Characterization and Preparation of Chitosan Nanoparticle to use as carriers of Hydrophobic Drug

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Abstract
The LM-WSFC with free amine group was purely prepared by salts removal method and identified by spectroscopic assay. The LM-WSFC nanoparticle modified hydrophobically would be used to both of intravenous injection and oral administration of hydrophobic drugs due to their adequate size for administration. We measured NMR, IR, and photon correlation spectroscopy (PCS) to investigate the characteristics of the nanoparticles. The synthesis of hydrophobically modified chitosan nanoparticles was identified from the results of $^1$H-NMR, IR spectrum. From the results of surface morphology observed by TEM and AFM, spherical nanoparticles were identified and its size was 30~100nm. At PCS measurement, the more increase the number of substitutive group, the more decrease the positive charge of nanoparticle surface. Resultantly, nanoparticles using hydrophobically modified chitosan were tested as a suitable device for the drug targeting system to the tumor cell.

1. Introduction
Drug delivery system is a device to carry the drug at wanted site for optimize the therapy of drug. The materials to utilize as drug carriers must have essentially characters such as biodegradable, biocompatible, and nontoxicity etc. In this point, chitosan, having the structure similiar to cellulose, extracted from crab, shrimp, is a suitable biomaterial to design the drug carrier. But, one of severe disadvantage for actual use is poor solubility in water or organic solvents. Thus, for the high solubility of chitosan, it was modified with PEG and modified chitosan formed the self-aggregate to utilize with drug and gene carrier. PEG-g-chitosan was investigated the potential as carriers for delivery of anionic drug by Tatsuro Ouchi et. al. Ick chan Kwon et. al showed the potential as drug carrier of self-aggregates formed by chitosan modified with hydrophobic groups. However, used chitosan had to dissolve in acetic acid which can be act as one of the major drawbacks due to bioactive agents and also don’t use the strong positive charge of chitosan. In this study, we prepared the LM-WSFC by novel method developed in our laboratory and identified by spectroscopic analysis. The LM-WSFC nanoparticles were sucessfully prepared as modify with hydrophobic group.
and hydrophilic group. The nanoparticles were formed as introduce hydrophilic group, PEG, and hydrophobic group, cholesterol, at chitosan chains. PEG chains can prevent cell adhesion by entropically driven steric repulsion and increasing hydrophilicity of carrier surfaces. The introduction of the cholesterol can enhance the association behaviour of chitosan and, stability and activity of the hydrophobic drug can be enhanced by formation of hydrophobic core of the chitosan-cholesterol derivatives.

It may be expected that hydrophobically modified nanoparticles are applicable to the targeting drug carriers.

2. Experimental

2-1. Materials
The LM-WSFC with Mw of 18,579 daltons and Dα of 93% was prepared by salts removal method. Methoxy poly(ethylene glycol) p-nitrophenyl carbonate (MPEG-pNP) as hydrophilic moiety was purchased from Sigma Co. and cholesteryl chloroformate as hydrophobic group was purchased from Aldrich co. Dialysis tubing (MWCO 8,000) was commercially obtained from Spectrum. Dimethylformamide (DMF) as reagent grade was used without further purification.

2-2. Preparation of LM-WSFC nanoparticles
This experiment was progressed with two steps. The first step is to formation the amide bond at between amine group of chitosan and carbonyl group of MPEG-pNP and the second step is to formation the other amide bond at between residue amine group of 1st-step product and carbonyl group of cholesteryl chloroformate. The LM-WSFC nanoparticles were synthesized by dialysis method with ratio of 2 molecular of MPEG-pNP and 2, 4, 6 molecular of cholesteryl chloroformate per 10 anhydroglucosamine units of chitosan, respectively.

2-3. Measurement of FT-IR and $^1$H-NMR spectroscopy
FT-IR(Shimadzu, FT-IR 8700) and $^1$H-NMR spectrometer(Bruker, DRX-500MHz) was used to identify the synthesis of chitosan nanoparticles modified with hydrophobic group and hydrophilic group. For $^1$H-NMR measurement, the chitosan nanoparticles was dissolved in CDCl$_3$ at a concentration of 10mg.ml$^{-1}$ and the spectra were performed at 353K.

2-4. Transmission electron microscope (TEM) observation
A drop of nanoparticles suspension containing 0.01% of phosphotungstic acid was placed on a carbon film coated on a copper grid for TEM. Observation was done at 80kV in a JEOL, JEM-2000 FX-II, Japan.

2-5. Atomic force microscope (AFM) observation
The morphology of chitosan nanoparticles was confirmed by AFM. Nanoparticle of 0.1mg/ml in distilled water was placed on a silicon wafer surface. AFM image was obtained by (PARK's Science, Autoprobe CP) at room temperature with cantilever oscillation frequencies between 12 and 24 kHz.

2-6. Photon correlation spectroscopy (PCS) measurements
PCS was measured with a Zetasizer 3000 (Malvern instruments, England) with
He-Ne laser beam at a wavelength of 633 nm at 25°C (scattering angle of 90°) for the determination of electrophoretic mobility.

3. Results and discussion

In this study, we prepared the LM-WSFC by novel method developed in our laboratory and identified by spectroscopic analysis. Using LM-WSFC, chitosan nanoparticles were prepared as introduce the hydrophobic and hydrophilic group at the C-2 position of chitosan by dialysis method to investigate the potential as drug delivery system.

The introduce of cholesterol as hydrophobic moieties were expected to increase the solubility of the hydrophobic drug and, by incorporation of PEG as hydrophilic group, steric stabilization of chitosan nanoparticles would be increased. Also, the introduction of the cholesterol can enhance the association behaviour of chitosan and, stability and activity of the hydrophobic drug can be enhanced by formation of hydrophobic core of the chitosan-cholesterol derivatives.

At the FT-IR spectrum of nanoparticles, the character peak of PEG by aliphatic –CH showed at 2880 cm⁻¹ and the C-O band of cholesteryl chloroformate was identified at 1110 cm⁻¹ sharply. The amide band by bonding formed between the amine group of chitosan and PEG or cholesteryl chloroformate was observed at 1640 cm⁻¹ and the absorption of amine group at 1590 cm⁻¹ was decreased due to formation of amide bonding by amine and carbonyl group. Also, from the results of ¹H-NMR data, the synthesis of chitosan nanoparticles was identified. The character peak by 4-H at cholesteryl chloroformate was observed at 1.8 ppm. Chitosan and PEG showed the character peak at 4.5, 4.3 ppm, respectively.

Fig. 1(a,b,c) shows TEM photographs of chitosan nanoparticles with the ratio of 10:2:2, 10:2:4, 10:2:6, the ratio for the units of glucosamine : PEG : cholesteryl chloroformate. The shape of the nanoparticles was spherical and the sizes were ranged about 30 nm~100 nm in diameter. Fig. 2. shows AFM observation of chitosan nanoparticles with the ratio of 10:2:2. The morphology and size of nanoparticles is agree with the TEM results. Table 1. shows the zeta potential of chitosan nanoparticles according to the number of group substituted at C-2 position of chitosan and the size of chitosan nanoparticle by TEM measurements. As results, the more increase the number of substitutive group, the more decrease the positive charge of nanoparticle surface. This means that the positive charge having amine group of chitosan was neutralized by substitution with other group.

In conclusion, it was showed that LM-WSFC prepared successfully in this work can form the modified chitosan nanoparticles for drug delivery system. Therefore, it was expected that chitosan nanoparticles modified with hydrophobic group can be increase the solubility of hydrophobic drug

Reference
Table 1. Particle size and zeta potential of Chitosan nanoparticles

<table>
<thead>
<tr>
<th>Chitosan nanoparticle</th>
<th>the number of substituted group per 10 units of glucosamine</th>
<th>Zeta potential (mV)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample 1</td>
<td>4</td>
<td>11.5</td>
<td>70~100</td>
</tr>
<tr>
<td>sample 2</td>
<td>6</td>
<td>3.2</td>
<td>50~80</td>
</tr>
<tr>
<td>sample 3</td>
<td>8</td>
<td>1.1</td>
<td>30~50</td>
</tr>
</tbody>
</table>

Fig. 1. TEM photographs of modified LM-WSFC according to the ratio of hydrophobic group
(Chitosan : PEG : Cholestryl chloroformate)

Fig. 2. The AFM observation