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Assessment of Palmitoyl and Sulphate Conjugated Glycol Chitosan for Development of Polymeric Micelles

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ABSTRACT

Introduction: Amphiphilic copolymers are capable of forming core shell-like structures at the critical micellar concentration (CMC); hence, they can serve as drug carriers. Thus, in the present work, polymeric micelles based on novel chitosan derivative were synthesized. ***Methods***: Block copolymer of palmitoyl glycol chitosan sulfate (PGCS) was prepared by grafting palmitoyl and sulfate groups serving as hydrophobic and hydrophilic fractions, respectively. Then, fourier transform infrared spectra (FTIR) and spectral changes in iodine/iodide mixture were carried out. ***Results***: FTIR studies confirmed the formation of palmitoyl glycol chitosan sulfate (PGCS) and spectral changes in iodine/iodide mixture indicated CMC which lies in the range of 0.003-0.2 mg/ml. ***Conclusion***: Therefore, our study indicated that polymeric micelles based on palmitoyl glycol chitosan sulphate could be used as a prospective carrier for water insoluble drugs.

Introduction

Amphiphilic copolymers have attracted a number of researchers due to their self-assembly into core-shell nanostructures in an aqueous solution. The core of micelles is formed by hydrophobic blocks of the copolymers, while hydrophilic blocks of the copolymers form the shell of the micelles and stabilize the micellar structure.[1,2]

Size of single molecule of micelles is 10 ~ 30 nm and the size of a micelles is approximately 200 nm having different shapes.[3] Amphiphilic copolymers form nanoscopic core shell structure above the critical micellar concentration (CMC). Here, the hydrophobic nucleus serves as a reservoir for hydrophobic drugs, while the hydrophilic part serves as border line between the bulk aqueous phase and the hydrophobic interior. Highly hydrated outer shells of the polymeric micelles can inhibit inter micellar aggregation of their hydrophobic inner cores. Consequently, the polymeric micelles maintain acceptable aqueous stability irrespective of high contents of hydrophobic drug incorporated into the hydrophobic core.[4] CMC of polymeric micelles can be determined by fluorescent probes,[5] methyl yellow solubilization, surface tension measurements, iodine solubilization[6] and other methods reported in the literature.

The CMC of polymeric micelles is typically on the order of 10^{-6} to 10^{-7} M, while that of low molecular weight surfactant micelles is on the order of 10^{-3} to 10^{-4} M.[7] Polymeric micelles with low CMC are preferred as drug carriers because they have higher stability over low molecular weight surfactant micelles. The higher stability is reflected by the fact that polymeric micelles with very low CMC are more resistant against dilution (e.g. after intravenous administration). Furthermore, polymeric micelles with a "frozen" glassy or crystalline core have greater kinetic stability than those with a liquid-like core, and they may remain intact or dissociate very slowly into monomers even below the CMC.[3,8]

In most cases, polymeric micellar systems consist of hydrophobic drugs and water-insoluble block copolymers and drug loading is performed with organic solvents such as o/w emulsion,[9] dialysis[4] and solid dispersion.[10] We can use polymeric micelles as efficient carriers for compounds which exhibit poor solubility, undesired pharmacokinetics and low stability in a physiological environment.[3]

The aim of present work was to develop Palmitoyl Glycol Chitosan Sulfate (PGCS) polymeric micelles based on glycol chitosan, N-hydroxysuccinimide ester and chlorsulphonic acid as an effective carrier of water

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insoluble drugs.

Material and method

Materials

Glycol chitosan (Sigma, Germany), palmitic acid N-hydroxysuccinimde ester (Fluka, Switzerland), dimethylformamide (Sigma-Aldrich, Germany), chlorosulfonic acid (Sigma-Aldrich, Germany), hydrochloric acid (BDH), sodium hydroxide (BDH), cellulose dialysis tubing (cut off Mw 6491 Sigma Aldrich, USA), potassium dihydrogen phosphate (Merck, Germany), iodine (Sigma-Aldrich, Germany) and potassium iodide (Sigma, Germany) were used. Any other chemicals used were of analytical grade.

Methods

Acid degradation of glycol chitosan

In the present work, polymers were synthesized by slight modification of a procedure reported earlier.[11] First degradation of glycol chitosan was carried out by dissolving two grams of glycol chitosan in 4M hydrochloric acid (150 ml) and then it was placed in a preheated water bath set at 50°C. After 48 h, the reaction was stopped by taking it out of water bath and afterwards the reaction mixture was dialyzed using cellulose dialysis tubing (cut off Mw 6491 Sigma, Aldrich, USA) against distilled water (5 liter with 6 changes over 24 h). At the end of the procedure, dialysate had a neutral pH. After freeze drying of dialysate, polymer was recovered as cream colored wool-like material.

Preparation of stock solutions

Stock solution of glycol chitosan

A 300 mg of glycol chitosan (GC) and 225.6 mg of sodium bicarbonate were dissolved in 30 ml of water.

Stock solution of palmitic acid N-hydroxysuccinimide

A 316.8 mg of palmitic acid N-Hydroxysuccinimide (P-NHS) was dissolved in 60 ml of ethanol by stirring it for 2 h.

Synthesis of PGCS polymer

Two polymers were synthesized simultaneously having optimum combination of hydrophilicity and hydrophobicity. The procedure was as follows: 3.3ml of GC and sodium bicarbonate stock solution (containing 10 mg/ml of GC and 7.52 mg/ml sodium bicarbonate) were taken in test tubes A and B. In second step, 3 and 5 ml of palmitic acid N-hydroxysuccinimide (P-NHS) stock solution (5.28 mg/ml) were added to test tubes A and B, respectively (Table 1). Both test tubes were then shaken for 16 h at room temperature and then heated at 85°C for 4 h to evaporate ethanol. The mixture in the test tubes were extracted with diethyl ether (3 × 5ml) to remove P-NHS and then with ethanol (3 × 5ml) to remove the polar contaminants and so sodium bicarbonate and succinyl compounds. Sulphonation of copolymers is carried out by first suspending them in 4 ml of dimethylformamide (DMF) and stirring overnight. Two dilutions of chlorosulfonic acid with DMF (1:2 and 1:3) were prepared under N_2 atmosphere. Dilution 1:2 was added to test tube "A" and dilution 1:3 was added to test tube "B". The reaction solutions were then neutralized with aqueous 20 % w/v NaOH to pH 7 and dialyzed against distilled water (800 ml with 3 changes) for 3 days and dialysate was lyophilized to get powder (Fig. 1).

Characterization

FTIR of PGCS

To characterize the functional groups (i.e. amine, carbonyl and sulphate of PGCS), FTIR spectroscopy was used (FTIR Shimadzu 8400 S). FTIR samples were prepared by KBr disks method (2 mg sample in 200 mg KBr). The scanning range was 4000-400 cm^{-1} and the resolution was 2cm^{-1}.[12]

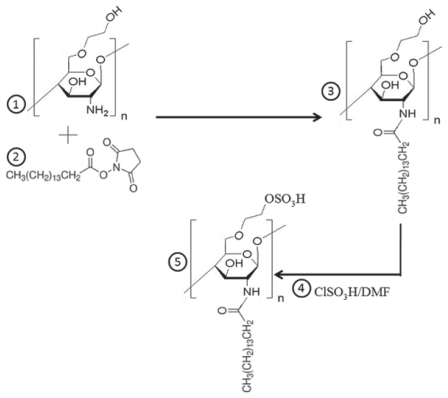

Fig. 1. Synthesis of palmitoyl glycol chitosan sulphate (PGCS). 1: Glycol chitosan (GC), 2: palmitic acid N-hydroxysuccinimde ester, 3: palmitoyl glycol chitosan, 4: chlorosulphonic acid/ dimethylformamide (DMF) 5: palmitoyl glycol chitosan sulphate

Determination of critical micelle concentration of PGCS

CMC of PGCS was determined at 25°C using the iodine/ iodide mixture. A stock solution containing 6.4 $\times 10^{-4}$ and 2 $\times$ 10^{-3} mol/L of iodine/iodide was prepared, respectively. One Milliliter of this solution was diluted with a polymer solution and the absorbance was measured in a UV-visible spectrophotometer (IRMECO U2020) at 360 nm.[6]

Results and discussion

FTIR of PGCS

Fig. 2 shows the FTIR spectra of PGCS. There were two prominent bands at ~1634 and ~1522 cm^{-1} which were assigned to the carbonyl stretching of amide I and amide II bands, respectively. Amide linkage is developed between the C=O of palmitoyl group and the amino group of chitosan, confirming the attachment of hydrophobic group to glycol chitosan. The amide I band region (~1634 cm^{-1}) consists of amide carbonyl C=O stretching with some contributions of C-N stretching and C-C-N deformation. The amide II (~1522 cm^{-1}) band involves both C-N stretching and C-N-H in plane bending.

Sulfonation of glycol chitosan was confirmed qualitatively by FTIR. This can be observed by S=O stretching of the

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Table 1. Quantities of reagents used for palmitoyl glycol chitosan sulphate (PGCS)

Formulation	Glycol chitosan (10 mg/ml)	P-NHS (5.28mg/ml)	$ClHSO_3$ (ml)
M_1	3.3 ml	3 ml	2 ml
M_2	3.3 ml	5 ml	3 ml

sulfonic groups at 1020 cm^{-1} in Fig. 2 which is in close agreement with 1028 cm^{-1} obtained by Johnson and co-workers who prepared sulfonated poly (arylene ether sulfones)[13] and C-S stretching from the sulfonic group at 638 cm^{-1}. Similar results were presented by Pooley *et al* who prepared salt, temperature and pH responsive hydrogels and found S=O and C-S stretching from sulfonic group at 1034 and 626 cm^{-1}, respectively.[14]

Critical micelle concentration

The critical micelle concentration is an important parameter of micelles for their application as a biomaterial. Therefore, in this work, CMC of polymeric micelles was determined at 37°C. Addition of polymers changes the absorption spectra of a solution containing a mixture of iodine and iodide. Below CMC, absorption spectra were similar to aqueous iodine and iodide (I_2/I^-) mixtures' spectra. But as the polymer concentration passed the CMC, abrupt decrease of absorbance was observed. It is assumed that the tri-iodide is displaced toward iodine and iodide as micelles are formed, due to solubilization of more hydrophobic iodine by the hydrophobic core of micelle. Therefore, polymer self-assembly leads to an abrupt absorbance decrease, allowing the CMC determination. Fig. 3 and Fig. 4 show the CMC of M_1 and M_2, respectively. CMC of polymeric micelles is 0.003 to 0.2 mg/ml. The low CMC values make them useful carriers for water insoluble drugs.

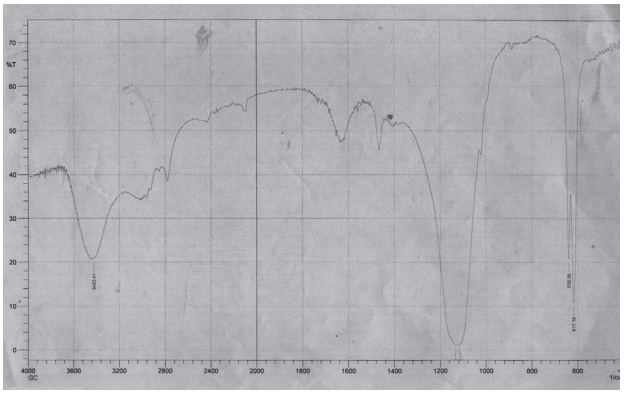

Fig. 2. FTIR spectra of PGCS

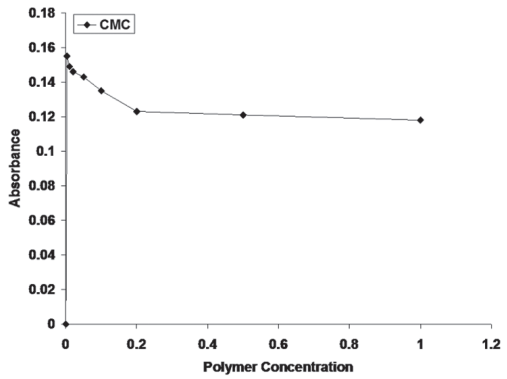

Fig. 3. Determination of CMC for M1 by I_2/I^- mixture

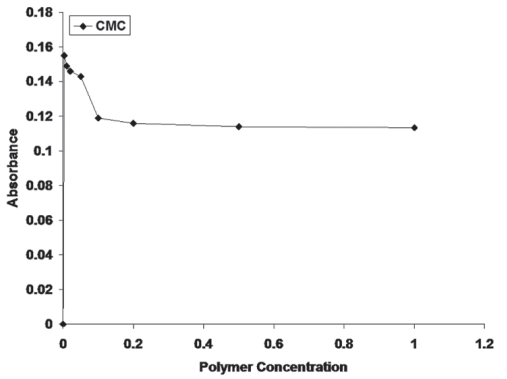

Fig. 4. Determination of CMC for M_2 by I_2/I^- mixture

Conclusion

Two samples of polymeric micelles containing palmitoyl group as hydrophobic moiety and sulfate group as hydrophilic moiety were successfully prepared. The formation of PGCS copolymer was confirmed by FTIR. The polymeric micelles show low CMC values and hence can be used as effective carriers for water insoluble drugs.

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Competing interests

Authors declared no competing interests.

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