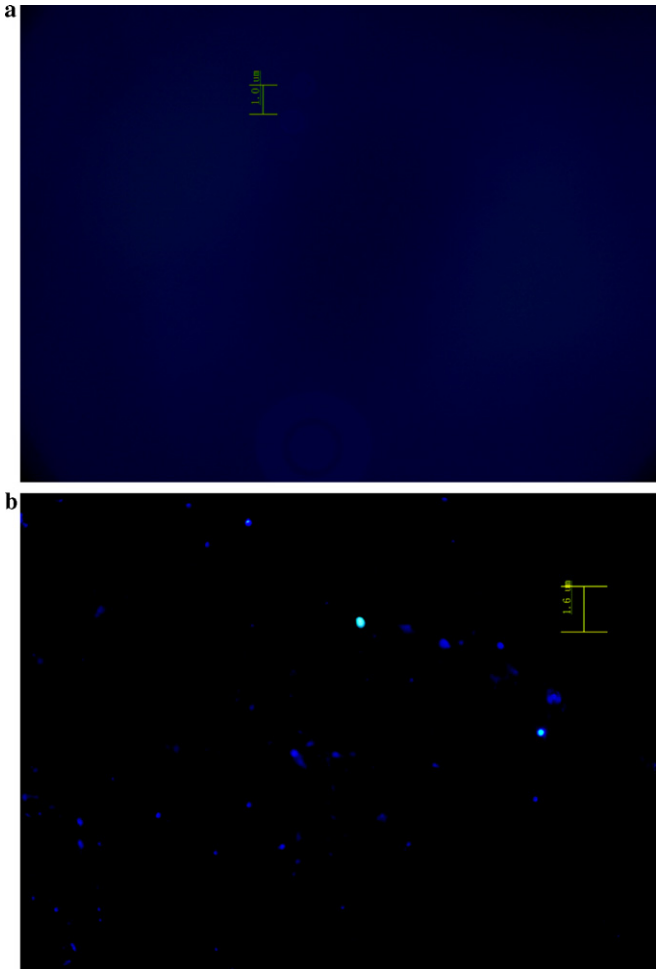


Fig. 9. Fluorescence micrographs of (a) pyrene (6×10^{-7} M) and mPEGOSC2000M (0.0003 mg/ml) in water (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively) and (b) pyrene (6×10^{-7} M) and mPEGOSC2000M (1 mg/ml) in water.

(Fig. 9). When the concentration of mPEGOSC2000M was below CMC, nearly no fluorescence spots was observed, however, when it was higher than CMC, clear fluorescence spots about 100 nm appeared, which resulted from the encapsulation of pyene into micelles formed by mPEGOSC2000M.

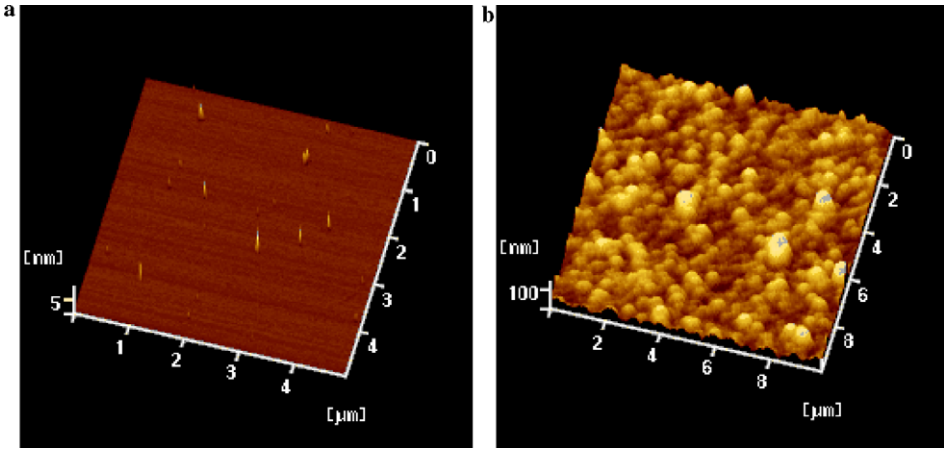


Fig. 10. Atomic force micrographs of (a) blank silicon slice and (b) paclitaxel-loading mPEGOSC2000M micelles (DS of mPEG group, octyl group and sulfate group is 0.10, 0.83 and 2.36, respectively).

Morphology of paclitaxel-loaded mPEGOSC2000M micelles was observed with AFM (Fig. 10). It was seen that the paclitaxel-loading mPEGOSC2000M micelles were spherical and the particle size was about 100 nm.

4. Conclusions

A series of novel *N*-mPEG-*N*-octyl-*O*-sulfate chitosan derivatives, which can form the micelles about 100–130 nm with potential brain-targeting characteristics, were prepared. The chemical structures of chitosan derivatives and some of their physical properties were characterized by ^{1}H NMR, ^{13}C NMR, FTIR, elemental analysis, WAXD and TG. The CMCs of the modified chitosans were found to be 0.011–0.079 mg/ml, and the log CMC was linear relative to four structure parameters, which were DS of chitosan unit, sulfate group, PEG unit and octyl group by mole per kilogram, respectively. The solubility of paclitaxel in mPEGOSC micellar solution was largely increased, and the highest concentration of paclitaxel in mPEGOSC2000H micellar solution was 3.94 mg/ml that was up to about 4000 times that of free paclitaxel in water (less than 0.001 mg/ml). So *N*-mPEG-*N*-octyl-*O*-sulfate chitosan may be used as a potential brain-targeting carrier for drugs with poor water solubility and experiments about their brain-targeting characteristics have been carrying on.

Acknowledgments

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